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Synthetic Vs Natural Nicotine: Investigation in the Indian Context

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Abstract: ***The objective of this study is to ascertain the enantiomeric composition and purity of synthetic nicotine samples in India, evaluating whether the surge in synthetic nicotine's popularity is paralleled by adequate knowledge of its pharmacological and toxicological effects. This research employs established US Pharmacopoeia [1] methods to analyse synthetic nicotine (resolved to the S-isomer) against Tobacco-Derived Natural Nicotine (TDN). Polarimetry and Gas Chromatography are the primary techniques utilized to determine the enantiomeric ratio and purity of nicotine in the samples. The study finds that Synthetic Nicotine, when resolved to the S-isomer, exhibits characteristics closely similar to TDN. Conversely, the racemic mixture of Synthetic Nicotine presents a balanced ratio of R and S isomers, as evidenced by the absence of optical rotation. These results suggest that Synthetic Nicotine has been accurately resolved to match the enantiomeric purity of natural nicotine. This research has implications for manufacturers, users, and policymakers. For manufacturers, it emphasizes the necessity for precise production techniques. For users, particularly in India, it emphasizes the importance of awareness about the contents of nicotine products. For policymakers, the study signals the urgent need for regulatory frameworks to manage the production and sale of synthetic nicotine, ensuring public health safety. This research fills a critical gap in the existing literature by providing empirical data on the enantiomeric composition of synthetic nicotine within the Indian context, a largely under-researched area. The findings and methodologies offer a valuable reference point for further studies and regulatory assessments.***

Keywords: Synthetic Nicotine, Enantiomeric Purity, Tobacco Derived Nicotine, Pharmacological Effects, India

I. INTRODUCTION

Nicotine, a predominant alkaloid extracted from the Nicotiana tabacum plant, is known for its unique chemical structure that includes pyridine and pyrrolidine nitrogen rings. The natural form of nicotine (TDN) primarily exists as the (S)-enantiomer, while synthetic nicotine (TFN/Syn) is a racemic mixture of both (S)- and (R)+ enantiomers [2]. The recent years have seen a surge in the use of synthetic nicotine, touted as a safer alternative to TDN. This trend is particularly significant in the United States and South Korea, with India, a major tobacco producer, emerging as a potential large market for synthetic nicotine [3]. The pharmacological and toxicological effects of these nicotine forms are subject to ongoing research. However, research on R-enantiomer present in synthetic nicotine remains limited.

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Studies indicate that the S-enantiomer in TDN and the R-enantiomer in synthetic nicotine exhibit different effects. For instance, a US study found that R-nicotine did not induce weight loss or trigger epinephrine release in rats, unlike its S-counterpart [4]. Similarly, oxidative and N-methylated metabolites were found in R-nicotine, whereas S-nicotine formed only oxidative metabolites in guinea pigs [5].

In India, changes in education, lifestyle, and cultural norms have led to varying patterns of nicotine consumption. Initially met with resistance, tobacco use increased with improved commercialisation. However, in recent years, there has been a decline in tobacco users by 34.1 per cent, reflecting a growing health awareness. India continues to be the second-largest tobacco producer, behind China, with approximately 0.45 million hectares of land under tobacco cultivation.

While substantial research on synthetic nicotine exists in countries like the US, UK, and Germany, there is a notable scarcity of such research in the Indian context [6]. Understanding the composition and effects of both natural and synthetic nicotine is crucial. An unregulated market of synthetic nicotine could lead to the threat of companies circumventing regulations, mislabelling products and undermining public health. Hence, it is essential to gain knowledge of the composition and effects of nicotine, derived from both natural and synthetic origins.

This knowledge can guide government and health organizations in policy-making, particularly in India, where research on this subject is limited [7].

To the best of the researcher's knowledge, there is very little research in this area in the Indian context. Hence, this study attempts to answer the following research question:

RQ: What is the enantiomeric composition and purity of synthetic nicotine samples in India?

