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Comparison of nicotine contents in local and imported cigarettes sold in Abidjan markets in Côte d'Ivoire: Lessons for regulation

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Tobacco use is one of the main leading causes of disease and death worldwide and according to the World Health Organization (WHO), constitutes a major issue to public health. Given the importance of nicotine as the major constituent of tobacco and its addictive potency on consumers, determining its content in cigarettes sold on Ivorian markets shows to be important. The aim of this study was to determine and compare nicotine content in imported and local brands of cigarettes marketed in the district of Abidjan. Nicotine was extracted from twenty-one brands of cigarettes with six being local and fifteen imported. Nicotine contents were measure by UV-Vis spectrophotometry at a wavelength of 508 nm. The assay method was linear on a concentration range of 0.2 to 1 mg/ml with a coefficient of determination of 0.9963 and a detection limit of 0.15 mg/ml. The average nicotine content in cigarettes of Ivory Coast brands was 1.73 ± 0.19 mg of nicotine/cigarette while that in imported brands was 2.25 ± 0.74 mg of nicotine/cigarette ($p < 0.05$). The lowest nicotine content was found in the local brand cigarette "Ex001", whereas the highest content was found in an imported brand cigarette "OR001 Pulse Bleu". The nicotine content of all tested cigarettes was higher than the European standard and a significative difference was found between the determined amounts of nicotine content in the cigarettes brands and the amounts displayed by the manufacturer on the cigarettes' packs. Monitoring of nicotine content in cigarettes sold on the Ivorian market should be considered in prevention strategies against smoking addiction and tobacco-related diseases.

Key words: UV-Vis Spectrophotometry, tobacco cigarettes, nicotine content, local market, Abidjan, Côte d'Ivoire.

INTRODUCTION

Tobacco use is one of the leading preventable causes of disease and death worldwide. According to the World

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Health Organization (WHO), tobacco represent a major issue to public health resulting in the death of more than half of the smokers (Maatouk and Barkallah, 2013). Elsewhere, Tobacco addiction in the world is responsible for 7 million of death yearly with 80% occurring in developing countries. Based on WHO projections, the number of annual deaths from 2020 to 2030 will reach ten million, with 70% coming from developing countries. Close to 80% of the billion smokers in the world live in countries with a low or intermediate income, particularly on the African continent (Amadou et al., 2019; Banque Mondiale, 2000; Marie-Hélène et al., 2018).

The health effects of tobacco smoke in humans are well known from both clinical and epidemiological studies. Smoked tobacco in any form causes up to 90% of lung cancer and is a significant risk factor for Chronic Obstructive Pulmonary Disease (COPD), Cerebral Vascular Accident (stroke) and hypertension (Naoussi Sango, 2010; Tachfouti, 2014). WHO states that 75% of cancers are either directly or indirectly related to smoking, including cancer of the throat and esophagus. In women, it is responsible for a decline in fertility, an increase in spontaneous abortions and a higher perinatal and neonatal mortality (Fofana, 2013; Naoussi Sango, 2010).

Many studies suggest a high transfer of nicotine from the blood stream, and therefore, testicular cells and spermatogenesis may be vulnerable to its effects (Kovac et al., 2015; Mabroukah Al-Darmoon et al., 2015; Price and Martinez, 2020; Tayoub et al., 2015).

Various tobacco products are consumed worldwide, but cigarette remains the most popular shape, mainly consumed as smoked tobacco (Amadou et al., 2019). Nicotine, also named as (S)-3-(1-méthylpyrrolidin-2-yl) pyridine, constitute about 1.5% of tobacco weight of sold cigarettes (Benowitz et al., 2009). It is the major active molecule in cigarette smoke and the principal alkaloid present in the leaves of *Nicotiana tabacum* (Taghavi et al., 2012; Tayoub et al., 2015). Nicotine is well known to be highly addictive and toxic depending on the level of exposure (Ali and Ibrahim, 2016; Carter et al., 2009; Johnson et al., 1973). Standard textbooks, databases, and safety sheets consistently state that the lethal dose for adults is 60 mg nicotine or less, leading to safety warnings that ingestion of five cigarettes could kill an adult (Hayes, 1982; Mayer, 2014).

