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Isolation of Nicotine by Chemical Methods: Application in Various Sample with Non- Aqueous Acid- Base Titration: A Review

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Abstract: *Determination of nicotine content This study aimed to quantify and compare the nicotine content in locally sourced tobacco leaves and commercially available cigarettes employing two distinct extraction methods. Ten samples were analysed: one from unprocessed tobacco leaves and nine from various cigarettes brands. Nicotine extraction is performed chemical and a non-aqueous acid alkali extraction technique. Quantitative analytical study revealed that unprocessed tobacco leaves contained higher nicotine concentration compared to processed tobacco in cigarettes. This study highlights the need for regulatory measure to monitor nicotine contents in tobacco products particularly local brand that use tobacco leaves directly. Among this method non aqueous acid -base titration yielded more efficient recovery of nicotine.*

Keywords: Nicotine**,** cigarette sample, isolation, acid-base titration, tobacco leaves, solution, review

I. INTRODUCTION

Nicotine is an alkaloid heterocyclic aromatic organic compound[1]. Nicotine is protonated in the plant and is present as the formate, maleate or acetate salts. Hence, nicotine is first treated with aqueous NaOH, to cleave the organic salts and release the free nicotine into the aqueous solution[2]. Nicotine is a readily water-soluble liquid which can be precipitated out of the aqueous solution in the form of the Di-picrate by addition of saturated picric acid solution[3]. Nicotine is an alkaloid (a substance with a basic charge), present in the leaves of several species of plants[4]. The primary commercial source of nicotine is by extraction from the dried leaves of tobacco plant (Nicotinia tabacum and N. rustica)[5].The chemical formula for nicotine is $C_{10}H_{14}N_2$, with a molecular mass of 162.23. In proper nomenclature, nicotine is 3-(1-Methyl-2-pyrrolidinyl) pyridine. Nicotine's structure was deduced by Pinner [6]. The nicotine is t is the main psychoactive compound in tobacco products and plays a significant role in addiction [7]. Due to its pharmacological effects on the central nervous system (CNS), nicotine has been widely studied in neuroscience, toxicology, and public health. Nicotine acts as an agonist at nicotinic acetylcholine receptors (nAChRs), leading to:

• Release of neurotransmitters like dopamine, serotonin, norepinephrine, and glutamate.
• Activation of the reward pathway in the brain, contributing to addiction.
• Increased heart rate, blood pressure, and cognitive alertness.

Nicotine is a highly addictive substance with both harmful and potential therapeutic effects. While its role in smoking-related diseases is well-established, research continues on its medical applications. Strict regulation and harm-reduction strategies are key in public health policies[8].The reported methods for estimation of nicotine suffer from such draw backs as high cost, multiple steps and also several clean-up steps (HPLC) [9]. They are time consuming and often poorly reproducible; some require toxic organic solvents [10]. Any method chosen for routine analysis should be reasonably simple, used materials should be readily available in the laboratory or readily obtainable and require a minimum amount of equipment. These objectives have been fulfilled by titrimetric Procedure[11]. Non-aqueous titrations are those in which the titrations of too weakly acidic or basic substances are carried out using non-aqueous solvents so as to get sharp end point. Such titrations can also be used for the titration of the substances not soluble in water[12]. The speed,

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precision and accuracy of the non-aqueous method are close to those of classical acidimetric and alkalimetric titrations[13]The apparatus involved are also same but moisture and carbon dioxide are to be avoided in non-aqueous methods because water, which is a weak base, can compete with the weak nitrogen base and the end point would not be sharp at all[14]. It has been observed through experiments that the moisture content in non-aqueous titrations should not be more than 0.05%.[15]

When a weakly basic drug is present, water (OH^-) acts as stronger base as compared to the former one and preferentially accepts proton from an acid. Thus, there is interference in the reaction of weak base with an acid. Similarly, when a weakly acidic drug is present, water (H^+) behaves like a strong acid as compared to the former one and preferentially donates proton to the base. Thus, there is interference in the reaction of weak acid with a base. Hence in the presence of water, titration of either weakly acidic substances with stronger base or weakly basic substances with stronger acid is not possible.[16]

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. The name derives from the word alkaline is due to nitrogen containing base. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, animals and are part of the group of natural products, also called secondary metabolites. Many alkaloids are purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anaesthetic and stimulant cocaine, the stimulant caffeine, nicotine, the analgesic morphine, or the antimalarial drug quinine. Some alkaloids have a bitter taste[17].

Nicotine is a pyridine alkaloid obtained from the dried leaves of tobacco plant *Nicotiana tabacum,* Family *Solanaceae*. Tobacco leaves contain 2 to 8% of nicotine combined as maleate or citrate.Nicotine is a colourless or pale yellow, very hygroscopic oily liquid with an unpleasant pungent odour and a sharp burning persistent taste.

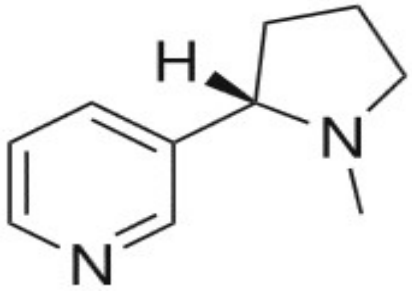

Fig. 1 Structure of Nicotine.