The rest of the paper is structured as follows. The next section provides a review of the existing literature on the differences between tobacco-derived nicotine (TDN) and tobacco-free nicotine (TFN), as well as the methods available to distinguish between the two. This is followed by a description of the materials and techniques used for the experiments under this study. The following section presents the findings and discussion of the experiment results. Lastly, the conclusion section discusses the study's limitations, implications, and scope for further research.

II. LITERATURE REVIEW

A. Nicotine

Nicotine, a dominant alkaloid derived from the Nicotiana tabacum plant, is notable for its complex chemical composition, comprising two nitrogen-containing rings, pyridine and pyrrolidine, linked by a carbon-carbon bond. The pyrrolidine ring

features a chiral carbon centre, which gives rise to two enantiomers of nicotine: (S)-(-)-nicotine and (R)-(+)-nicotine. These enantiomers exhibit distinct properties and implications in both natural and synthetic forms of nicotine [2].

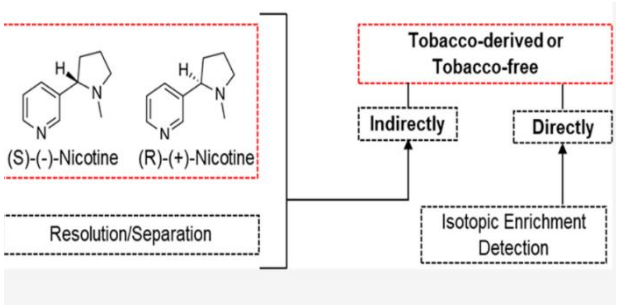
Tobacco Derived Nicotine (TDN), the natural form of nicotine, predominantly exists as the (S)-enantiomer. However, the (R)-enantiomer comprises only a minor portion (0.02% to 0.46%) of the total nicotine content. In contrast, Tobacco-Free Nicotine (TFN), also known as synthetic nicotine, is synthesised to produce a racemic mixture of both enantiomers, with the possibility of refining to achieve ~approximately 99 per cent (S)-nicotine. This refining process, however, increases the production cost [2].

The advancement in the synthesis of synthetic nicotine, also known as Tobacco-Free Nicotine (TFN), has been notable, particularly in the development of various synthetic methods. These methods are designed to produce nicotine that is free from tobacco-derived impurities, offering a purer form of the compound. Three significant methods have been developed over the years:

Ethyl Nicotinate Pathway (NGL Method): This method, patented by NGL, begins with ethyl nicotinate, an ester of nicotinic acid (also known as niacin). The process involves several steps to transform this compound into a tobacco alkaloid called myosmine. Myosmine is then reacted with N-vinyl-2-pyrrolidinone, leading to the formation of nornicotine. Nicotine undergoes a methylation step, resulting in the production of a racemic mixture of (S)- and (R)-nicotine enantiomers. This method is notable for its use of readily available synthetic bulk chemicals and its ability to produce a 50/50 mix of the nicotine enantiomers [2].

CNT's Stereoselective Purification Process: The method developed by Contraf-Nicotex-Tobacco (CNT) in Germany also starts with ethyl nicotinate. The process involves a reaction with n-vinylpyrrolidone nicotinic acid. What makes this method stand out is the subsequent step of stereoselective purification using L-0,0'-dibenzoyl tartaric acid. This approach allows for a more targeted production of nicotine, focusing on creating a specific enantiomer rather than a racemic mixture. This method highlights the advancements in stereoselective synthesis, which are critical for producing substances with specific pharmacological properties [3].

Zanoprima's Biotechnological Process: Zanoprima's patented process represents a significant leap in the synthesis of S-nicotine, employing a biotechnological approach. This method begins with the conversion of myosmine to S-nornicotine using a specific recombinant enzyme, a NADH/NADPH-dependent imine reductase. The choice of enzyme and its specific action on myosmine is crucial for ensuring the production of S-nornicotine with high stereoselectivity. The final step involves methylation, which transforms S-nornicotine into S-nicotine. This biotechnological approach is notable for its precision and efficiency in producing a specific enantiomer of nicotine [3].



