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ANALYSIS OF NICOTINE IN HAND SWABS OF TOBACCO WORKERS USING THIN LAYER CHROMATOGRAPHY

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ABSTRACT

The objective of the study was to assess the presence of Nicotine in samples collected from Tobacco workers through swabs. To achieve this, numerous samples were taken and subjected to analysis using TLC with silica gel as the stationary phase and Methanol: Chloroform: Ammonia (5:3:2) as the mobile phase. Following the chromatographic process, the samples were observed under a UV cabinet, and subsequent visualization involved the preparation and application of spraying reagents. The presence of Nicotine was confirmed by the appearance of orange bands or spots upon the application of Dragondorff reagent.

Keywords: Heavy metal, Soil, Contamination, Health, AAS. etc.

INTRODUCTION

Nicotine was detected at specific ppm levels in tomato, potato peel, aubergine, and green chilli. Detection of Nicotine occurred in Solanaceae species but not in non-Solanaceae species [1]. Nicotine, one of the toxic components of Tobacco, is known to easily cross the Blood-brain barrier [2].

Associations between Nicotine and lung cancer, peripheral arterial disease, and over five million premature deaths have been established [3]. Tobacco manufacturing generates approximately 200 million tons of waste or by-products, posing a significant environmental threat and leading to pollution [4].

Upon entering the human body, Nicotine, a major component of Tobacco, undergoes metabolism into various chemicals [5]. Nicotine comprises around 4000 active compounds responsible for a range of diseases, including atherosclerosis, cancer, and neurohormonal changes [6].

Nicotine, a colourless to pale yellow oily substance, is derived from Nicotiana tabacum plant leaves. It acts as a stimulant, engaging the peripheral and central nervous systems [7]. Nicotine serves as a defensive response in Tobacco and other Nicotiana species against insects and predators [8].

TLC is commonly utilized for Drug analysis [9]. Tobacco's potential alternate use as dietary supplements and animal feed has also been studied [10]. Densitometers in TLC, like solid-state detectors, obviate the need for cells, thereby reducing maintenance requirements. This is particularly beneficial for developing nations, aiding in identifying substandard and illicit distribution[11].

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The challenge lies in effectively separating alkaloids of varying polarity, necessitating a specialized TLC system [12].

Regular exposure to smokers puts individuals at risk of becoming passive or second-hand smokers (SHS), which can lead to lung abnormalities [13]. The TLC method is cost-effective and efficient for Nicotine sample analysis, encompassing various samples including cigarettes, even facilitating the detection of counterfeit cigarettes [14].A new High-Performance Thin-Layer Chromatography (HPTLC) method was developed and validated for the precise determination of nicotine in tobacco extracts [15].Studies also exist concerning the determination of nicotine and cotinine in plasma, along with quantification of nicotine in urine [16][17].

Plants from the Nicotiana genus exhibit high nicotine levels, whereas Solanum genus plants contain minimal amounts [18]. Another study outlined Nicotine analysis via silica-coated TLC plates with a petroleum ether-acetone-diethyl amine mobile phase ratio of (7.6:2:0.4) v/v [19].

METHODOLOGY

Sample Collection

For this study, a total of 100 samples were collected. Hand swabs of workers were obtained from two distinct industries located in the Narsinghpur and Jabalpur regions of Madhya Pradesh. Cotton soaked in distilled water was utilized for collection, and swabs were taken. The collected samples were air-dried thoroughly to prevent fungal growth, followed by careful packaging in plastic zipper bags. Subsequently, the samples underwent analysis using the TLC (Thin Layer Chromatography) method.

Chemicals Used - The chemicals employed include Chloroform, Methanol, Ammonia, distilled Water, Potassium Iodide, Bismuth Nitrate, and Glacial Acetic Acid.

Equipment Used - The equipment utilized comprised TLC plates, a UV Cabinet, and a Weighing Balance.



Figure 1: UV Cabinet.

Standard Preparation - Standard solutions were prepared by adding 5 ml of methanol to Tobacco and subsequently extracting the content through filtration.

Instrumentation - The analysis was conducted using the TLC method, a chromatographic technique based on the differential affinities of compounds for the mobile and stationary phases.

Thin Layer Chromatography–The separation principle underpins TLC. The separation is based on the chemicals' relative affinity for both phases. The mobile phase compounds travel across the surface of the stationary phase. The movement occurs in such a way that the compounds with a higher affinity for the stationary phase are displaced.

PROCEDURE

Sample Pre-treatment - Prior to analysis, samples were treated with methanol and heated at 80°C for about 5 minutes. After extraction and concentration, samples were spotted onto a TLC plate using a micropipette. Silica gel G-coated glass slides (75*25mm, thickness 1.1-1.2 mm) acted as the stationary phase, with a mobile phase of Chloroform-Methanol-Ammonia (6:5:2 v/v/v). During TLC, slides were saturated with solvent for 10-15 minutes, followed by observation under UV Cabinet. Dragondorff's Reagent was prepared by combining bismuth nitrate, glacial acetic acid, potassium iodide, and water, and then sprayed on the TLC plate to reveal orange spots or bands.

Measurement and Analysis - The Retention factor (Rf) was calculated by dividing the distance travelled by the solvent by the distance travelled by the sample. The mean Rf values were then determined and utilized for report preparation.

RESULTS AND DISCUSSIONS

The presence of nicotine was confirmed in certain samples through TLC and Dragondorff reagent in analysis of swabs obtained from tobacco workers. The application of the reagent resulted in the formation of orange bands or spots, indicating the presence of nicotine. The quantitative data from this study will offer valuable insights into the extent of nicotine exposure among tobacco workers, facilitating the establishment of appropriate workplace health and safety measures.



Figure 2: Separation of nicotine using TLC

Table 1. Detection of Nicotine in Swab Samples from Tobacco Workers

S . No.	Sample Sr. no. (n=100)	Results	Mean of Rf values	Median of Rf values	Mode of Rf values
1	60 samples	Negative	-	-	-
2	40 samples	Positive	0.65	20	0.63,0.72

Negative results were obtained upon examination of the first sixty samples, as no spotting or tailing was observed. Positive results were achieved for the remaining forty samples. Mean, median, and mode were determined for the positively yielding samples. The calculated average Rf value was 0.65.

CONCLUSION

The study successfully determines the presence of nicotine in swabs collected from tobacco workers using Thin Layer Chromatography with silica gel as the stationary phase and Methanol: Chloroform: Ammonia (5:3:2) as the mobile phase. Dragondorff reagent is then applied for visualization. These findings contribute to our comprehension of nicotine exposure in tobacco workers, highlighting the importance of implementing measures to safeguard their health. Future research can explore additional aspects of tobacco worker safety and potential long-term health effects.

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