It gradually becomes brownish on exposure to air or light[18]. Nicotine is soluble in water, alcohol, chloroform, ether, kerosene, petroleum ether and fixed oils. Nicotine is a ganglionic cholinergic receptor agonist with complex pharmacological activities that includes effects mediated by binding receptors in autonomic ganglia, adrenal Madula, the neuromuscular junction and the brain. Nicotine causes psychological and physical dependence during chronic uses.[18] Cigarette smoking remains a leading cause of preventable disease and premature death in the India and other countries. On average 4,35,000 people in the India die prematurely from smoking-related diseases such as cancer, cardiovascular disease and pulmonary disease each year; overall, smoking causes 1 in 5 deaths. The chance that a lifelong smoker will die prematurely from a complication of smoking is approximately 50%. Cigarette smoking is a risk factor for respiratory tract and other infections, osteoporosis, reproductive disorders, adverse postoperative events and delayed wound healing, duodenal and gastric ulcers and diabetes. Conditioning is a major factor that causes relapse to drug use after a period of cessation. It must be addressed as a component of counselling and behavioural therapy for drug addiction.[19]

Exploration of the potential of tobacco as a raw material of a hygienic product is one alternative solution for the sustainability of tobacco cultivation while increasing the empowerment of tobacco farmers. Nicotine is an alkaloid found predominantly in tobacco leaves [20]. Various solvent extraction can be used to isolate nicotine in tobacco leaves. Nicotine is widely used in fine chemical, pharmaceutical and agriculture industries, and in the tobacco industry itself as an essential cigarette additive [21]. One potential for tobacco is the conversion of nicotine from tobacco leaves into

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nicotinic acid or niacin. Niacin a compound of Vitamin B3 which is widely used as medicine, vitamins, and cosmetics. This compound has a pyridine group and a carboxylate group as a side group so that the synthesis of nicotinic acid from nicotine from tobacco leaves can be done[22].

Experimental methodology and Processes:

Materials and Methods

Ten samples of common brands were purchased from the local market and compared to a raw tobacco sample local brand.Diethyl Ether (98%) (Fluka), Potassium Hydroxide (Sigma Aldrich), concentrated sulfuric acid 98% (Sigma Aldrich), Barium hydroxide, Perchloric acid, Potassium hydrogen phthalate. Crystal violet indicator.

Method- 1

i) Into an Erlenmeyer flask accurately weigh a 6-gm sample of tobacco (6-9 cigarettes without the paper and filter components). Record this data in your lab notebook as well as the brand name of the cigarettes.

(ii) To the flasks add approximately 50 ml of the saturated aqueous $Ba(OH)_2$ solution and 2 gm of granular Ba(OH)2. Ensure that the tobacco is thoroughly wetted. Into the flask, pipet 100.00 ml of toluene, add a stirring bar, stopper the flask, and magnetically stir for 20 minutes.

(iii) After 20 minutes filter most of the organic layer through a Whatman No.2folded filter paper into another clean, DRY Erlenmeyer flask. The aqueous layer should not be poured into the filter.

(iv) Into a clean, DRY Erlenmeyer flask pipet 20.00 ml of the filtered solution. Add 4-5 drops of crystal violet indicator. Using your burette filled with your standardized 0.1M HCIO, titrate to the characteristic greenish yellow endpoint. Repeat Step 4 two more times for reproducibility. (repeat the same procedure for next Nine samples).

Calculations:

1] Normality of perchloric acid

10 cm^3 of 0.1N potassium hydrogen phthalate
= x cm^3 of perchloric acid

$$\text{Normality of perchloric acid} = \frac{10X0.1}{10}$$
$$= 0.1 \text{ AN}$$

2] Since,1 mole of nicotine = 1 mole of perchloric acid.

1000 cm^3 of 1N $HClO_4$ = 162.23 g/mol of nicotine

X cm^3 of 0.1N $HClO_4 = \frac{162.23 \text{ X } 16 \text{ X } 0.1}{1000}$ x is (constant burette reading)

= Y. gm of Nicotine.

Method -2

Moisten about 50 gm of powdered drug with sufficient quantity of 20% alc. KOH solution to liberate alkaloidal base. Dry it at below 60°C.

Place the powder in flask containing 100ml of solvent ether and heat on water bath under refluxing condition for 10 minutes.

Filter and concentrate the filtrate to one fourth volume.

iv) Treat the ethereal extract with 20 ml of dilute sulphuric acid twice.

To the aqueous layer, add sufficient quantity of 5% NaOH solution.

vii) Extract free base with solvent ether twice. Concentrate ethereal extract and dissolve in about 5ml of water and filter. To the filtrate, add picric acid dropwise till complete precipitation of nicotine picrate take place. Keep the solution in cold condition for 15 minutes.

viii) Dry the product, weigh and determine its M.P. (repeat the same procedure for next Nine samples).