[Fig.1: Analytical Methods on Nicotine Enantiomers and Sources]

Source: Salam et al.[2]

These methods collectively demonstrate the versatility and innovation in the field of synthetic nicotine production. From chemical synthesis to biotechnological approaches, the development of these methods not only offers a way to produce nicotine without the need for tobacco but also enables the creation of specific enantiomers, opening new possibilities in the fields of pharmacology and toxicology.

Synthetic nicotine is marketed in both racemic and enriched forms. The racemic form is more cost-effective as it does not require enantiomeric enrichment, making it a viable option for manufacturers [6]. A key advantage of TFN products over TDN is the absence of impurities typically associated with tobacco extraction processes. TFN is considered a purer and cleaner substance, free from harmful toxins that occur during tobacco extraction [9].

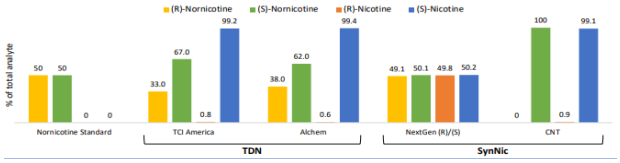
B. Differences Between TDN and TFN

Chemical Composition: Tobacco-Derived Nicotine (TDN) and Tobacco Free Nicotine (TFN) differ significantly in their chemical compositions. While TDN predominantly exists as the (S)-enantiomer, TFN is synthesised as a racemic mixture, containing equal amounts of the (S)- and (R)-nicotine enantiomers. The production methods for TFN, such as those developed by NGL and CNT, aim to create a product devoid of the residual impurities found in TDN. These impurities in TDN, including tobacco-specific nitrosamines (TSNAs), metals, and other metabolites, are inherent to the tobacco leaf origins and the extraction process. In contrast, TFN may contain synthetic process impurities like precursors or residual solvents [6];[5].

Pharmacological and Toxicological Properties: The pharmacological and toxicological differences between the (S)- and (R)- enantiomers of nicotine are substantial. (S)-Nicotine, which is majorly present in TDN, is known to be more harmful than its (R)- counterpart. This is evident in its more decisive agonist action on nicotine receptors and its association with various adverse health effects such as weight loss and stimulation of epinephrine release in animal studies. On the other hand, (R)-nicotine, which constitutes 50 percent of the content in racemic TFN, shows comparatively milder pharmacological effects and does not exhibit the same level of potency in terms of receptor activation or the health effects [3];[8].

Enantiomeric Distribution and Analysis: The difference in the enantiomeric distribution between TDN and TFN is crucial from a

pharmacological perspective. Chiral chromatography, which resolves enantiomeric mixtures based on their interactions with a chiral stationary phase, facilitates the quantification of the relative amounts of each enantiomer. This analytical technique reveals that if the nicotine enantiomeric ratio differs significantly from the respective nicotine R/S ratios, it indicates TDN origin. Conversely, a well-matched nornicotine to nicotine ratio (50:50) suggests the presence of TFN. This method of analysis is essential in differentiating between the sources of nicotine and understanding their respective pharmacological effects [5].



[Fig.2: Comparison of the R/S-Nornicotine Ratio to the R/S-Nicotine Ratio]

Source: (Cheetham et al.) [6]

The differences between TDN and TFN extend beyond their chemical compositions to include significant variations in their pharmacological and toxicological profiles. Understanding these differences is crucial for assessing their respective health impacts and regulatory implications. While TFN is marketed for its purportedly cleaner profile, the pharmacological impact of its enantiomeric composition, particularly the effects of (R)-nicotine, warrants further investigation.

C. Discerning the Source of Nicotine

The recent introduction of products like Puffbar's disposable electronic cigarettes (EC), which contain synthetic nicotine, highlights the growing use of synthetic nicotine in commercial products. This rise has also brought attention to the unclear regulatory status of such products. The challenge lies in the lack of established methods to accurately distinguish between Tobacco-Derived Nicotine (TDN) and Tobacco-Free Nicotine (TFN). This ambiguity could expose users to unexpected levels of R-nicotine or lower levels of S-nicotine than they are accustomed to, potentially leading to the consumption of higher total nicotine content [3].

