Comparative Thin Layer Chromatographic Detection of Nicotine from Cerumen of Smokers and Non-Smokers

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***Abstract*—Nicotine, a major psychoactive alkaloid found in tobacco, is widely used as a biomarker to assess tobacco exposure in both clinical and forensic settings. Conventional biological matrices such as blood, urine, saliva, and hair have limitations including invasiveness, short detection windows, and complex analytical requirements. This study explores cerumen (earwax) as an alternative biological matrix for nicotine detection due to its non-invasive collection, lipid-rich composition, and ability to retain substances over extended periods. Thin Layer Chromatography (TLC), a simple and cost-effective analytical technique, was employed to comparatively detect nicotine in cerumen samples obtained from smokers and non-smokers. Samples were processed through solvent extraction and analyzed using silica gel TLC plates with appropriate mobile phases. Visualization was carried out using ultraviolet light and chemical reagents. The results revealed clear nicotine spots in smokers' samples with Rf values closely matching standard nicotine, whereas non-smokers exhibited negligible or faint traces. This comparative analysis demonstrates the effectiveness of TLC as a qualitative screening tool and highlights cerumen as a promising matrix for long-term nicotine exposure assessment, contributing to advancements in forensic toxicology and public health monitoring.**

***Index Terms*—Nicotine Detection; Thin Layer Chromatography; Cerumen Analysis; Forensic Toxicology.**

I. INTRODUCTION

Tobacco consumption remains one of the leading causes of preventable morbidity and mortality worldwide. At the core of tobacco addiction lies nicotine, a potent alkaloid responsible for dependence and various physiological effects. Upon inhalation, nicotine rapidly enters the bloodstream, reaches the brain, and stimulates nicotinic acetylcholine receptors, leading to dopamine release and reinforcing addictive behavior. Despite its short half-life, nicotine and its metabolites can be detected in biological matrices, making them useful indicators of tobacco exposure. Traditional matrices such as blood and urine are widely used for nicotine detection. However, these methods often present challenges such as invasive collection procedures, susceptibility to contamination, and limited detection windows. Hair analysis offers long-term detection but requires sophisticated instrumentation and complex preparation. These limitations have driven the search for alternative biological matrices that are non-invasive, stable, and capable of long-term retention of analytes.

Cerumen, commonly known as earwax, has emerged as a promising candidate in this regard. Produced by sebaceous and ceruminous glands in the external auditory canal, cerumen is a lipid-rich substance composed of fatty acids, alcohols, cholesterol, and keratinized cells. Its hydrophobic nature allows it to accumulate lipophilic substances such as nicotine over extended periods. Additionally, cerumen is less prone to environmental contamination compared to other matrices, making it suitable for forensic investigations. Recent studies have highlighted the potential of cerumen in detecting drugs, environmental pollutants, and disease biomarkers. Its ability to reflect both endogenous and exogenous compounds position it as a valuable tool in clinical diagnostics and forensic toxicology. However, systematic research on nicotine detection in cerumen remains limited.

Thin Layer Chromatography (TLC) is a widely used analytical technique known for its simplicity,

affordability, and rapid analysis. It operates on the principle of differential adsorption of compounds between a stationary phase and a mobile phase. TLC is particularly useful for preliminary screening of organic compounds, making it suitable for comparative studies. This study aims to evaluate the effectiveness of TLC in detecting nicotine in cerumen samples collected from smokers and non-smokers. By comparing chromatographic profiles, the study seeks to establish cerumen as a reliable biological matrix and demonstrate the utility of TLC in forensic and clinical applications.

II. GLOBAL BURDEN OF SMOKING AND PUBLIC HEALTH IMPLICATIONS

Smoking is a global public health crisis, contributing to millions of deaths annually. It is associated with a wide range of diseases including cardiovascular disorders, respiratory illnesses, and various forms of cancer. The addictive nature of nicotine makes cessation difficult, thereby prolonging exposure and increasing health risks.

The burden of smoking is particularly significant in low- and middle-income countries, where healthcare systems are often ill-equipped to manage tobacco-related diseases. In addition to direct health impacts, smoking imposes substantial economic costs due to healthcare expenditure and loss of productivity. Second-hand smoke exposure further complicates the issue, affecting non-smokers including children and pregnant women. This highlights the need for reliable methods to assess tobacco exposure not only in active smokers but also in passive smokers.

Biomonitoring plays a crucial role in evaluating exposure levels and assessing the effectiveness of public health interventions. Accurate detection of nicotine and its metabolites can aid in smoking cessation programs, policy implementation, and epidemiological studies. The development of non-invasive and cost-effective detection methods is essential for large-scale screening and monitoring. In this context, cerumen analysis using TLC offers a practical approach that can be implemented even in resource-limited settings.

