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Evaluation & comparison of Nicotine quantification in smokeless tobacco products

Research Article

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Abstract

Introduction: Smokeless tobacco products (STPS) represent a significant health risk and have been associated with oral and pancreatic cancers, oral lesions, coronary artery and peripheral vascular disease and adverse pregnancy outcomes. So, the aim of the present study is to perform quantitative determination of nicotine, the main alkaloid of smokeless product (Gutkha) available in Vadodara, Gujarat. Method: Collection of sample was done from local tobacco selling shopkeeper from Vadodara i.e. vimal, RMD, pan vilas, rajnigandha and raag. All the samples included in the study the same products available everywhere in Gujarat. The quantification of nicotine was done by High Performance Thin Layer Chromatography (HPTLC) using the mobile phase toluene: ethyl acetate: Diethylamine (6:4:0.5). Spectrodensitometric measurement was carried out at absorption maximum 254 nm. Results: Five different smokeless tobacco samples were estimated using HPTLC method. Nicotine content was found to be 2.45% in Vimal, 3.11% in RMD, 2.60%- Pan Vilas, 3.06%- Rajigandha,3.32%- Raag. Conclusion: A considerable variation of nicotine content was found among the five investigated smokeless tobacco product where sample raag revealed the highest amount of nicotine than the other samples. The nicotine concentration of commercially available chewing tobacco products was found to be much lower than that of the smoking form of tobacco, but the higher average daily consumption made it comparable to the smoking form.

Key Words: Smokeless tobacco, HPTLC, Gutkha.

Introduction

Tobacco is obtained from two vegetal species, namely *Nicotiana tabacum* and *Nicotiana rustica*, both of them native from Peruvian and Ecuadorian Andes. It has been used in many ways: smoked in pipes, inhaled, chewed, eaten, ingested as tea, used for intestinal lavage (clyster), scraped on the skin to fight louses, instilled as eye drops, and used in ointments, analgesics, and antiseptics. The main acute effects of nicotine (Figure 1) on the cardiovascular system are as follows: peripheral vasoconstriction, increased systemic arterial pressure, and increased cardiac frequency. In the nerve endings, it stimulates release of the neurotransmitters acetylcholine, dopamine (DA), glutamate, serotonin, and gamma-aminobutyric acid (GABA) (1). Nicotine acts on the so-called nicotinic receptors. Cholinergic synapses may contain nicotine or muscarinic receptors. Those containing nicotinic receptors occur at all excitatory neuromuscular junctions in vertebrates and at numerous sites in the nervous system. Nicotine is the major alkaloid of tobacco. The fatal dose for humans is about 100 mg. It may be oxidized to nicotinic acid (niacin) by the action of chromic acid. Nicotine is a tertiary amine displaying a pyridine and a pyrrolidine rings, namely, 3-(N-Methyl-2-pyrrolidyl) pyridine (2).

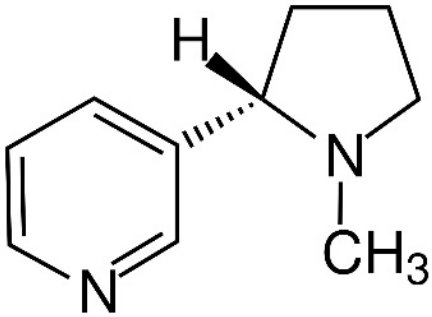

Figure 1 Structure of nicotine

Smokeless tobacco products (STPS) represent a significant health risk and have been associated with oral and pancreatic cancers, oral lesions, coronary artery and peripheral vascular disease and adverse pregnancy outcomes. Approximately 28 carcinogens have been identified in STPs so far. Tobacco-specific nitrosamines (TSNAs) are considered a potent class of carcinogens in STPs.(2) During the STP curing process, TSNAs form in the leaves and increase if the tobacco is subsequently fermented (3). Levels of TSNAs are also dependent on other factors, such as the basic pH level and the nitrite/ nitrate content of the product (4). The moisture content of the product and the related increase in microbial action are other causes of increased TSNA content in STPs.