Currently, commercial products containing TDN are authorized by the USFDA, while those marketed as containing TFN have not yet completed the Premarket Tobacco Product Application (PMTA) process. The FDA defines a tobacco product as one that is made or derived from tobacco and intended for human consumption. This definition includes components, parts, or accessories of a tobacco product, except for raw materials other than tobacco used in manufacturing. Manufacturers of TDN products face expensive and complex PMTA processes. In contrast, those marketing TFN products may choose to forgo a portion of their profits to remain in the market without regulatory hindrance [3]; [5].

There is a concern that companies might mix synthetic nicotine with TDN to increase the concentration of R-nicotine, thereby circumventing tobacco product regulations. Such a practice could misrepresent the product as containing only partially enriched synthetic nicotine, as determined by chiral analysis. Therefore, there is a significant economic and regulatory need to differentiate TDN and TFN analytically in commercial products [6].

The development and application of analytical methods that can discern the (R)-/(S)-nicotine ratio in TFN and TDN products are crucial for accurate analysis. These methods would not only aid in regulatory compliance but also ensure product safety and consumer awareness. Given the distinct pharmacological and toxicological profiles of the R- and S-enantiomers of nicotine, accurately identifying the source of nicotine in products is crucial for public health and regulatory purposes [2].

The ability to discern the source of nicotine in commercial products is vital for regulatory compliance, public health, and consumer safety. The distinction between TDN and TFN has significant implications, ranging from the pharmacological effects on users to the regulatory framework governing these products. As the market for synthetic nicotine grows, the development of robust analytical methods to accurately identify the source of nicotine becomes increasingly essential.

D. Methods to Distinguish TDN and TFN

Polarimetry: In the realm of analytical techniques, polarimetry is a prominent method for identifying the presence of (R)- and (S)-nicotine. This technique relies on the principle of optical activity, where the optical rotation detected by a polarimeter is used to determine the enantiomeric excess and specific rotation of the samples. Notably, (S)-nicotine and (R)-nicotine, being optically active enantiomers, exhibit specific rotations of −169° and +169° respectively. When applied to TFN e-liquids, this method can reveal a racemic mixture through an α value of 0.0°. However, polarimetry faces limitations in its reliance on the optical activity of enantiomers, which may not always yield clear differentiation, particularly in complex sample matrices.

Nuclear Magnetic Resonance (NMR): Nuclear Magnetic Resonance, particularly 1H NMR spectroscopy, is widely recognized for its ability to identify and quantify nicotine enantiomers. Its application in this context involves assessing the interaction of nicotine with a chiral complexing agent, leading to distinct peaks in the NMR spectrum that indicate the presence of different enantiomers. The efficacy of this technique was demonstrated by Duell et al. [10], who successfully used it to identify the (R)-nicotine to (S)-nicotine ratios in Puff Bar e-liquids, thereby indirectly assessing the nicotine source as TFN or TDN. Despite its effectiveness, NMR's indirect approach to nicotine source assessment and the complexity involved in interpreting NMR spectra present challenges.

Gas Chromatography (GC) and Liquid Chromatography (LC): In the evolving landscape of analytical chemistry, Gas Chromatography (GC) and Liquid Chromatography (LC) have been instrumental in differentiating between TDN and TFN in e-liquids. A notable application of GC in this context was the method employed by Duell et al. [10] utilizing a Beta DEX 120 GC column. In their study, e-liquids were also assessed using a chiral GC column (CHIRALDEX G-TA). However, this approach encountered significant challenges, particularly

concerning the excessively long retention times of the isomers and the inability to achieve adequate separation of the peaks. These limitations made quantitative analysis particularly demanding, prompting the need for an alternative method.