Moreover, nicotine counts among the 4000 chemicals, such as carbon monoxide, hydrogen cyanide, B[a]Pyrène or heavy metals, ranked as toxic chemical products or carcinogenic found in tobacco leaves and in cigarette smoke. Consequently, nicotine appears to be a good indicator of the addictive potency of cigarette smoke, but also a promising tracer of exposure to the other toxic or carcinogenic substances co-existing in tobacco cigarettes smoke (Balfour, 2004; Borgerding and Klus, 2005; Carter et al., 2009; Johnson et al., 1973; Tayoub et al., 2015).

In Côte d'Ivoire, a wide range of tobacco cigarettes brands, imported, locally manufactured or even smuggled, are sold on the local market. Teenagers are the largest users of cigarettes with a smoking prevalence of 21% (Marie-Hélène et al., 2018). As a result, smoking is responsible for more than 5,000 deaths per year and was incriminated in 90% of lung cancers in 2011-2012. The cost related to the diseases caused by smoking would be 27 billion in Ivory Coast (Amadou et al., 2019).

Unfortunately, data available on the nicotine content of tobacco cigarettes smoked by the Ivorian population is scarce. This situation is an important hurdle in the assessment of exposure and toxicological risks associated with tobacco cigarettes smoking in this young population.

Several analytical methods have been reported for the determination of nicotine in tobacco. However, all these approaches based on liquid or gas chromatography and mass spectrophotometry are very expensive and require highly experimented staff (Ali and Ibrahim, 2016). The first aim of this study was to evaluate the analytical performances of a simple, economical and sensitive UV-Visible spectrophotometric method for the quantification of nicotine in tobacco cigarettes. Then, the method was applied for the determination and comparison of nicotine content in different cigarettes brands sold on the local market in the District of Abidjan.

MATERIALS AND METHODS

Reagents

A stock solution of NaOH 10 M was prepared by dissolving 40 g of sodium hydroxide in 100 ml of distilled water. An amount of 10.95 g zinc acetate was dissolved in 1.5 ml of concentrated acetic acid, the volume was completed to 50 ml with distilled water. Then, 1.06 g of $K_4Fe(CN)_6$ was dissolved in 10 ml of distilled water to prepare 10.6% (w/v) potassium hexacyanoferrate(II) solution. All working solutions were prepared according to the method developed by Al-Thamrah (Al-Tamrah, 1999).

Sodium hypochlorite (NaClO)

Sodium hypochlorite (3.10^{-3} M) was prepared daily by diluting 56.07 µl sodium hypochlorite (12°; 0.535 mol/l) in distilled water and adjusted to the mark with distilled water in a 10 ml volumetric flask. It was stored in a dark bottle and protected from light.

Methyl orange

Stock solution (3.10^{-3} M) of methyl orange was prepared by dissolving 0.05 mg of methyl orange in distilled water and diluted up to the mark with distilled water in a 50 ml volumetric flask.

Table 1. Description of the cigarettes's brands sampled in the district of Abidjan.

Brand	Brand codes	Site of manufacturing
Local brands	F001 (Rouge/ Lights /Duo)	Sitab (Imperial Brands)
	Ex001	Sitab (Imperial Brands)
	N001 (Rouge/Bleu)	Sitab (Imperial Brands)
Imported brands	CA001 (Original/ Click Bleu/Click Violet/Gold)	British American Tobacco (BAT)
	W001 Light	Japan Tobacco (JT) International
	M001 (Gold/Red)	Philip Morris
	DI001 (Rouge Select and Rouge)	British American Tobacco (BAT)
	DA001 Gold	Imperial Brands
	LM Blue	Philip Morris
	OR001 (Double Apple Marula/Pulse Bleu/Pulse Orange)	Trade mark Owner in Germany

Hydrochloric acid

Hydrochloric acid (0.25 M) was prepared daily by diluting 0.414 ml hydrochloric acid (37%, 1.19 g/ml) in distilled water and diluted to the mark with distilled water in a 20 ml volumetric flask.

Nicotine standard solution

Stock solution (1 mg/ml) of nicotine (99.1%, Sigma Aldrich-Germany) was prepared by dissolving 50 µl in a small portion of distilled water then made up it to 50 ml in a volumetric flask. It was stored in a dark bottle and protected from light. Desired concentrations were obtained by diluting the stock solution.

Instruments

Ultraviolet-visible double beam spectrophotometer from ANALYTIK JENA, Specord 100 Plus model (Germany) was used at fixed wavelength of 508 nm with 1 cm quartz cells.