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One of the most obvious orthogonal features of the two techniques is the primary use of reversed phases in HPLC (High Performance Liquid Chromatography) versus unmodified silica gel in HPTLC, resulting in partition chromatography and adsorption chromatography respectively. Unlike other methods, HPTLC (High Performance Thin Layer Chromatography) produces visible chromatograms complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously, so that reference and test samples can be compared for identification. Similarities and differences are immediately apparent and with the help of the image comparison (4). Several chromatograms can be compared directly, even from different plates. In addition to the visible chromatograms, analog peak data are also available from the chromatogram. They can be evaluated either by the image-based software Videoscan or by scanning densitometry with TLC Scanner, measuring the absorption and/or fluorescence of the substances on the plate. TLC is an offline technique: the subsequent steps are relatively independent, allowing parallel treatment of multiple samples during chromatography derivatization and detection. Some of the steps can be repeated independently of others, for example in post chromatographic derivatization, some reagents can be applied in sequence allowing multiple derivatization and thus multiple detection of the same sample (4, 5). In view of the above article describes the key features of traditional thin layer chromatography and modern HPTLC advantages.

The HPTLC fingerprint is also suitable for rapid and simple authentication and comparison of the suitable difference among samples with identical plant resource but different geographic locations, also allow the hyphenation of a high resolution planar separation with modern mass spectrometers for identification and quantitation of substances. Among the modern Analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. HPTLC is playing an important role in today analytical world, not in competition to HPLC but as a complementary method (6). In this study we aim to evaluate and compare nicotine & packaging disclosure in various smokeless products available in Vadodara, Gujarat.

Materials and Methods

Standards and chemicals

Standard Nicotine 99% was purchased from Sigma Aldrich, USA. All the reagents were HPLC grade and purchased from Merk, India.

Collection of Samples

Collection of samples was done from local tobacco selling shopkeeper from Vadodara. All the samples included in the study the same products available everywhere in Gujarat. 10 samples each will be required as 1 sample of each consists of 1 gm of gutkha for sample solution. Hence, 50 samples were required. Packaging disclosure of each sample label will also be checked.

Samples to be used are:

A. Vimal
B. RMD
C. Pan Vilas
D. Rajnigandha
E. Raag

Instrumentation and Chromatographic Conditions

A CAMAG automatic TLC applicator (Linomat V) was used for application of the samples with a 100 µL syringe (Hamilton). Plates were developed in a glass twin-trough chamber (20 × 10 × 4 cm; CAMAG, Switzerland). Samples were spotted on 0.2¬mm thick HPTLC plates (20 × 10 cm) pre-coated with silica gel 60 F254 (Cat. No. 1.05548, E. Merck, Darmstadt, Germany). Densitometry scanning was carried out by a CAMAG Scanner 3. Detection of the spots was done under ultraviolet (UV) light. Data were analyzed by integrated software of winCATS (CAMAG).

Preparation of sample solution

Accurately weighed 10 gm of marketed samples was macerated in 100 mL of methanol for 24 hrs. The extracts were filtered. Filtrate is evaporated on water bath till the dry extract remains. This dry extract will be stored and further used for the entire study.

Preparation of standard Nicotine

To establish linearity, various working standards solutions were prepared using different concentration range from standard nicotine stock (1 mg/mL) in six working standard solution to follow the Lambert-Beer law.

Construction calibration curve of Nicotine

Working Standard nicotine solution corresponding to 1.5, 2, 2.5, 3, 3.5 and 4 µg nicotine were prepared from stock solution. These concentrations were applied to a plate (20×10 cm) in triplicates of 6 mm band length with 4 mm between each two bands. The chamber was saturated with the mobile phase for 30 min before developing the plates. The mobile phase consisted of a mixture of Toluene: Ethyle acetate: Diethyleamine (6:4:0.5, v/v/v). The plates were developed to height of 8.0 cm at 25 ± 2°C and 45% RH. After drying the plates were placed at room temperature under current of air for 15 min. Each sample was scanned at absorbance maxima (254 nm). The peak areas were noted, and calibration was plotted with concentration vs. peak areas. The calibration curve was constructed in accordance with the fulfillment of ICH Q2B1 guideline[5].