Addressing these challenges, the focus shifted to Liquid Chromatography. In 2019, Ji et al [11] pioneered a swift and sensitive ultra-performance LC-MS/MS method. Their approach involved the use of a Chiralpak AGP column and an isocratic elution program consisting of ammonium formate, ammonium hydroxide, and methanol as the mobile phase. This method proved remarkably efficient, successfully resolving enantiomers in tobacco leaves and various tobacco products within just 10 minutes. Further demonstrating the versatility of LC, the team employed a triple quadrupole MS method that effectively identified the concentrations of nicotine enantiomers in TFN products. Interestingly, while TFN products were found to consist of a racemic mixture, only minimal amounts of (R)-nicotine were detected in TDN products.

Zhang et al. [5] further contributed to this field by developing a normal phase LC procedure to distinguish nicotine enantiomers in various samples, thereby determining the nicotine source. Their findings showed that small quantities of (R)-nicotine, ranging from 0.02% to 0.76%, were present in the samples, considerably lower than those found in TFN e-liquids. Consequently, a general conclusion emerged: a nicotine sample can be considered TDN if the (R)-nicotine ratio is around 1 percent, while it is assumed to be TFN if the ratio is 50 percent or 100 percent (indicative of a racemic mixture).

However, an intriguing scenario arises if TFN is synthesised to become 99% (S)-nicotine. In such cases, the ratio of (R)-nicotine present in the sample is virtually eliminated as a method to assign the nicotine source, rendering TDN and TFN indistinguishable using the previously defined methods. This development underscores the continuous need for innovation and adaptation in analytical techniques to keep pace with the evolving complexities of nicotine products.

Multidimensional Gas Chromatography (MDGC): The fully automated multidimensional gas chromatography (MDGC) system represents a significant advancement in the field of chromatographic analysis. This system integrates both GC and LC methods by employing a megabore precolumn coupled with a chiral cyclodextrin-based analytical column. The innovation of MDGC lies in its ability to simultaneously harness the strengths of both GC and LC, thereby enhancing analytical capabilities. Specifically, this system is designed to detect the enantiomeric compositions of various alkaloids such as anatabine, nornicotine, and anabasine, which are commonly found in commercial tobacco products.

MDGC allows for a comprehensive analysis that combines the selectivity and sensitivity of GC with the versatility of LC. This approach is beneficial in the complex matrices of tobacco products, where different alkaloids and their enantiomers must be accurately identified and quantified. Moreover, the integration of MDGC with a reverse-phase ultra-performance liquid chromatography/mass spectrometry/mass spectrometry (UPLC/MS/MS) approach, as developed by Perfetti et al. [5], further enhances its analytical power, enabling the precise determination of these compounds in tobacco products.

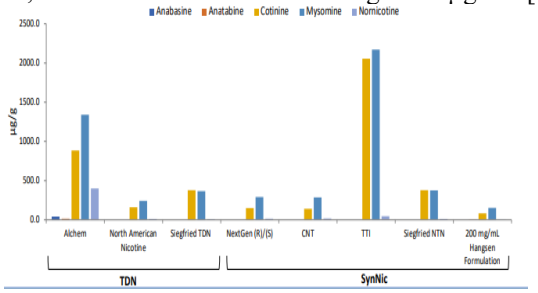
The primary concern with this method is the complexity and cost of the setup. Additionally, the combination of different chromatographic techniques, although advantageous for comprehensive analysis, also means that the process may be more time-consuming and technically demanding compared to simpler chromatographic methods.

Chiral Supercritical Fluid Chromatography (Chiral-SFC): Chiral Supercritical Fluid Chromatography (Chiral-SFC), coupled with UV diode array detection (DAD-UV), has emerged as a pivotal analytical tool for detecting the enantiomeric ratios of R- and S-nicotine, as well as R and S-nornicotine in various commercial products containing Tobacco-Derived Nicotine (TDN) and Synthetic Nicotine (SyN). As outlined in the work of Perfetti et al. [5], this method has demonstrated its utility in accurately determining the specific enantiomeric compositions of these compounds in a range of nicotine-rich products.

The significance of Chiral-SFC extends beyond merely identifying nicotine enantiomers. Nicotine, a minor alkaloid and a key intermediate in Tobacco-Free Nicotine (TFN) synthesis, plays a crucial role in defining the nature of nicotine-rich samples. Its detection, therefore, is not only essential but also indispensable in such analyses. Chiral-SFC, with its UV detection capability, is adept at detecting the enantiomeric ratios of both nicotine and nornicotine, offering a comprehensive insight into the composition of the samples under study.

