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Evaluation and Method Validation of Common Psychoactive Substances: Current Issues and Future Directions (Nicotine)

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Abstract: ***Nicotine is one of the most commonly used psychoactive alkaloids worldwide. It is still very important in forensic science because it can be found in cigarettes, smokeless tobacco, nicotine replacement products, electronic nicotine delivery systems, and biological samples in poisoning or exposure cases. In forensic and analytical settings, working with nicotine requires clear differences between preliminary alkaloid screening, confirmatory identification, and validated quantitative methods. Classical colour and precipitation tests can suggest that an alkaloid is present but cannot establish its specific identity with scientific certainty.***

This paper reviews the history and physical properties of nicotine as well as chemical test and also define the causes or adverse effect of regular consumption of tobacco and different type of cigarette.

The study also highlights current challenges such as matrix interference, variability in tobacco products, and limitations in field-level detection. Future directions emphasize advanced techniques like HPTLC, portable detection systems, and improved forensic applications.

Keyword: Nicotine, psychoactive substance, Alkaloid detection Test, Chromatographic Techniques, Method Validation, Hazardous Effect form Daily consumption, Future Directions.

I. INTRODUCTION

Nicotine is a natural compound mostly found in tobacco plants (Nicotiana tabacum) and is what makes tobacco products psychoactive and addictive. It is a volatile, oily liquid made up of a pyridine and pyrrolidine ring, and it acts as a weak base. Nicotine quickly enters the body through inhalation, ingestion, or skin contact, with smoking being the most common way people are exposed. Once it's in the body, it stimulates nicotinic acetylcholine receptors in the central nervous system. This stimulation causes the release of neurotransmitters like dopamine, creating feelings of pleasure and reinforcement. Because of this process, nicotine is highly addictive and can lead to dependence when used repeatedly. While it can be used safely in controlled doses for quitting smoking, long-term exposure is linked to various health risks, including effects on the heart and nervous system. Because of its wide use and significant impact on health, nicotine continues to be an important topic in research areas like pharmacology, toxicology, and forensic science.

II. HISTORY

Nicotine is a natural compound found in tobacco plants (Nicotiana tabacum). Indigenous peoples of the Americas have used these plants for medicinal and ceremonial purposes for thousands of years. Christopher Columbus and his crew introduced tobacco to Europe in the late 15th century. The name "nicotine" comes from Jean Nicot, who promoted tobacco in France in the 16th century for its supposed health benefits. In 1828, German chemists Wilhelm Heinrich Posselt and Karl Ludwig Reimann first isolated nicotine from tobacco leaves. Since then, researchers have studied nicotine extensively for its effects on the body. It is now known as the main addictive substance in tobacco products.

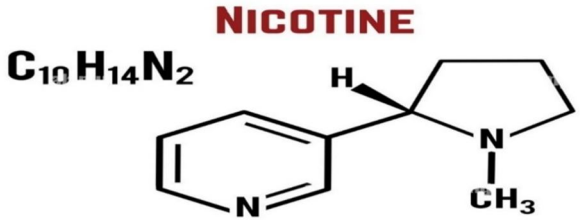


Fig.1 Structure of Nicotine

III. PHYSICAL PROPERTIES

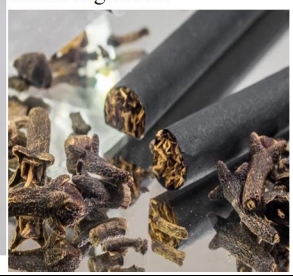
Parameter	Marlboro Cigarettes	Gold Flake Cigarette	Flake Cigarette	Clove Cigarettes
				