Samples collection

A stratified sampling technique was applied to ensure a good representation of cigarettes' retailers in the District of Abidjan. First, Abidjan was divided in 10 municipalities and 3 municipalities were selected based on the population size. Then, in each selected municipality, we randomly selected 3 tobacco retailers and bought one pack of all the cigarettes brands available in the retailer stock. As a result, each cigarette brand available on the local market at the time of the study was represented in our final sample.

Twenty-one different brands of cigarettes, six local brands and fifteen imported brands available on the local market were randomly chosen to evaluate their nicotine content. Local and imported brands of cigarettes packs with 20 sticks per pack were bought from local market in the 3 more popular areas in the District of Abidjan. Details on cigarettes sampled for the study are summarized in the Table 1.

Extraction of nicotine

The extraction method of nicotine from cigarettes applied in this work was carried out according to the method presented by Suryani et al. (2012) with slight modifications. The main modification concerned the optimization of the extraction solvent (methanol) volume to be added to the sample. Ten sticks of cigarettes from each of the various samples without rolling paper were weighed and pulverized in a blender. Each of the samples was placed in a 100 ml beaker with 25 ml methanol and was stirred with a magnetic agitator for 30 min. Distilled water of 25 ml was added, followed by 1 ml of 2 M sodium hydroxide, and was stirred into the solution for another 30 min. The mixture was heated in a water bath for 6 min without leaving it to boil. It was then cooled and filtered through a filter paper into a 50 ml volumetric flask (filtrate 1). Then, 1 ml of zinc acetate and 1 ml potassium hexacyanoferrate (II) were added into the filtrate, swirled, and shook slowly to mix. Distilled water was added to complete the volume to the mark. The mixture was centrifuged at 3.600 rpm for 5 min. The supernatant was collected into a 50 ml beaker, and the residue was discarded. Activated carbon of 1 mg was added, mixed thoroughly, and allowed to settle for 2 min before 5 ml of 0.01 M sodium hydroxide was added. The mixture was filtered through a filter paper into a 50 ml volumetric flask. Finally, distilled water was added to complete the volume to the mark (filtrate 2).

Calibration curve for nicotine quantification

Five standard solutions of nicotine were prepared by dilution of standard stock solution with distilled water (1 mg/ml) at increasing concentration within the range of 0.2 to 1 mg/ml. The absorbance (A) of each of the five standards of nicotine solutions were individually measured by UV-Vis spectrophotometry at the wavelength of 508 nm. Then, the values of absorbances were plotted against corresponding concentration to obtain the calibration curve.

Determination of nicotine content in cigarettes samples

Nicotine contents in the extracted solution using the aforementioned procedure for different brands of cigarette samples were determined using UV-Vis spectrophotometer by the measurement of specific absorbance at a wavelength of 508 nm. The assay method used in this work was carried out based on the method presented by Ali et al. with slight modifications in order to

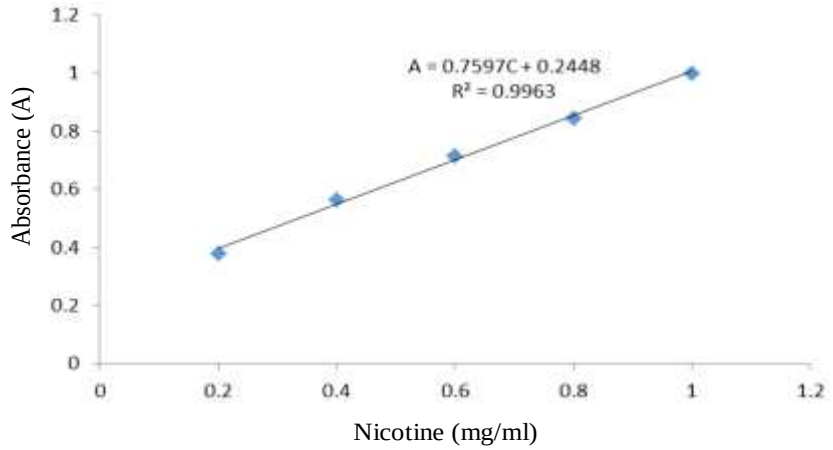

Figure 1. Calibration curve of nicotine standard.

optimize the concentration of the colored indicator methyl orange (Ali and Ibrahim, 2016). Accordingly, 1 ml of 3.10^{-3} M of NaClO and 3 ml of 1 mg/ml nicotine/the filtrate 2 were added to a 25 ml volumetric flask and allowed for 1 min (time 1), followed by the addition of 1 ml of 1.10^{-3} M methyl orange and allowed again for 1 min (time 2). Finally, 1 ml of 0.25 M HCl was added and allowed for 1 min (time 3) and the solution was diluted to the mark with distilled water. Then the absorbance of the final solution was measured at 508 nm against a blank, and the concentration derived from de calibration curve.