Quantification of the nicotine present from marketed products

The analysis of nicotine will be performed on High Performance Thin Layer Chromatography (HPTLC) aluminum plates pre-coated with silica gel as stationary phase. Development was carried out in a horizontal chamber under sandwich configuration with the mobile phase toluene: ethyl acetate: Diethylamide (6:4:0.5, v/v/v)(7) at room temperature (25 ± 2°C). A

Thin Layer Chromatographic scanner was used for Spectro densitometric scanning and analysis in the absorbance mode at 254 nm, which was provided compact spots for nicotine. The percentage content of nicotine was calculated from standard curve.

Results

In the present investigation, we estimate the quantity of nicotine in *Nicotiana tabacum* and all five marketed gutkha products. Toluene: Ethyle acetate: Diethyleamine (6:4:0.5, v/v/v) was considered as most appropriate mobile phase as obtained sharp bands with clear resolution at R_f 0.41±0.01in the system.

The linearity of the HPTLC method for quantification of nicotine was evaluated by analyzing a series of different concentrations of the standard nicotine where six different concentrations of the standard nicotine solutions were used in order to cover the range of 1.5 µg - 4 µg/spot; each concentration was applied to a plate (20×10 cm) in triplicates of 6 mm band length with a distance of 4 mm between each two bands. The distance from the plate side edge was 10mm and from the bottom of the plate was also 10 mm. The application rate was 15 mL; the bands were developed using Toluene: Ethyl Acetate: Diethylamine (6:4:0.5, v/v/v). The developing mixture used was found to be suitable for selective separation of nicotine from other alkaloids and matrix components when tested on a representative sample. The development time was 12 min, the plates were air dried for 10 min, and standard zones were quantified by linear at λ 254 nm, the wavelength corresponding to the maximum sensitivity of nicotine. The presented method exhibited strict linearity between the peak area and the concentration over the range with a correlation coefficient (R^2) of 0.9975. Linear regression equation was found to be y= 318.2+956.4x, where y is the spot area and x is the concentration in µg/mL Showed in figure 2. The area under curve of chromatogram peaks is represented in table 1.

Figure 2 The calibration curves for nicotine in the range of 1.5 – 4. µg/mL

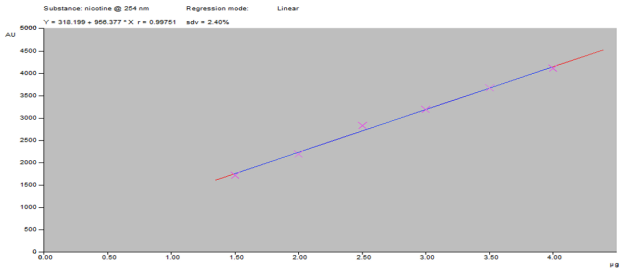


Table 1: The area under curve of chromatogram peaks of different concentration of standard nicotine

Concentration (µg/mL)	AU
1.5	1709.55
2	2192.87
2.5	2831.45
3	3187.98
3.5	3665.69
4	4101.87

In the selected mobile phase Toluene: Ethyle acetate: Diethyleamine (6:4:0.5, v/v/v) nicotine was characterized by R_f value as 0.41±0.01 confirmed the presence of nicotine (Figure 3A). On scanning under UV, the spot of nicotine was found to have λ_{max} of 254 nm. Figure 3 depicted the chromatographs of nicotine includes (A) integrated peak for the nicotine and (B) 3D chromatogram of nicotine.