Appearance	Cylindrical, white paper-wrapped tobacco rod with filter tip	Uniform, well-finished cylindrical rod	Less uniform, slightly rough finishing	Cylindrical, paper-wrapped cigarette containing tobacco mixed with clove buds
Length	Typically, 84 mm (King Size) or 100 mm (Long Size)	70 mm (regular), 84–85 mm (king size)	Usually ~65–75 mm (varies)	Typically, 70–100 mm
Diameter	Approximately 7.8–8.0 mm	~7–8 mm	~6–8 mm (variable)	Approximately 7–8 mm
Weight	~0.8 to 1.2 grams per cigarette	0.6-1.1grams	0.5-0.9 grams (variable)	~1.0 to 1.5 grams per cigarette
Colour (Body)	White (paper wrapping)	Clean white	Dull white or slightly yellowish	White to brownish paper
Colour (Filter)	Light brown/tan (cellulose acetate filter)	Light golden brown	Dark brown or mixed	Brown/tan (if filtered) or absent in traditional types
pH of Smoke	Slightly acidic to neutral (approx. 5.5–6.5)	Slightly acidic (~5.5–6.2)	Slightly acidic but more variable	Slightly acidic (approx. 5.0–6.0)
Filter Type	Cellulose acetate filter with plasticizer (triacetin)	Present (cellulose acetate)	May or may not be present	Dark Gray, more compact ash
Packing Density	Firmly packed tobacco column	Higher and Uniform	Loose or uneven packing	Moderately dense with visible clove fragments
Smoke Colour	Bluish-white smoke	light grey (cleaner)	darker, denser	Dense, whitish smoke

Table 1. Physical properties of Different Cigarettes.

Parameter	Cigar	Connect Cigarette	Tobacco
			
Appearance	Thick, brown, leaf-wrapped	Slim, white paper with filter	Loose, shredded/flakes
Length	100–200 mm	~70–85 mm	Variable
Diameter	8–20 mm	~7–8 mm	Irregular
Paper Quality	No paper (tobacco leaf)	Processed cigarette paper	Not applicable
Filter Tip	Absent	Present	Not applicable
Tobacco Colour	Dark brown	Light to medium brown	Light to dark brown
Tobacco Cut	Long filler	Fine cut	Fine / ribbon / flake
Weight	5–20 g	~0.6–0.8 g	According to analysis 1 -5g
Filter Colour	Not applicable	White/brown tip	Not applicable
Packing Density	High	Medium	Low–medium
pH of Smoke	Alkaline (8–9)	Slightly acidic (5.5–6.5)	5.5–7

Table 2. Physical properties of Cigar, Connect Cigarettes, Tobacco

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IV. CHEMICAL PROPERTIES

Types	Brand Examples	Major Chemicals	Approx. Composition	Health Effects
Cigarette (Filtered) 	Marlboro, Gold Flake, Classic, Navy Cut, Wills Filter, Insignia, Benson & Hedges, Dunhill, Parliament, Camel, Lucky Strike	Nicotine ($C_{10}H_{14}N_2$), Carbon Monoxide (CO), Formaldehyde (Methanal, CH_2O), Benzene (C_6H_6), Tobacco-Specific Nitrosamines (NNK: Nicotine-derived Nitrosamine Ketone, NNN: N Nitrosonornicotine)	Nicotine (absorbed): 1.0–1.8 mg/cig (~1–3%) Tar: 6–22 mg/cig CO: 5–17 mg/cig	Lung cancer, COPD, cardiovascular disease
Cigarette (Unfiltered) 	Charminar, Scissors, No Filter Red, Red & White PlainCohiba, Davidoff, Montecristo, Romeo y Julieta, Partagas, Hoyo de Monterrey, Punch, Bolivar	Nicotine, Polycyclic Aromatic Hydrocarbons (Benzo[a]pyrene), Hydrogen Cyanide (HCN)	Nicotine in tobacco: ~15 20 mg/g (~1.5 2%) Tar: 15–25 mg/cig (higher than filtered)	Higher cancer risk, severe lung damage
Cigar 	Cohiba, Davidoff, Montecristo, Romeo y Julieta, Partagas, Hoyo de Monterrey, Punch, Bolivar	Nicotine, Ammonia (NH_3), Nitrosamines, Phenolic compounds	Nicotine in tobacco: ~3 5% Nicotine delivery: 1–3.5 mg/unit	Oral, throat cancer, addiction
Jarda (Smokeless Tobacco) 	Baba Zarda, Tulsi Zarda, Shimla Zarda, Dilruba Zarda, Rajnigandha Zarda	Nicotine, Total alkaloids, oisture, Carbohydrates / Sugars, Proteins & Amino acids, Ash (minerals), Essential oils / flavors, Additives (lime, spices, dyes), Tobacco-specific nitrosamines (TSNAs), Heavy metals (Pb, Cd, As)	Nicotine 0.5– 5% Total alkaloids 2 – 8% Moisture 10 – 20% Carbohydrates / Sugars 5 – 15% Proteins & Amino acids 1 – 3% Ash (minerals) 10 – 20% Essential oils / flavors 1 – 5% Additives (lime, spices, dyes) Variable Tobacco specific itrosamines (TSNAs)	Oral cavity—Oral cancer, leukoplakia, gum damage Cardiovascul ar — High BP, increased heart rate Digestive system — Irritation, reduced appetite Liver & Kidney — Toxic damage Pregnancy — Low birth weight, fetal problems General - Increased cancer risk