Analytical performances of the method

As we slightly modified the method presented by Ali et al. (2016), the following analytical performances were assessed: precision, accuracy, linearity, limits of detection (LOD) and quantification (LOQ). Precision was expressed as a relative standard deviation (%RSD). Accuracy was assessed by determination of recovery after spiking a tobacco matrix with known concentration of nicotine. Linearity was evaluated through the coefficient of determination (R^2). The LOD and LOQ were calculated using the signal-to-noise technique.

Statistical analysis

The comparison of the average nicotine content, expressed in mg/cigarette, between imported and local brands of cigarette, has been done by the non-parametric Mann-Withney test, with a confidence interval of 95% using SPSS software. Comparison between average nicotine in local and imported cigarettes was graphically represented by a boxplot.

RESULTS

Analytical performances of the method

Standard solutions of nicotine were used to evaluate the analytical performance of the quantification method. The calibration curve was constructed between the absorbances and concentrations of nicotine standards (mg/ml). The method showed a good linear response with a determination coefficient R^2 = 0.9963 over the range from 0.2 to 1 mg/ml (Figure 1). The intra and inter day precision of the method, evaluated for 0.2, 0.6 and 1 mg/ml, was also good as confirmed by RSD varying between 0.011 and 5.59%. The accuracy, evaluated by standard addition, was estimated to 88 and 106%, respectively for 0.4 and 0.6 mg/ml. The limit of detection was established at 0.15 mg/ml and the limit of quantification was 0.46 mg/ml.

Nicotine content in cigarettes samples collected in the district of Abidjan

The content and percentage of nicotine in each cigarette, in six local brands and fifteen imported brands of cigarettes are presented in Tables 2 and 3 respectively. Globally, the average nicotine content in local brand cigarettes, which is 1.73 mg/cigarette ± 0.19 (range: 1.5-2.03 mg/cigarette), was significantly different (p- value = 0.045) compared to the average nicotine content in imported brands, namely 2.25 mg/cigarette ± 0.74 (range: 1.514 - 4.04 mg/cigarette) (figure 2). Each cigarette contained an average of 0.25 and 0.43% nicotine for local and imported brands of cigarettes, respectively (Tables 2 and 3).

Figure 3 shows the distribution of nicotine content expressed in mg/cigarette by commercial brand of cigarette. Findings highlight the great variation in nicotine concentration among the different local and imported brands. The lower nicotine concentration was found in the local cigarette brand "*Ex001"* (1.5 mg/stick) and the

Table 2. Nicotine and tobacco content as well as percentage of nicotine content in each local brands of cigarette.

Brands of cigarettes	Nicotine content			Tobacco content/cigarette (g)
	Nicotine (mg/cigarette)	Nicotine (mg/g tobacco)	Nicotine/cigarette (%)	
F001 Rouge	1.509 ± 0.002	1.96 ± 0.002	0.197 ± 0.0002	0.76
F001 Duo	1.90 ± 0.0008	2.62 ± 0.001	0.26 ± 0.0001	0.72
F001 Lights	2.03 ± 0.002	3.10 ± 0.003	0.31 ± 0.0003	0.65
Ex001	1.5 ± 0.0002	2.14 ± 0.0003	0.21 ± 3.36E-05	0.69
N001 Bleu	1.73 ± 0.0002	2.63 ± 0.0003	0.26 ± 3.79E-05	0.65
N001 Rouge	1.74 ± 0.0002	2.56 ± 0.0003	0.25 ± 3.54E-05	0.68

Table 3. Nicotine and tobacco content as well as percentage of nicotine content in each imported brands of cigarette.