Figure 3. The chromatogram obtained at the evaluation of nicotine using HPTLC; A) Integrated peak for the nicotine; B) 3D chromatogram of nicotine

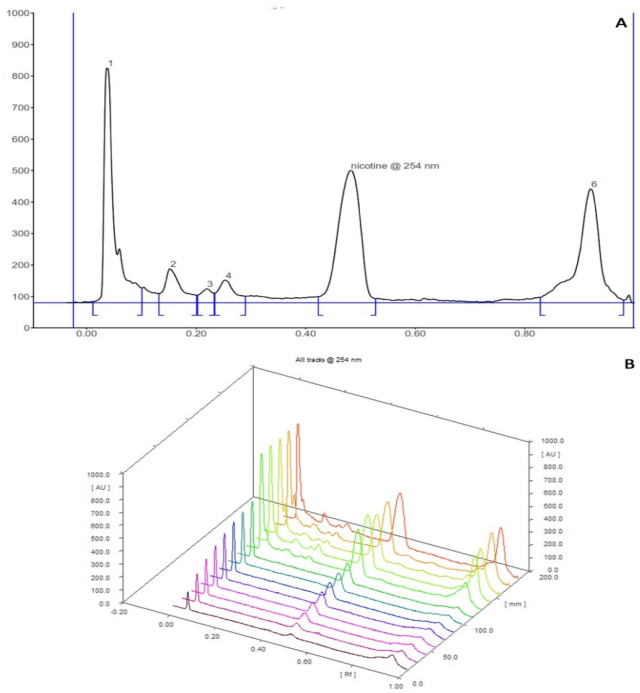
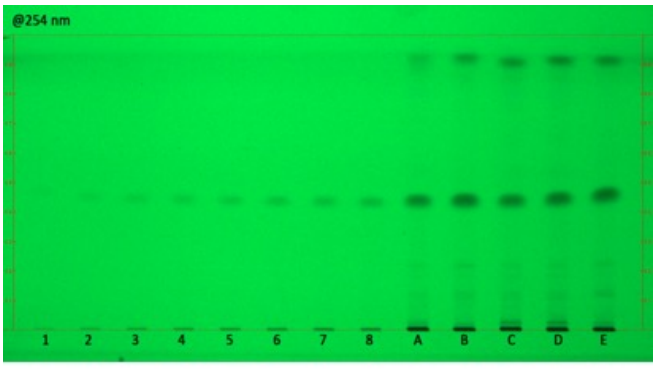


Figure 4. HPTLC chromatogram of standard nicotine and six different brands of gutkhas. Spot 1-8 indicate the various concentration range of nicotine (1.5 µg to 4.0 µg/spot) spot A-E represent the various smokeless tobacco products: Vimal, RMD, Pan Villas, Rajnigandh and Raag, respectively



Five different smokeless tobacco samples vimal, RMD, Pan Villas, Rajnigandha, and Rag were estimated using HPTLC method. The HPTLC method allows for the determination of nicotine in the examined brands of gutkha without any interference with minor tobacco alkaloids as complete separation of nicotine was achieved. Upon application of the suggested procedure, a considerable variation of nicotine content was noticed

among the nine investigated brands of gutkhas and the chromatograms are illustrated in Figure 4.

In present investigation the percentage level of Nicotine content was found to be 2.45% in Vimal, 3.11% in RMD, 2.60%- Pan Vilas, 3.06%- Rajigandha,3.32%- Raag, where samples Vimal and Raag revealed the highest amounts of nicotine. The percentage level of nicotine content was calculated based on the linearity equation as

$$Y = 318.199 + 956.377 * X \quad and \; r = 0.99751$$

where X is the concentration of nicotine in sample and Y is the area under curve (AUC) of the peak.

Discussion

The present investigation aimed to estimate the percentage nicotine content in marketed smokeless tobacco products using the simple, reproducible and cost-effective high performance thin layer chromatography. This experiment supports to improve the method of analysis for analytical toxicological study for nicotine intoxication which is frequently reported. This method also supports in the view of development of faster, efficient, sensitive and cost-effective laboratory method.