Table 3. Chemical properties of Nicotine Products

V. MICROSCOPIC EXAMINATION

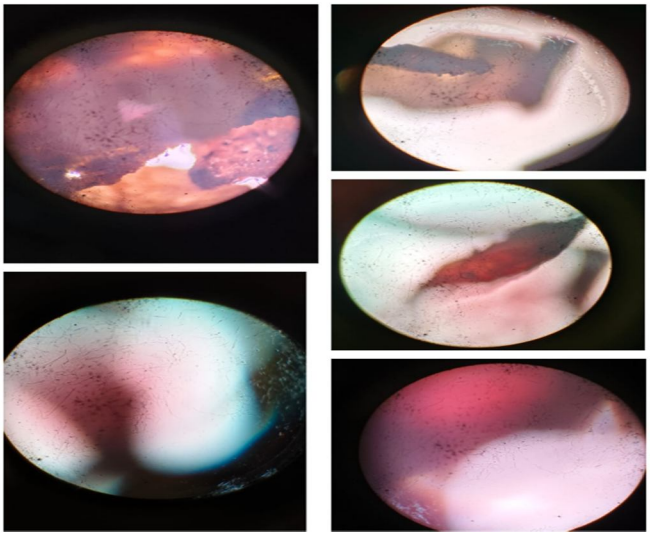

Fig.2.Microscopic view of nicotine products

Fig.3. Extraction of Nicotine

VI. METHODOLOGY

A. Sample Preparation

Nicotine was extracted from tobacco using an alkaline liquid-liquid extraction method. First, take 1 gram of tobacco and make a finely powder by using mortar pestle. Then, suspend the powdered tobacco in 10–15 mL of distilled water. Make the solution alkaline by adding 2–3 mL of 10% sodium hydroxide solution. This helps c0onvert nicotine into its free base form. Next, transfer the mixture to a separating funnel and extract it with 15–20 mL of chloroform. Collect the organic phase and repeat the extraction several times to maximize nicotine recovery. Filter the combined organic extracts to remove solid impurities. This give nicotine, which appears as a pale-yellow oily residue and is suitable for further analytical testing.

VII. COLOUR TEST FOR ALKALOIDS

A. General Principle

Dragendorff's, Mayer's, and Wagner's tests are classical preliminary alkaloid tests used to indicate the presence of basic nitrogenous alkaloids in an extract. A positive result supports the inference that an alkaloid may be present and justifies moving to confirmatory testing, but it is not by itself sufficient for definitive forensic identification.