Brands of cigarettes	Nicotine content			Tobacco content/cigarette (g)
	Nicotine (mg/cigarette)	Nicotine (mg/g tobacco)	Nicotine/Cigarette (%)	
CA001 Original	2.013 ± 0.0003	3.12 ± 0.0005	0.31 ± 5.27E-05	0.64
CA001 Click Bleu	2.19 ± 0.0004	3.42 ± 0.0006	0.34 ± 6.71E-05	0.63
CA001 Click Violet	2.43 ± 0.007	4.12 ± 0.012	0.41 ± 0.0012	0.59
CA001 Gold	2.10 ± 0.0001	3.43 ± 0.0002	0.34 ± 2.90E-05	0.61
MA001 Rouge	2.26 ± 0.0002	3.84 ± 0.0004	0.38 ± 4.74E-05	0.58
MA001 Gold	1.78 ± 0.0002	2.71 ± 0.0003	0.27 ± 3.82E-05	0.65
LM Blue	1.63 ± 0.0001	2.23 ± 0.0002	0.22 ± 2.05E-05	0.73
W001 Lights	2.13 ± 0.0002	3.68 ± 0.0004	0.36 ± 4.89E-05	0.57
DA001 Gold	1.52 ± 0.0001	2.20 ± 0.0001	0.22 ± 1.14E-05	0.69
DI001 Rouge Select	1.514 ± 0.0001	2.04 ± 0.0001	0.20 ± 1.99E-05	0.74
DI001 Rouge	1.519 ± 0.0002	1.95 ± 0.0002	0.19 ± 2.71E-05	0.77
OR001 Pulse Orange	3.86 ± 0.001	10.48 ± 0.002	1.04 ± 0.0002	0.3
OR001 Pulse Bleu	4.04 ± 0.0007	11.74 ± 0.002	1.17 ± 0.0002	0.34
OR001 Double Apple	2.07 ± 0.0003	4.24 ± 0.0004	0.42 ± 4.59E-05	0.48
OR001 Marula	2.71 ± 0.0003	5.43 ± 0.0006	0.54 ± 6.6E-05	0.49

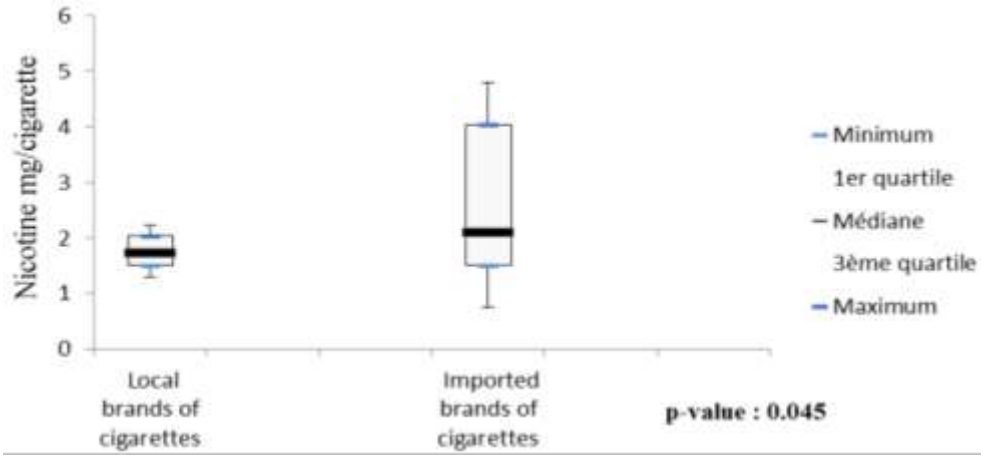

Figure 2. Comparison of the average nicotine content in local and imported brands of cigarettes.

