Comparative Assessment of Nicotine Content in some Cigaratte (Tobacco) in Benin City, Nigeria

¹Oluwatosin A. Akinyemi, ²Amraibure Odia, ³Inegbenose Godwin Ihimire, ¹Harrison Onome Tighiri

¹Department of Environmental Sciences, Cyprus International University, Haspolat-Lefkosa, Via Mersin 10, Turkey, ²Chemistry Department, Ambrose Alli University, P.M.B., 14, Ekpoma, Edo State, Nigeria, ³Biochemistry Department, Ambrose Alli University, P.M.B., 14, Ekpoma, Edo State, Nigeria.

Abstract – The objective of this study is to provide information on the level of nicotine content of various brands of cigarettes sold in Nigeria using Benin city cigarette market as a case study. Twelve brands of cigarettes were purchased from the open market in Benin City, Edo State Nigeria for their Nicotine content. Nicotine was extracted with organic solvent from the respective brands and percentage nicotine content determined by titration with 0.1M H₂SO₄. Mean values of 1.27 – 3.62% were observed in the products. The mean comparison reveals statistical significance (P<0.05) difference in the level in the brands values observed compared with those reported with HPLC/mass spectrometry or spectrophotometric methods determined. The study suggests that the procedure can be reliably used in quality control.

Keywords - Cigarettes, Nicotine, HPLC, Mass Spectrometry, Quality Control, Benin City, Nigeria

---- ♦

1 INTRODUCTION

IN recent years, the tobacco industry has aggressively targeted African nations, described by the industry as "exciting prospects" for hooking a new generation of smokers. This attention from tobacco companies is alarming in countries where many people do not realize the harms of tobacco use. Nigeria's GATS report shows that 20 percent of Nigerians do not know that smoking causes serious illness and only half know that smoking causes stroke [1].

The Nigeria report also indicates a high level of risk to those who do not understand the risks of breathing secondhand smoke. One in four Nigerians does not believe that secondhand smoke is harmful to non-smokers. Secondhand smoke – a deadly mixture of more than 7,000 chemicals, at least 69 of which cause cancer – is a known cause of death and disease in non-smokers. The only way to effectively protect the public from the dangers of secondhand smoke is through the implementation of 100 percent smoke-free laws [1].

Nigeria ratified the World Health Organization's Framework Convention on Tobacco Control (WHO FCTC) in 2005. Under the treaty, Nigeria is obligated to implement methods proven to reduce tobacco use including smoke-free public places, increased tobacco taxes, bans on tobacco advertising, promotion and sponsorship and effective warning labels on tobacco products. Implementing these policies will not only fulfill Nigeria's obligations to the treaty, but will protect Nigerians from the devastating social and economic burden of tobacco use – the world's leading cause of preventable death [1]. Cigarette commonly referred to a small roll of finely cut tobacco leaves wrapped in a cylinder of thin paper for smoking [2]; [3]. About half cigarette smokers die of tobacco-related diseases and loss on average of 14 days of life (Doll et al., 2004). Use of cigarette use by pregnant women has also been shown to cause birth defects including mental and physical disabilities.

Tobacco leaf contains 0.6 - 3% of their dry weight of nicotine. In edible plants, nicotine occurs in the range of $2 - 7 \Box g/kg$. It functions as an antiherbivore chemical. In low doses, an average cigarette yields about 1mg of absorbed nicotine. At high doses, 30 - 60mg is yielded and this could have fatal effect [4]; [5]. Nicotine has been reported to have stimulant effect responsible for the dependence forming properties of tobacco smoking [6]. Nicotine addiction has historically been the hardest addiction to break and has been associated with inhibition of chromatin-modifying enzyme which increases the ability of cocaine to cause an addiction [7]; [8]; [9]; [10].

Nicotine is an alkaloid with chemical formula of $C_{10}H_{14}N_2$. In proper nomenclature, nicotine is 3-(1-methyl-2-pyrrolidinyl) pyridine [11]. It is a bicyclic compound with a pyridine cycle and a pyrrolidine cycle. In nature, nicotine exists in S-shape which is levogyre. In processed tobacco leaf for cigarette, nicotine has been found to the extent of 10mg [12]; [7]; [13]; [14]. In smokers, 1 – 2mg of nicotine has been found after each cigarette. This has been found to be enough to make someone addicted to smoking [7]; [15]. As a result of the problematic nature of nicotine in tobacco in cigarette, various assessment procedures have been devised. The major problems in the usage of this include requirement for expensive instrumentation and high-level trained personnel among other associated with high contending issues of stable electricity supply in most developing countries. In realisation of this, the study is aimed at providing an inexpensive, less vigorous approach in the monitoring of nicotine content in cigarette or in processing tobacco for cigarette manufacture. Extraction with organic solvent and titrimetric approach was used in determination. Highly diluted titrant was used to enhance the level of detection. The result was compared with those earlier reported for similar product done with higher precision instrumentation.