1) DragenDorff's test

DragenDorff's reagent is an alkaloid-detecting reagent prepared from a bismuth salt and potassium iodide in acidic medium, producing a reactive iodobismuth complex that forms an orange to orange-red or reddish-brown precipitate with many protonated alkaloids. One practical description states that the reagent is prepared by combining potassium iodide with bismuth subnitrate in dilute acid, and the reaction with alkaloids forms an ion-pair precipitate. In nicotine analysis, addition of Drage Dorff's reagent to an appropriately acidified extract may therefore indicate an alkaloid reaction consistent with nicotine, but because other alkaloids and basic compounds can also respond, the result is presumptive only.

2) Mayer's Test

Mayer's reagent is commonly described as potassium mercuric iodide reagent and is prepared from mercuric chloride and potassium iodide in water. Standard laboratory descriptions list approximately 1.358 g mercuric chloride and 5 g potassium iodide in 100 mL water for preparation. When added to an alkaloid-containing extract, it typically gives a cream or pale precipitate; in the nicotine context, this indicates the presence of an alkaloidal base but not uniquely nicotine behaves as a protonated basic nitrogen compound in acidic solution, and Mayer's reagent provides a heavy-metal iodide complex that forms an insoluble salt with the alkaloid. The practical limitations should also be stated, including falseositives with other alkaloids and lower evidential value than chromatographic identification.

3) Wagner's Test

Wagner's reagent is a solution of iodine in potassium iodide and is used to precipitate alkaloids from solution. A commonly cited preparation is 2 g iodine plus 6 g potassium iodide in 100 mL water. In the presence of alkaloids, brown, reddish-brown, needle

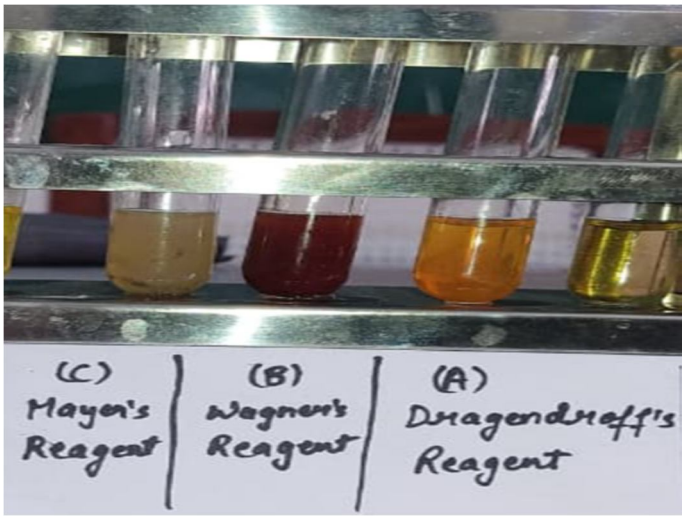

Fig.4. Color Test of Alkaloids

B. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) analysis of nicotine was conducted using silica gel 60 F_{254} as the stationary phase. For non-flavoured cigarette samples such as Marlboro, Gold Flake, Connect, and Classic, a mobile phase made of dichloromethane and methanol was used. In contrast, for clove and flavoured cigarette samples, a more polar solvent system with ethyl acetate, methanol, ammonia, and water was employed to improve separation due to the presence of extra flavouring compounds. For raw tobacco samples, a simpler solvent system of ethyl acetate and ammonia was used. like, or related precipitates may be observed depending on concentration and matrix. The changes in solvent systems were essential to reduce matrix interference and to achieve clear separation of nicotine in different sample types and their result image and table is shown in below.

S. No.	Sample Type	Sample Name	Solvent System	Ratio	Rf Value
1	Non-flavoured Cigarette	Marlboro	Dichloromethane: Methanol	9: 1	0.23
2	Non-flavoured Cigarette	Gold Flake	Dichloromethane: Methanol	9: 1	0.58
3	Non-flavoured Cigarette	Connect	Dichloromethane: Methanol	9: 1	0.3
4	Non-flavoured Cigarette	Classic	Dichloromethane: Methanol	9: 1	0.20
5	Flavoured Cigarette	Clove Cigarette	Ethyl acetate: Methanol: Ammonia: Water	5: 3: 0.2: 1.8	0.51
6	Flavoured Cigarette	Flake	Ethyl acetate: Methanol: Ammonia: Water	5: 3: 0.2: 1.8	0.47
7	Tobacco	Raw Tobacco	Ethyl acetate: Ammonia	9: 1	0.50