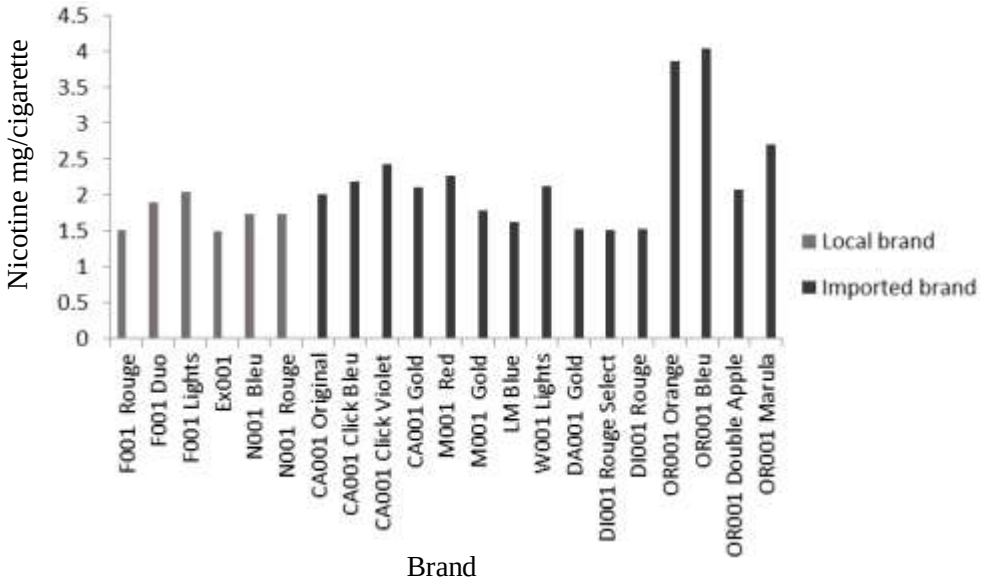

Figure 3. Nicotine content in different local and imported brands of cigarettes.

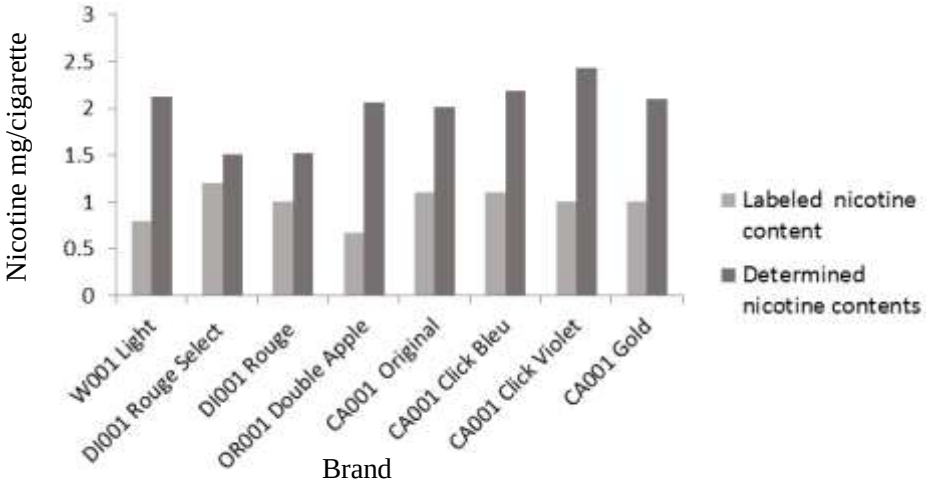

Figure 4. Difference between labeled and determined nicotine contents in some imported brands of cigarettes.

higher concentration in the imported trademark "*OR001 Pulse Bleu*" (4.04 mg/stick). However, the amount of nicotine in 1 g of those cigarettes shows, respectively 2.14 mg and 11.74 mg/g of tobacco.

Interestingly, it was found that three brands, one local and two imported, labelled as "light" had higher nicotine concentrations (F001 light = 2.03 mg/stick; CA001 Gold = 2.10; W001 light = 2.13 mg/stick) than many other regular cigarette brands. In addition, comparison between the nicotine content indicated on some imported cigarettes packaging and the measured nicotine content showed that labelling underestimated the real nicotine concentration in 8 imported brands (Figure 4).

DISCUSSION

Six local brands of cigarettes and fifteen imported brands of cigarettes were analyzed for their nicotine content. Cigarettes from the local brand "Ex001", contained the

lowest nicotine content whereas those from the local brand "F001 Light" had the highest nicotine content. The latter represents indeed 45% of the Ivorian tobacco market according to the Abidjan newspaper published on 11 January 2018 (Ouattara, 2018). Therefore, the high market rate of this brand of cigarettes may expose the Ivorian population to a more consequent risk of consumption and addiction, especially since the population exposed tends to be young with a tobacco prevalence of 21% in Ivorian teenagers (Marie-Hélène et al., 2018). It is now well established that the earlier a person begins to smoke, the more difficult it is to quit. The increased use of tobacco cigarettes in teenagers negatively impacts the individual, family and nation. Young smokers are more likely to be caught in the vicious circle of poverty because of the amount of money spent on tobacco products, increased health expenditure due to tobacco-related diseases, and premature death in their active life (Ahmad et al., 2004).