Cigarette consumption in African countries is on a rampant increase both locally made and imported ones. Many Western governments and health authorities now try to persuade people not to smoke because of the health related side effects and socioeconomic problems. In some developed countries, consumption has already begun to fall and as a result, tobacco companies have started to diversify and intensify promotion of cigarettes and the growth of tobacco in the third world. In recent years, the burden of tobacco use has increasingly shifted to low- and middle-income countries, with evidence that tobacco use in Africa is growing by 4.3 percent each year. Currently, 80 percent of the world's smokers live in these countries - identified by the tobacco industry as growth markets for deadly tobacco products. If current trends continue, tobacco use will kill more than eight million people each year by 2030, with 80 percent of these deaths in low- and middleincome countries. These deaths are entirely preventable if countries like Nigeria remain committed to sound tobacco control policies [1]. Hence, the objective of this study is to provide information on the level of nicotine content of various brands of cigarettes sold in Nigeria using Benin City cigarette market as a case study.

2 MATERIALS AND METHODS

2.1 Samples Collection

Samples were purchased from major marketing centres in Ring Road/ Lagos Street, Benin City, Edo State, Nigeria.Twelve samples of different brands produced by four different manufacturers were used: Sweet Menthol (A); St. Moritz (B); Aspen (C); London (D); Standard (E); Rothmans (F); Ace (G); Green Spot (H); Benson & Hedges (I); Gold Bond (J); Paul Mall (K); Titan Phillies (L). The reagent/chemicals used were all of analytical grade.

2.2 Extraction of Nicotine Content from Samples

Modified procedure reported by Ihimire (1984) was used. 3g of sample was added to a 250ml flask containing 1g barium hydroxide except for Titan Phillies sample where only 1g was taken. Subsequently 15ml of saturated barium hydroxide solution was added. The flask was swirled until the tobacco was thoroughly wet. Then 100ml of 9:1 mixture of benzene and chloroform mixture was added. This mixture was shaken at intervals of 5 minutes for four times (20 minutes). Then, 2g of

celite was added to the mixture before allowing the mixture to separate into 2 liquid layers. The organic layer containing nicotine was filtered through fluted no. 2 filter paper into a dry flask containing 2g of anhydrous magnesium sulphate. After 5 minutes, the solution was filtered again to obtain the nicotine extracted solution.

2.3 Determination of Nicotine Content

Modified procedure earlier reported by [16] was used. The filtrate obtained above was titrated with 0.1N sulphuric acid after addition of 2 drops of methyl violet indictor solution (0.2% in chlorobenzene). The colour changed from violet to green. Triplicate assessment was done and nicotine content was calculated from the relationship:

% Nicotine = (Titre value / 2) X 0.16213

Triplicate results for each analyte were subjected to one analysis of varians and Turkey Krammer multiple comparison test (SAS, 2001). Results were compared at 95% confidence limit (P< 0.05).

3 RESULTS AND DISCUSSION

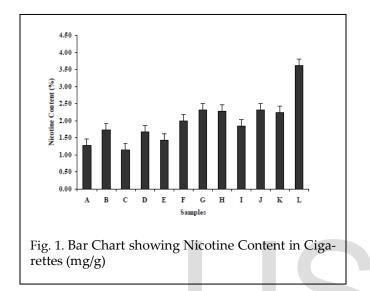
In Table 3.1 below, the result of analysis of nicotine content in different brands of cigarettes are presented. The order of presentation did not correlate with order of listing of producer as reported above. Each result is presented as mean value of triplicate analysis \pm standard error of mean of each determination. The highest nicotine content was observed in sample L (3.62%). The content in the respective brands were statistically significantly (P < 0.05) different except for mean comparisons for G vs J or H vs K. These recorded comparable nicotine content in their samples. The least nicotine content was observed in sample C.