Table 4. RF Value of Separated Component from Different Nicotine By-Product through Thin layer Chromatography

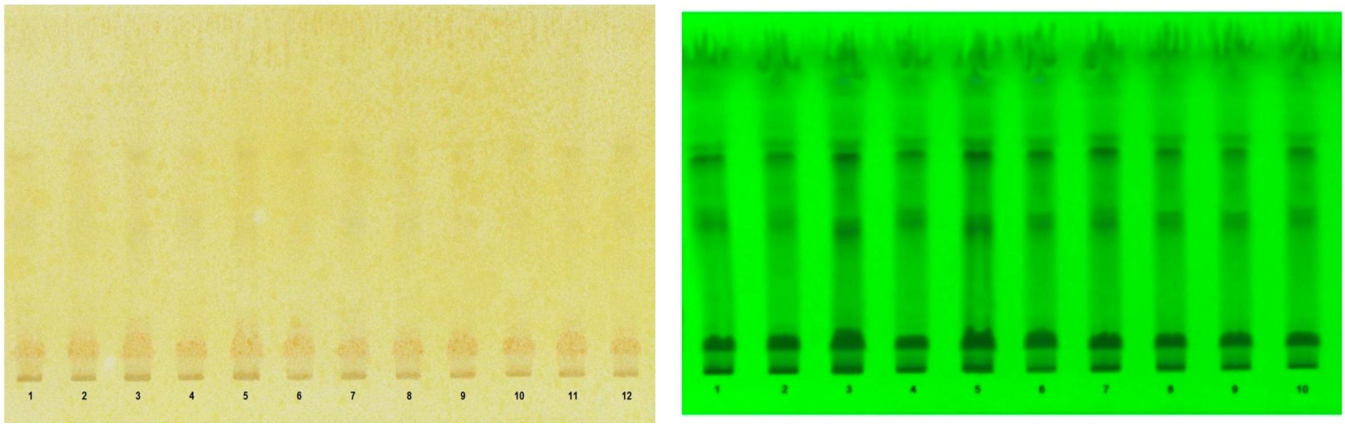

Fig.5. Visualization of separated component of nicotine through thin layer chromatography with the help of visible light (after spraying dragendroff's reagent) and UV light.

C. UV Spectrometer

Nicotine estimation in tobacco is used UV spectrophotometric principles based on its absorption at 261 nm. Methanol was chosen as the extraction and dilution solvent because it dissolves nicotine well and has a low UV cut-off. Nicotine was accurately weighed and transferred into a 100 mL volumetric flask this will form 100 ppm stock solution. A 2-12 µg/ml of the Standard solution of Nicotine was prepared by using stock solution and examined. A standard calibration curve was made using nicotine solutions with concentrations between 2 and 12 µg/ml. This curve showed a good linear relationship according to Beer-Lambert law. The absorbance of the prepared solutions was measured at 261 nm, and the nicotine content was estimated using the calibration curve. Nicotine 50 µg/ml solution was scanned in UV spectrophotometer within the wavelength range of 200–400 nm. Methanol solvent was used as blank.