Smokeless tobacco products have lower nicotine content, both per pack and per gram of tobacco consistent with earlier reports (8, 9). However, it was observed that users often abuse it more frequently than smoked tobacco products. Oral use of tobacco has an increased risk of cancers and squamous cell and verrucous carcinomas of oral cavity and pharynx according to by Shah (10). The quality of the product, additives, and nicotine content can all influence the patterns and consequences of use of this diverse range of tobacco products (11). From the public health perspective, this work can be further expanded to quantification of nitrosamines and polycyclic aromatic hydrocarbons and disease markers as well as gaseous toxins of each product (carbon monoxide, tar, and other oxides). To ensure the consumer's right to be informed about the health-risk from a product, there is a need to monitor and regulate the emission and ingredients among all smokeless tobacco products used in the country. In addition, awareness should be created in the general public about the harmful effects of these products (11). This study also has implications for effective pharmacological management for tobacco cessation, which have been shown to improve treatment rates in India and elsewhere. Our findings suggested that Nicotine content was found to be 2.45% in Vimal, 3.11% in RMD, 2.60%-in Pan Vilas, 3.06%-in Rajigandha, 3.32%- in Raag. These findings are likely to be relevant to many other countries in the region which also have multiple tobacco products.

Conclusion

The nicotine concentration of commercially available chewing tobacco products was found to be much lower than that of the smoking form of tobacco, but the higher average daily consumption made it comparable to the smoking form. The above information is likely to be useful in the evaluation, interpretation, and comparison of data on tobacco chewing related cancers among Eastern and Western populations. Indian chewing tobacco users are often also smokers; the excessive use of chewing tobacco and concurrent smoking of bidis (which require deep inhalations to prevent it from getting extinguished) reverse smoking in certain populations, and the slightly increased nicotine concentration in Indian tobacco products, all contribute to the increased incidence of oral cancer in India when compared to West, where the incidence of lung cancer is more than that of oral cancer.

References

1. Musk A.W., Klerk N.H., History of tobacco and health. Respirology. Sep, 2003; 8(3); 286-90.
2. Nagashima S., Simultaneous reaction rate spectrophotometric determination of cyanide and thiocyanate by use of the pyridine-barbituric acid method. Anal Chem. 1984; 56(11); 1944-7.
3. Cogliano V., Straif K., Baan R., Grosse Y., Secretan B., Ghissassi F.E., Smokeless tobacco and tobacco-related nitrosamines. Lancet Oncol. 2004; 5(12); 708.
4. WHO. Tobacco Habits Other than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines. In: Res. IJC, editor. Lyon, France: World Health Organisation, ; 1985. p. 37-141.
5. Guideline I.H.T., editor Validation of analytical procedures: text and methodology Q2 (R1). ICH, Geneva, Switzerland; 2005.
6. Sharma P., Murthy P., Shivhare P., Nicotine quantity and packaging disclosure in smoked and smokeless tobacco products in India. Indian J Pharmacol. Jul-Aug, 2015; 47(4); 440-3.
7. Paillat L., Périchet C., Lavoine S., Meierhenrich U., Fernandez X., Validated high-performance thin-layer chromatography method for the determination of nicotine in tobacco (Nicotiana tabacum L.) extracts. J Planar Chromatogr - Mod TLC. 2012; 25(1); 23-9.
8. Smith R.A., Lawson T., Johansson S.L., Detection of DNA adducts by 32P postlabeling following chronic exposure of rats to snuff. Cancer Lett. Dec 16, 1997; 121(1); 11-7.
9. Mishra G.A., Pimple S.A., Shastri S.S., An overview of the tobacco problem in India. Indian J Med Paediatr Oncol. Jul, 2012; 33(3); 139-45.
10. Sushma C., Sharang C., Pan masala advertisements are surrogate for tobacco products. Indian J Cancer. Apr-Jun, 2005; 42(2); 94-8.
11. Shah N., Sharma P.P., Role of chewing and smoking habits in the etiology of oral submucous fibrosis (OSF): a case-control study. J Oral Pathol Med. Nov, 1998; 27(10); 475-9.

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