II. RESULT AND DISCUSSION

In this researchreview, nicotine has been isolated and analysed from ten different tobacco products using two different methods. The results of nicotine analysis are presented in the table below. The data shows that nicotine content varies

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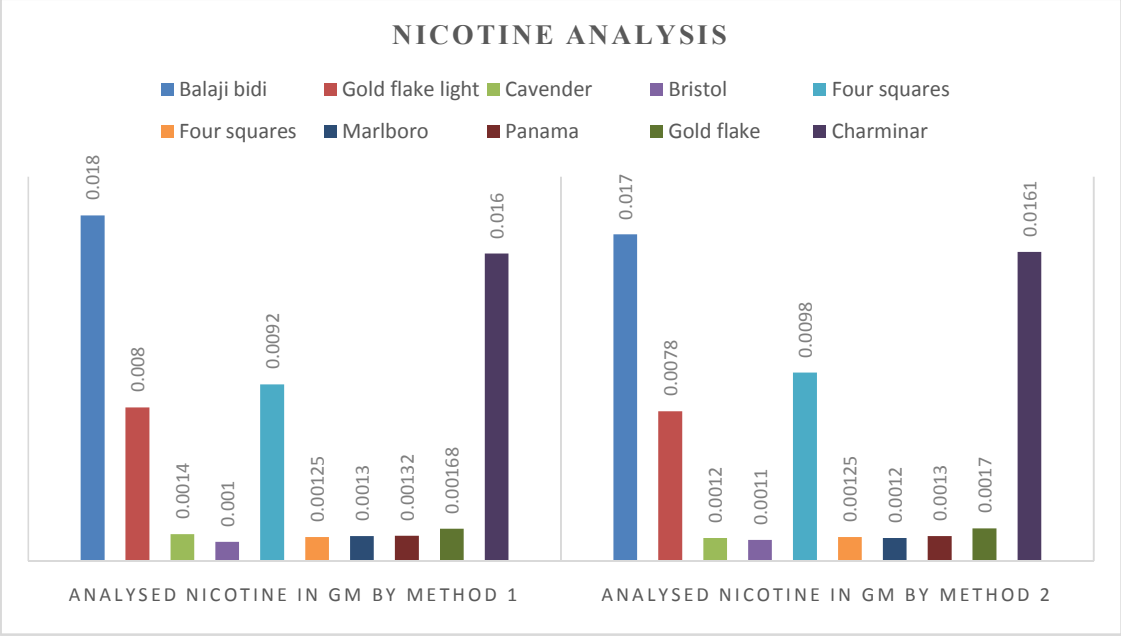
across different products. Among the analysed samples, the highest nicotine content was observed in Balaji bidi (0.018 g by Method 1, 0.017 g by Method 2), followed by Charminar (0.016 g, 0.0161 g). Gold Flake Light had the lowest nicotine content (0.008 g, 0.0078 g), followed by Bristol (0.001 g, 0.0011 g).

A comparison between the two analytical methods indicates a high degree of consistency, as both methods yielded closely matching nicotine values. However, slight variations are observed, which could be attributed to differences in extraction efficiency, instrument sensitivity, or sample preparation techniques.The results confirm that cigarette and bidi brands contain varying levels of nicotine, which may influence their addictive potential. The findings emphasize the importance of understanding nicotine content for regulatory and health impact assessments.

Table-1 shows analysed nicotine content in different samples using different methods in gm.

Sr. No	Name of the product	Analysed Nicotine in gm by Method 1	Analysed Nicotine in gm by Method 2
1	Balaji bidi	0.018	0.017
2	Gold flake light	0.008	0.0078
3	Cavender	0.0014	0.0012
4	Bristol	0.001	0.0011
5	Four squares	0.0092	0.0098
6	Four squares	0.00125	0.00125
7	Marlboro	0.00130	0.00120
8	Panama	0.00132	0.00130
9	Gold flake	0.00168	0.0017
10	Charminar	0.016	0.0161



Graph-1 Nicotine Content analysis by two methods.

III. CONCLUSION

In this section,non-aqueous titrations are widely used in the Pharmacopoeias for the assay of many drug substances based on the properties of the drug which is either weakly acidic or weak bases.

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In this study, nicotine content was analysed in 10 different cigarette samples, including both commercial and local brands, using two distinct analytical methods: a chemical method and a nonaqueous method. The results revealed that the Balaji Bidi local cigarette brand exhibited the highest nicotine content among all tested samples. This suggests that local brands may have different formulations, tobacco blends, or processing techniques that contribute to elevated nicotine levels.

The comparison between the chemical and nonaqueous methods provided valuable insights into their accuracy and efficiency in nicotine quantification. Both methods effectively determine nicotine levels, but further studies may be needed to validate their precision and reproducibility across a wider range of samples.

The significantly higher nicotine content in the local brand raises concerns regarding potential health risks, addiction potential, and regulatory considerations. Future studies could explore the impact of such high nicotine levels on consumer health, smoking behaviour, and possible measures for nicotine regulation in locally produced cigarettes or tobacco product.

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