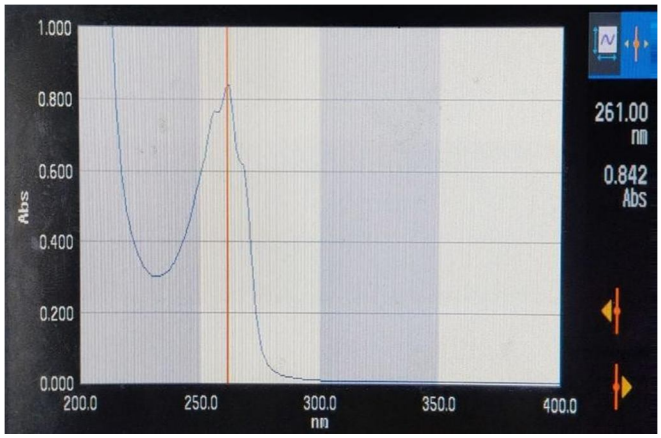

Fig.6: Maximum wavelength at 261nm

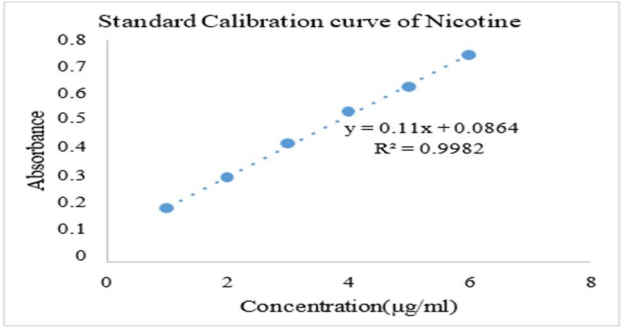

Fig.7.Standard calibration curve of Nicotine

Sr no.	Conc.(µg/ml)	Absorbance
1	2	0.191
2	4	0.302
3	6	0.426
4	8	0.539
5	10	0.628
6	12	0.743z

Table5. Absorbance of Different Concentration of Nicotine

Conc.(µg/ml)	Absorbance at 259nm	Absorbance at 260nm	Absorbance At 262nm	Absorbance at 263nm	Statistical Analysis
4	0.207	0.212	0.210	0.208	Mean = 0.3406
6	0.335	0.341	0.338	0.334	SD = 0.003091
8	0.472	0.481	0.480	0.478	%RSD = 0.93%

Table6. Different concentrations of Nicotine solution like (4 µg/ml,6 µg/ml,8 µg/ml) was analysed at different wavelength includes 259nm, 260nm, 261nm, 262nm, 263nm to determine robustness of the method.

VIII. METHOD VALIDATION

The analytical method developed for determining nicotine was thoroughly validated to ensure its reliability and suitability for routine analysis. The method showed excellent linearity of concentration range of 2 to 12µg/ml, with a strong correlation between absorbance and concentration. Accuracy was confirmed through recovery studies, which yielded results within acceptable limits, showing that the measured values were close to the true concentration. The method also demonstrated high precision, as indicated by 0.93%RSD values, reflecting good repeatability and reproducibility. The calculated limit of detection (LOD) and limit of quantification (LOQ) confirmed that the method has enough sensitivity to detect and measure nicotine at low concentrations. Additionally, the method exhibited satisfactory specificity, allowing for reliable identification of nicotine in the presence of interfering substances commonly found in tobacco products. Together, these validation parameters show that the method is accurate, precise, sensitive, and scientifically sound, making it well-suited for analytical, forensic, and research purposes.

IX. CURRENT ISSUES

1) Complexity of Tobacco Matrix: Tobacco is a chemically complex material. It contains not only nicotine but also related alkaloids like nor nicotine, anabasine, and anatabine, as well as tar, resins, sugars, and additives. These components can interfere with analytical signals during detection and quantification. Such interference reduces the selectivity and accuracy of methods like UV spectrophotometry and TLC. This makes it difficult to isolate nicotine specifically without prior purification or specialized equipment.

2) Lack of Specificity in Preliminary Tests: Traditional alkaloid tests like Dragendorff's, Mayer's, and Wagner's rely on general reactions with nitrogen-containing compounds. While they are helpful for quick screening, they do not differentiate nicotine from other alkaloids in the sample. This lack of specificity may result in false-positive results, limiting their use in confirmatory analysis and forensic investigations.

3) Limited Sensitivity of Conventional Techniques: Methods like Thin Layer Chromatography (TLC) and UV-Visible spectrophotometry are common because they are simple and affordable. However, they have limited sensitivity. These techniques are generally unsuitable for detecting nicotine at trace levels, especially in biological samples or environmental contexts, where highly sensitive methods are necessary.