The local brand of cigarettes marketed as “light” (“F001 light”) revealed a measured nicotine content (2.03 mg/cigarette) higher than normal cigarettes (1.90 and 1.509 mg/cigarette for the “F001 Duo” and “F001 Rouge”, respectively). Consequently, people who smoke light cigarettes, unknowingly, are exposed to a higher level of nicotine than whose smoking normal cigarettes; which contributes in strengthening their addiction and increasing their exposure to carcinogenic compounds found in tobacco smoke (Mahmood and Zaman, 2010). Moreover, tobacco use increases the risk of developing severe forms of Covid-19 in smokers (Olds and Kabbani, 2020). Emergent evidence supports the involvement of smoking as a key predisposing factor for COVID-19-related illness severity and mortality based on recent study of 1,590 patients from 575 hospitals in China (Guan et al., 2020). Consequently, in the present Covid pandemic context, reinforcement of regulation on nicotine content in cigarettes appears to be relevant. The method we have validated could be applied for quality control of cigarettes produced or imported in Côte d’Ivoire.

Among the imported brands, cigarette "DI001" contained the lowest nicotine contents while cigarette "CA001 violet" and "OR001 Pulse Bleu" contained the highest nicotine contents (2.43 and 4.04 mg nicotine/cigarette, respectively). These cigarettes brands are cheap and mostly consumed by low-income customers and teenagers. The price of these cigarettes’ packs would rather depend on the tax paid than the nicotine contents contained within: "DI001" (1.95 mg of nicotine/g of tobacco) is sold for 1000 FCFA or 1.85 USD, whereas the "M001" brand (3.84 mg de nicotine/g of tobacco) is sold for 700 FCFA (1.25 USD). The relation between the high concentration of nicotine in cigarettes and the low selling prices, also reported by Suryani (2012), contributes to tobacco addiction in young consumers. Furthermore, selling at a unit price the cigarettes (25 FCFA in Côte d’Ivoire) is a factor favoring the growth of tobacco use among teenagers (Boli, 2018; Suryani et al., 2012).

From the twenty-one brands of cigarettes studied, only eight displayed the nicotine contents on their packs (38.09%). Our results showed that the actual nicotine contents were higher than those claimed by the manufacturer of all the brands involved. These results match with those obtained by Musa et al. (2017) in Nigeria, where out of fifteen brands of cigarettes analyzed, thirteen had nicotine contents displayed on their pack different from the actual nicotine content by more than 23% (Musa et al., 2017). In addition, these imported cigarettes from Switzerland or Europe, where the maximal amount of nicotine a cigarette can contain is 1 mg according to the policy 2014/40/UE of the European parliament and the Council of the European Union (Official Journal of the European Union, 2014), all have nicotine amounts higher than 1 mg in our study.

In order to compare the results obtained by the method used in the present study, the brands "LM" and "M001" were used. Results showed that nicotine contents found in the brands analyzed (2.23 and 3.84 mg/g of tobacco for "LM" and "M001", respectively) closely met those found by Mabroukah et al. (2015) in Libya (2.61 and 4.87 mg/g of tobacco for "LM" and "M001", respectively) (Mabroukah Al-Darmoon et al., 2015). A different technique, specifically LCMSMS, was used by Vlase et al. (2005) to determine the nicotine content in the same cigarettes’ brands. The results found for "LM" and "MA001" were superior to those of our study. Nicotine contents were 9.16 and 9.86 mg/g of tobacco in cigarettes analyzed by Vlase meanwhile they were 2.23 mg and 3.84 mg/g of tobacco, respectively for the cigarettes analyzed in this study (Vlase et al., 2005). These differences in the content may be due to either the method used to determine the nicotine content, or the weight of each cigarette stick which depends on the quality of tobacco leaves used, the process of manufacturing and packaging of tobacco in cigarette form (Mabroukah Al-Darmoon et al., 2015).

Conclusion

Results of this study revealed that the local brands of cigarette contained less nicotine than the imported brands. Nevertheless, all these cigarettes had nicotine contents above 1 mg/cigarette. Eight out of the twenty-one brands of cigarette studied displayed nicotine contents on their packs. Values claimed by the manufacturer were lower than those obtained in our study. Based on the results obtained by the present

study, we suggest that nicotine content in those cigarettes should be lowered to the recommended level in order to reduce the risk of addiction and exposure to carcinogenic compounds found in cigarette smoke responsible for tobacco-linked pathologies. The method for the quantification of nicotine content in tobacco we have developed in the National Laboratory of Public Health could be applied routinely for the quality control of cigarettes sold on the local market.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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