Original Research Article

Extraction and analysis of nicotine (3-(1-methyl-2-pyrrolidinyl)pyridine) from cigarettes using small volume liquid extraction and ultrasonic bath system technique

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ABSTRACT

The tobacco (Nicotiana tabacum) plant belongs to solanaceae family. It is an annual herbaceous plant. Over 0.45 million hectares of land are used for growing tobacco commercially, with a total production of over 750 million kg of tobacco. According to the Global adult tobacco survey held in the year 2016-2017, there are nearly 267 million adults (above 15 years of age) in India abusing tobacco. Nicotine can be extracted from biological samples by liquid-liquid extraction, small volume liquid extraction, etc. Thin-layer chromatography is a very effective technique as it can be employed for many samples simultaneously, which is cost-effective and easy to perform without any errors. A new technique, the Ultrasonic bath technique (Sonication technique) is used for the same. Four different types of solvents were used for extraction purposes out of which two gave favorable results. This methodology is proven to be cost-effective, consumes fewer chemicals, less manpower, and is effective.

1. Introduction

The tobacco (Nicotiana tabacum) plant belongs to solanaceae family. It is an annual herbaceous plant.[1,2] The leaves of Nicotiana are commercially grown and processed to form consumable tobacco. N.tabacum requires an optimum temperature of 20-30°C, a humidity of 80-85%, and nitrogen deficit soil for growing it desirably. India is the second-largest producer of tobacco after China and Brazil.[3] Over 0.45 million hectares of land is used for growing tobacco commercially, with a total production of over 750 million kg of tobacco. Abusing of tobacco can be done in different ways like in cigarettes, cigars, electronic cigarettes, water pipes, and a no. of smokeless products.[4,5] Smokeless tobacco products are mostly abused in India. According to the Global adult tobacco survey held in the year 2016-2017, there are nearly 267 million adults (above 15 years of age) in India abusing tobacco.[6,7]

Nicotine($C_{10}H_{14}N_2$) also known as 3-(1-methyl-2-pyrrolidinyl)pyridine which is a plant alkaloid with a molecular weight of 162.23 g/mol.[2,8] It is denser than water, having a pale-yellow to dark brown color, when subjected to heat it gives a fish-like odor, with a boiling point of 246.66°C and a melting point of -43.33°C.[9,10] Nicotine belongs to the tertiary amine group and possesses pyridine and pyrrolidine rings, nicotine has the potential to cross the blood-brain barrier as well as several other biological membranes.[3] The liver effectively metabolizes the absorbed nicotine and converts it into cotinine N'-oxides and nicotine N'-oxide. Long-term abuse of tobacco

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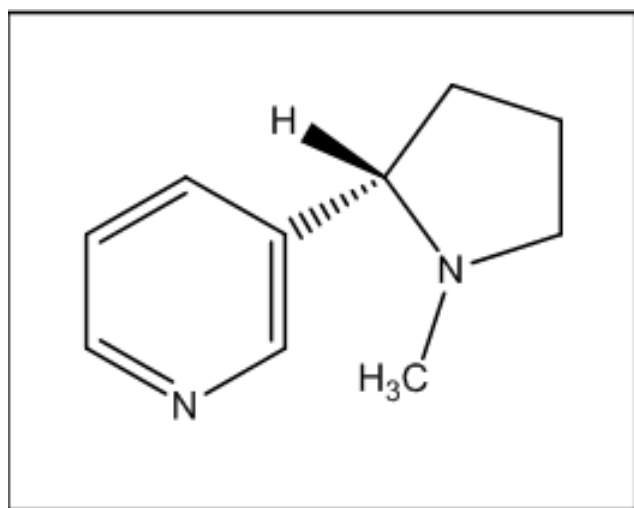
Fig. 1: Chemical structure of Nicotine

has been linked to an elevated risk of malignant cancers, low birth weight of infants, chronic obstructive pulmonary disease, and atherosclerosis.[3] Studies have shown in recent times that nicotine can be used as a therapeutic drug, in cases of neurological diseases like Alzheimer's.[2] Nicotine also shows some synergistic effects with Arsenic, their co-exposure can lead to oxidative stress, lung cancer as well as impaired arsenic metabolism.

Tobacco leaves have three kinds of alkaloids: Nicotine, indole, and D-limone. Nicotine exposure to the human body is evaluated by screening the amount of nicotine and its metabolites in biological samples such as urine and blood.[11] Nicotine can be extracted from biological samples by liquid-liquid extraction, small volume liquid extraction, etc. Nicotine can be extracted from leaves of tobacco using liquid-liquid extraction by dissolving it in NaOH.[12]

Chromatography is a technique widely used for separation. Chromatographic techniques use a stationary phase (ex. Silica, cellulose, etc.) and a mobile phase based on the compound to be separated.[13] It works on the affinity of the compound towards the stationary or mobile phase. When the sample completes its run on the stationary phase a solvent front is marked and then the plate is visualized under UV-chamber or sprayed with a reagent to fix the spots, and then the Rf (Retention factor) is calculated.[14] Thin-layer chromatography is a very effective technique as it can be employed for many samples simultaneously, it is cost-effective and easy to perform without any errors. Thin-layer chromatography is widely utilized for qualitative analysis of compounds but this technique also has some drawbacks like, it can give false results at low temperatures.[14]