4) Variability in Nicotine Content: The concentration of nicotine varies greatly depending on the type of tobacco, cultivation conditions, processing methods, and the presence of additives. This variability impacts the reproducibility of results and complicates standardization, particularly when comparing different brands or types of samples.

5) Interference from Co-existing Compounds: Along with other alkaloids, substances such as compounds similar to caffeine, polyphenols, and combustion products can disrupt analytical measurements. These interferences may change absorbance values in spectrophotometry or affect separation in chromatographic methods, leading to inaccurate quantification.
6) Lack of Portable Detection Methods: Most reliable nicotine detection methods require laboratory instruments like spectrophotometers or chromatographic systems. There is a shortage of portable, quick, and user-friendly detection tools, which limits on-site analysis in forensic investigations, environmental monitoring, and regulatory checks.
7) 7.Limitations in Method Validation Across Laboratories: Differences in analytical procedures, calibration standards, and validation methods across laboratories result in inconsistent outcomes. The absence of universally accepted validation guidelines hampers the comparability and reliability of nicotine analysis.

X. FUTURE DIRECTIONS

1) Adoption of Advanced Analytical Techniques: Future research should focus on using highly sensitive and selective methods like LC-MS/MS and GC-MS. These techniques allow accurate identification and quantification of nicotine even at trace levels and can effectively differentiate it from similar compounds, improving analytical accuracy in complex samples.
2) Development of Portable and Rapid Detection Systems: There is an increasing demand for portable analytical devices, such as biosensors and handheld detectors, that can deliver fast and on-site nicotine detection. These systems will be very useful for forensic fieldwork, environmental monitoring, and regulatory enforcement.
3) 3.Integration of Artificial Intelligence and Automation: Using artificial intelligence (AI) and machine learning in analytical chemistry can improve data analysis, spectral interpretation, and pattern recognition. Automated systems can lessen human error, enhance efficiency, and allow real-time monitoring of nicotine levels.
4) Standardization of Analytical Methods: Future efforts should work toward establishing globally accepted validation protocols and standard operating procedures. Standardization will ensure consistency, reproducibility, and reliability of results across different laboratories and research studies.
5) Enhancement of Sample Preparation Techniques: Better extraction and purification methods, such as solid-phase extraction (SPE) and microextraction techniques, can lower matrix interference and increase detection sensitivity. Improved sample preparation will significantly boost the overall performance of analytical methods.
6) Expansion into Biological and Clinical Analysis: Future studies should broaden nicotine analysis to biological samples like blood, urine, and saliva. Monitoring nicotine and its metabolites will provide useful insights into toxicology, addiction studies, and clinical diagnostics, expanding its use beyond tobacco analysis.
7) 7.Real-Time Monitoring and Environmental Analysis: Advancements in sensor technology may enable real-time monitoring of nicotine exposure in air, water, and biological systems. This will be especially valuable for assessing passive smoking exposure and environmental contamination.

XI. CONCLUSION

This study evaluates nicotine as a widely used psychoactive substance through various analytical techniques. The use of classical alkaloid tests, Thin Layer Chromatography, and UV-Visible spectrophotometry shows that nicotine can be identified and measured effectively. These methods are cost-effective and widely available. The successful validation of the method relies on parameters like linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), confirming its reliability for routine analytical and forensic use.

However, the study also points out key limitations, including matrix interference, lack of specificity in preliminary tests, and lower sensitivity of traditional techniques. These issues highlight the need for ongoing improvements in analytical methods. Currently, integrating high-sensitivity techniques such as LC-MS/MS, better sample preparation methods, and portable detection systems can greatly improve the accuracy and usefulness of nicotine analysis.

Overall, this research lays a solid analytical groundwork and suggests that future work should focus on enhancing sensitivity, specificity, and real-time detection capabilities. By incorporating modern technologies and standardized validation protocols, nicotine analysis can be further improved to meet the changing needs of forensic, clinical, and pharmaceutical sciences.