Fig 1 is a bar chart illustrating comparatively the nicotine contents in the respectively brands studied. Values for the respective brands as determined in this study are within 8 – 20mg/g reported for the same brands of cigarettes determined with high performance liquid chromatography coupled with mass spectrometric method [17]. The values also compared with those determined with titrimetric or spectrophotometeric procedure for some brands of cigarettes sold in Lagos [16].

Nicotine (3– 1–methyl–2–pyrrolidinyl pyridine) is the most abundant of the volatile alkaloids in tobacco leaf [17]; [7]. It acts on nicotinic cholinergic receptors, affecting most organ systems in the body and is a highly addictive drug [5]; [18]; [19].

The amount of information regarding nicotine content in cigarettes is slight and only a few studies have been done to bring light to this subject especially in Nigeria [16]; [20]; [21]. There are described HPLC methods for the determination of nicotine from pharmaceutical formulations [22]. Also, HPLC-tandem mass spectrometry methods have been reported for determiInternational Journal of Scientific & Engineering Research, Volume 6, Issue 5, May-2015 ISSN 2229-5518

nation of nicotine in biological samples [23]; [24]. In this study, cognisance was taken of inability of most developing countries to purchase modern instruments for laboratory use. In instances where these are purchased, adequate manpower, effective operating working conditions and other variables makes substantial difficulty in usage. The approach used for this study is simple, though painstaking but has proved to be very reliable. Hence considering the deleterious consequences of nicotine, the usage of this process in quality control is recommended.



4 CONCLUSION AND RECOMMENDATION

Monitoring nicotine content when processing tobacco or in already processed tobacco products provides critical information for product quality usage. The result as obtained with this procedure correlates with those of high precision instrument. Hence the procedure can be adopted for quality control or product monitoring by legislative body. The cost for this purpose is not outrageous as what is used in most developed economy. The result obtained after patiently carrying out the procedure as shown is as good as those with high-precision instrumental approach.

In developing countries like ours where high precision instruments are expensive and rear to buy, even when they can be purchased, adequate man-power to operate and maintain these instruments properly cannot be guaranteed. Hence, this titration method is recommended.

ACKNOWLEDGMENT

I will like to sincerely thank everyone that was part of this research process especially my Lecturer Dr. Mrs. Amraibure Odia and Dr. Inegbenose Godwin Ihimire who were of great assistance to me with the information and data required and also guided me in the writing of the paper. My sincere appreciation to my dear mother who has being a source of strength financially and morally. My heart felt gratitude goes to my lovely wife Prudence Oluwatosin and everyone that has sup-

 TABLE 1

 NICOTINE CONTENTS OF VARIOUS SAMPLED CIGARATTES

Sample	Nicotine Content (%)
А	1.27 ± 0.00
В	1.72 ± 0.01
С	1.14 ± 0.01
D	1.67 ± 0.00
E	1.42 ± 0.00
F	1.99 ± 0.00
G	2.32 ± 0.00
Н	2.27 ± 0.01
Ι	1.84 ± 0.01
1	2.32 ± 0.01
К	2.24 ± 0.01
L	3.62 ± 0.00

Sweet Menthol (A); St. Moritz (B); Aspen (C); London (D); Standard (E); Rothmans (F); Ace (G); Green Spot (H); Benson & Hedges (I); Gold Bond (J); Paul Mall (K); Titan Phillies (L)Results = Mean ± SEM of Triplicate Determinations

ported me on this research once more, thanks and God bless.

REFERENCES

- [1] http://global.tobaccofreekids.org/en/media_center/detail/2013_07_11_ nigeria
- [2] H., Douglas, *Tobacco*. Online Etymology Dictionary. www.etymonline.com, Retrieved 05/10/2012, 04:50pm 2001.
- [3] E.M., David, <u>"How Cigarettes are Made"</u>. USA: Philip Morris Richmond Offices, 1994.
- [4] A.L., Brody, M.A., Mandelkern, E.D., London, R.E., Olmstead, J., Farahi, D., Scheibal, J., Jou, V., Allen, E., Tiongson, S.I., Chefer, "Cigarette Smoking Saturates Brain α4β2 Nicotinic Acetylcholine Receptors". Archives of General Psychiatry 63(8): 808 – 816, 2006.
- [5] N.L., Benowitz, "Pharmacology of Nicotine: Addiction and Therapeutics". Annu. Rev. Pharmacol. Toxicol. 36: 597 – 613, 1996.
- [6] I., Ujváry, "Nicotine and Other Insecticidal Alkaloids". In: Yamamoto, I., Casida, J. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. Tokyo: Springer-Verlag, pp. 29–69, 1999.
- [7] A., Rodgman, T.A., Perfetti, <u>The Chemical Components of Tobacco and</u> <u>Tobacco Smoke</u>. Boca Raton, FL: CRC Press, pp. 36 – 38, 2009.
- [8] WHO, <u>WHO Report on the Global Tobacco Epidemic 2008: Foreword and Summary</u>. Geneva: <u>World Health Organization</u>, p. 8, 2006.