III. BIOLOGICAL MONITORING OF NICOTINE

Biological monitoring involves the measurement of chemicals or their metabolites in biological samples to assess exposure. For nicotine, commonly used matrices include blood, urine, saliva, and hair. Blood analysis provides accurate information about recent exposure but requires invasive sampling and trained personnel. Urine analysis is less invasive but reflects only short-term exposure and may be influenced by renal function. Saliva testing is convenient but subject to variability due to pH and flow rate. Hair analysis offers long-term exposure data but involves complex analytical procedures and expensive equipment. These limitations necessitate the exploration of alternative matrices. Cerumen presents several advantages including ease of collection, stability, and resistance to contamination. Its composition allows for the accumulation of lipophilic substances, making it suitable for detecting nicotine. The integration of cerumen into biomonitoring practices could enhance the accuracy and accessibility of nicotine detection, particularly in forensic and clinical contexts.

IV. CERUMEN AS A BIOLOGICAL MATRIX

Cerumen is a unique biological substance with significant analytical potential. It is composed of secretions from sebaceous and ceruminous glands, along with shed epithelial cells. Its lipid-rich nature enables it to trap and retain various compounds over time. Unlike blood and urine, cerumen provides a longer detection window, making it suitable for assessing chronic exposure. Its non-invasive collection method eliminates the need for medical personnel and reduces the risk of infection.

Studies have demonstrated the presence of drugs, heavy metals, and volatile organic compounds in cerumen. Its similarity to keratinous matrices like hair suggests a comparable mechanism of drug incorporation through blood circulation. Despite its potential, cerumen remains underutilized in analytical research. Establishing baseline profiles and standardizing analytical methods are essential steps toward its widespread adoption.

V. ANALYTICAL TECHNIQUES FOR NICOTINE DETECTION

Various analytical techniques have been employed for nicotine detection, including Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), and Mass Spectrometry (MS). These

methods offer high sensitivity and specificity but require expensive instrumentation and skilled personnel. Thin Layer Chromatography (TLC) provides a simpler alternative. It involves the separation of compounds on a stationary phase using a mobile phase. The movement of compounds is characterized by the Retention Factor (Rf), which is used for identification.

TLC is particularly advantageous for preliminary screening due to its low cost, ease of operation, and ability to analyze multiple samples simultaneously. While it may not match the sensitivity of advanced techniques, it is highly effective for qualitative analysis.

In this study, TLC is used to detect nicotine in cerumen samples, demonstrating its applicability in forensic investigations and routine laboratory analysis.

VI. METHODOLOGY: SAMPLE COLLECTION AND TLC PROCEDURE

The study involved the collection of cerumen samples from adult participants categorized as smokers and non-smokers based on self-reported history. Ethical considerations were maintained throughout the process. Nicotine extraction was performed using an organic solvent system under controlled conditions. Standard nicotine solutions were prepared to establish reference Rf values. Samples were applied onto pre-coated silica gel TLC plates and developed in an optimized mobile phase. The plates were then visualized under ultraviolet light and treated with Dragendorff's reagent for confirmation. This methodology ensures accurate identification of nicotine and allows for comparative analysis between different sample groups.

VII. RESULTS AND COMPARATIVE ANALYSIS

The results demonstrated distinct chromatographic spots corresponding to nicotine in samples obtained from smokers. The Rf values closely matched those of the standard nicotine solution, confirming the presence of nicotine.

In contrast, cerumen samples from non-smokers showed either no detectable nicotine or faint traces, indicating minimal exposure. The intensity and frequency of spots were significantly higher in smokers, highlighting the effectiveness of TLC in differentiating between the two groups. These findings validate the use of cerumen as a reliable matrix and TLC as an effective screening tool for nicotine detection.

VIII. DISCUSSION AND FORENSIC IMPLICATIONS

The study highlights the potential of cerumen analysis in forensic toxicology. Its ability to retain substances over time makes it suitable for retrospective analysis of exposure.

TLC serves as a practical tool for preliminary screening, especially in settings with limited resources. The combination of cerumen and TLC offers a cost-effective approach for monitoring smoking behavior and environmental exposure.

However, further research is needed to standardize methods and improve sensitivity. Integration with advanced techniques could enhance accuracy and broaden applications.

IX. CONCLUSION

This study establishes cerumen as a promising biological matrix for nicotine detection and demonstrates the effectiveness of Thin Layer Chromatography as a qualitative analytical technique. The comparative analysis between smokers and non-smokers reveals significant differences in nicotine presence, supporting the reliability of the method.

The non-invasive nature of cerumen collection, combined with its ability to retain substances over extended periods, makes it an ideal candidate for long-term exposure assessment. TLC, with its simplicity and affordability, provides a practical solution for preliminary screening in forensic and clinical settings.

The findings contribute to the growing body of research on alternative biological matrices and highlight the need for further exploration and standardization. Future studies could focus on quantitative analysis and integration with advanced techniques to enhance accuracy.

Overall, this research underscores the potential of cerumen-based analysis in advancing forensic toxicology and public health monitoring, offering a novel approach to understanding and addressing tobacco exposure.

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