This study aims to devise an effective procedure for the extraction of Nicotine from commercially available cigarettes and examine its effectiveness by using a thin-layer chromatographic analysis technique to extract it against a nicotine standard. Study on Nicotine holds a great deal of importance as it is a highly abused compound throughout the world and can lead to furthermore studies.[15]

2. Material and Methods

2.1. Glassware

Superior quality glassware was used for the experiment. Borosilicate glass measuring cylinder of 10 ml volume (Borosil), GC vials (Agilent), Glass funnel (Borosil), Glass beaker (Borosil), Glass rod, and TLC developing chamber of 20 x 20 cm dimensions (Borosil) were used.

2.2. Chemicals

All the reagents, chemicals, and solvents used were highly pure and experimental grade. Chemicals used were Sodium Sulphate Anhydrous, Potassium Iodide, Bismuth Subnitrate, Tartaric acid, and Sulfuric acid (Merck India limited). Different Solvents utilized to devise solvent system were Methanol, Ethyl acetate, Ammonia (Glaxo Smithkline pharmaceuticals limited Mumbai), and double-distilled water. TLC silica gel 60 F_{254} coated silica plates (Merck Millipore) was used.

Crude tobacco samples were utilized for the experiment. Nicotine standard was used from TCI (Tokyo Chemical Industry) Co. Ltd.

2.3. Methodology

2.3.1. Extraction of micotine
Nicotine was extracted from cigarettes employing two different extraction procedures:

2.3.2. Small volume liquid extraction
1g of tobacco sample was collected in a GC vial and 10g NaOH was added, the vial was vigorously shaken. The sample was subjected to a sonicator for 20 minutes. The content was filtered and the filtrate was collected without impurities. Nicotine extract was taken out for repetition. About 3-5 ml ultrapure water was added. The above content was taken in a separating funnel with filtrate and diethyl ether in equal proportions (1:1) was added. The content was filtered and the ethereal layer was collected, the above-collected layer was subjected to Sodium Sulphate Anhydrous (to absorb any kind of moisture) this process was repeatedly done 2-3 times and the remaining ethereal layer was collected in a glass bowl. The final content was left for 2-3 hours for evaporating until 1-2 ml is left.

2.3.3. Using ultrasonic bath system (Sonicator)
Four different solvents were used for this extraction procedure. Solvents taken were Methanol, Chloroform, Diethyl Ether, and Ether along with Ammonia (4.5:0.5). 1g of tobacco sample was taken in four different GC vials and

Table 1: Different TLC Solvent System

	Chloroform	Methanol	Diethyl ether	Diethyl ether + Ammonia
Ethyl acetate: Methanol: Ammonia: Water (8:2.5:0.02:1.58)	Yes	No	No	Yes
Methanol: Ammonia (9:1)	Yes	No	No	Yes

were marked as 1, 2, 3, and 4. 5ml Methanol was added to vial 1, 5ml chloroform in vial 2, 5ml Diethyl ether in vial 3, and 4.5ml Diethyl ether along with 0.5ml Ammonia was added to vial 4. The vials were packed and they were exposed to a sonicator for 20 minutes. The filtrate was collected and TLC was run.

2.3.3.1. Spotting the sample and Standard on the TLC plate . The extracted Nicotine sample from both the extraction procedures was spotted on the Silica gel 60 F_{254} TLC plates using a jet capillary alongside that Nicotine standard was also loaded.

2.3.3.2. Developing the TLC plates. The Spotted plate was placed in the TLC chamber which was prepared and saturated with the appropriate solvent system (mobile phase). The plate was allowed to rest until the mobile phase runs to the height of 75mm from the loading point. The plate was removed from the TLC chamber and solvent front was marked and the plate was air-dried.

Solvent system tested were

1. Ethyl acetate: Methanol: Ammonia: Water(8:2.5:0.02:1.58).
2. Methanol: Ammonia (9:1).

2.3.3.3. Visualisation of TLC plate. The TLC plates were air-dried and sprayed with dragendroff's reagent.

3. Result and Discussion

Nicotine can be extracted from tobacco in cigarettes using several techniques. Some key points like ease, less manpower, cost-effectiveness, eco-friendliness, and reproducibility should be considered as well. Both the techniques employed during the experiment have shown favorable results. Nicotine was best extracted using an Ultrasonic bath system in vial 2 containing 5 ml chloroform and vial 4 containing 4.5ml Diethyl ether along with 0.5ml Ammonia.

4. Conclusion

An effective methodology was developed to extract nicotine using Small Volume Liquid Extraction technique and Ultrasonic Bath System Technique. Out of which Ultrasonic bath technique was proven better.

5. Conflict of Interest

The authors declare that there is no conflict of interest.

6. Source of Funding

None.

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