http://www.ijser.org

up- [9] R., West, S., Shiffman, Fast Facts: Smoking Cessation. UK: Health Press

International Journal of Scientific & Engineering Research, Volume 6, Issue 5, May-2015 ISSN 2229-5518

Ltd., p. 28, 2007.

- [10] A.P., Raval, A., Bhatt, I., Saul, "Chronic Nicotine Exposure Inhibits 17-β-estradiol-mediated Protection of the Hippocampal CA1 Region against Cerebral Ischemia in Female Rats". *Neurosci. Lett.* 458 (2): 65– 9, 2009.
- [11] P., Phyllis, "Nicotine". Berichte der Deutschen Chemischen Gesellschaft 29: 294, 1999.
- [12] H.K., John, Tobaccoism or How Tobacco Kills. Michigan: The Modern Medicine Publishing Co., p. 15, 1992.
- [13] J., Goodman, Tobacco in History and Culture: An Encyclopedia. Detroit: Thomson Gale, pp. 15 – 18, 2005.
- [14] N.P., Robert, "The History of the Discovery of the Cigarette–Lung Cancer Link: Evidentiary Traditions, Corporate Denial, Global Toll". *Tobacco Control 21*: 87 – 91, 2012.
- [15] A.S., Villégier, G., Blanc, J., Glowinski, J.P., Tassin, "Transient Behavioural Sensitisation to Nicotine becomes long-lasting with Monoamine Oxidases Inhibitors". *Pharmacol. Biochem. Behav.* 76 (2): 267–74, 2003.
- [16] I.G., Ihimire, Determination of Nicotine in some Cigarettes marketed in Lagos. HND Thesis, Department of Science Technology, Yaba College of Technology, Yaba Lagos, 1984.
- [17] V., Laurian, F., Lorena, M., Ioana, E.L., Sorin, "Determination of Nicotine from Tobacco by LCMSMS". *Studia Universitatis Babeşbolyai*, *Physica L(4b)*: 19 – 24 2005.
- [18] P.E.T., Douben, An Ecotoxicological Perspective. New Jersey: John Wiley and Sons, pp. 85 – 89, 2003.
- [19] R., Doll, R., Peto, J., Boreham, I., Sutherland, <u>"Mortality in Relation to Smoking: 50 Years' Observations on Male British Doctors"</u>. BMJ 328(7455): 1519, 2004.
- [20] G.F., Gause, "Chapter V: Analysis of various Biological Processes by the Study of the differential Action of Optical Isomers". In: Luyet, B. J. Optical Activity and Living Matter. A Series of Monographs on General Physiology. Normandy, Missouri: Biodynamica, 1941.
- [21] Y.S., Ding, M.J., Yan, R.B., Jain, E., Lopp, A., Tavakoli, G.M., Polzin, S.B., Stanfill, D.L., Ashley, C.H., Watson, "Determination of 14 Polycyclic Aromatic Hydrocarbons in Mainstream Smoke from US Brand and non-US Brand Cigarettes". *Environmental Science and Technology* 40(4): 1133 – 1138, 2006.
- [22] R.T., Kaustubh, B.K., Ritesh, G.A., Sanjay, "Validated High Performance Liquid Chromatographic Method for Analysis of Nicotine in Pure form and from Formulations". *Journal of Pharmaceutical and Biomedical Analysis* 32(3): 441 – 450, 2003.
- [23] T., Apani, T., Johnsson, K., Reijula, "Analysis of Nicotine, 3-Hydroxycotinine, Cotinine, and Caffeine in Urine of Passive Smokers by HPLC-Tandem Mass Spectrometry". *Clinical Chemistry* 45: 2164– 2172, 1999.
- [24] G.D., Byrd, R.A., Davis, M.W., Ogden, "A Rapid LC–MS–MS Method for the Determination of Nicotine and Cotinine in Serum and Saliva Samples from Smokers: Validation and Comparison with a Radioimmunoassay Method". *Journal of Chromatographic Science* 43(3): 133 – 140, 2005.