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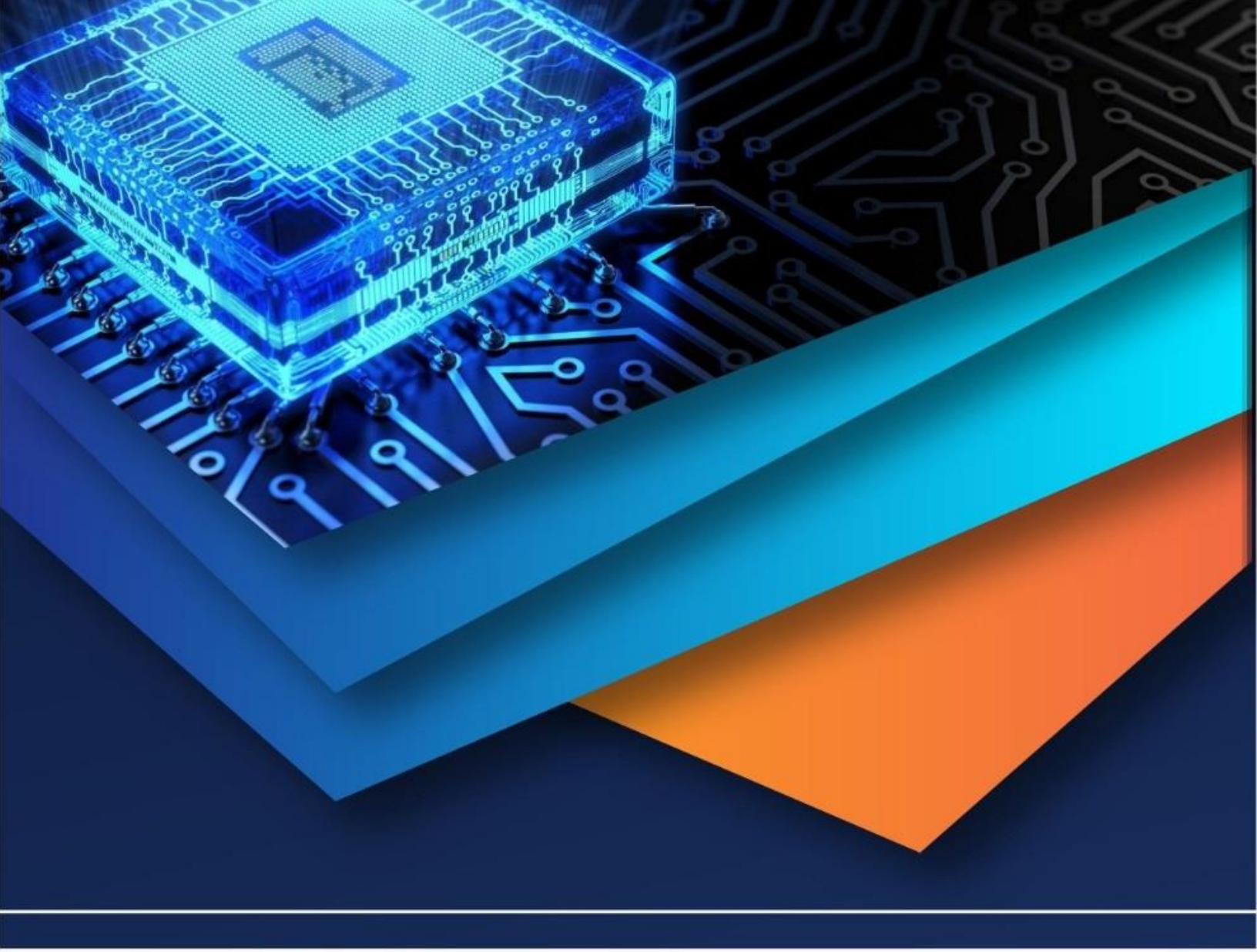

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