Moreover, the detection of nornicotine assumes greater importance when considering the degradation pathways of nicotine. According to research by Cheetham et al. [6], nornicotine is identified as one of the seven nicotine degradants, which also include anabasine, anatabine, cotinine, nicotine-N-oxide, β-nicotyrine, and myosmine. The presence of nornicotine in a sample has been associated with TDN, primarily because TFN samples have not been found to contain significant levels of this degradant. This distinction is critical as nicotine serves as a standard degradation product for both TDN and TFN, thus playing a pivotal role in distinguishing between these two sources.

Chiral-SFC with UV diode array detection (DAD-UV) is highly effective for detecting the enantiomeric ratios of R- and S-nicotine and R- and S-nornicotine in various commercial products of TDN and SyN. It can help obtain baseline separations of these enantiomers in under seven minutes, with detection limits in the range of 5 µg/mL [5].



[Fig.3: Nicotine Degradants]

Source: (Cheetham et al.) [6]

Isotope Ratio Mass Spectrometry (IRMS): Isotope Ratio Mass Spectrometry (IRMS) is a

pivotal tool in the realm of analytical chemistry, particularly in its application for analysing isotope ratios at natural abundance. This technique offers insights into the isotope contents of various elements, including 2H/1H, 13C/12C, 15N/14N, 18O/16O, and 34S/32S. Its significance is especially pronounced in the analysis of synthetic nicotine, where the use of different starting reactants leads to distinct 2H/1H isotopic ratios. For instance, reactants such as 3-pyridinecarboxaldehyde, 3-(3-pyridyl) allyl carbonates for the pyridine ring, and Allyldiisopropylaminoboran and azide anion for the pyrrolidine ring, each result in unique isotopic ratios.

This ability to distinguish compounds based on their origin and synthetic pathways is instrumental in tobacco analysis, where nicotine serves as a model molecule for comparison and authentication. An innovative approach in this regard involves the use of NMR spectroscopy to differentiate Tobacco-Derived Nicotine (TDN) from Tobacco-Free Nicotine (TFN). This differentiation is achieved through the direct comparison of 2H/1H SPIR values derived from 1H and 2H NMR spectra. Studies, such as those conducted by Bin Liu and colleagues, have shown that the 2H/1H SPIR values are slightly higher in synthetic origins ((R)- and rac-Nicotine) compared to natural nicotine (p- and (S)- Nicotine), except for the site 5′α. This finding suggests that enriched 2H values in TFN products can effectively distinguish them from natural compounds.

Furthermore, IRMS allows for the detection of subtle changes in the natural abundance of isotope composition at specific molecular sites. The measurement of 2H/1H NMR SPIR values is instrumental in detecting differences in the isotopic composition of nicotine, depending on its origin and synthetic pathway. This capability extends to identifying adulterated mixtures, distinguishing them from 100% natural nicotine. As a result, IRMS emerges as a critical tool not only for authenticating nicotine sources but also for detecting adulteration in nicotine products [9].

14C Analysis: Radiocarbon analysis, also known as 14C analysis, utilising accelerator mass spectrometry, is a crucial tool in accurately determining nicotine sources and identifying misbranded products labelled as TFN. This method revolves around the study of naturally occurring carbon isotopes, specifically carbon-12 (12C), carbon-13 (13C), and the radiocarbon isotope carbon-14 (14C), which undergoes radioactive decay. The decay property of 14C is particularly significant for distinguishing between biologically derived materials and those derived from fossil sources. This differentiation is vital, considering the need to authenticate the origins of substances used in various nicotine products.

Beyond the isotopic analysis, another aspect that can aid in differentiating between TDN and TFN is the impurity profile of these sources, as suggested by Cheetham et al. The presence or absence of specific impurities can serve as an indicator for identifying the source of nicotine. For instance, nornicotine, formed through the enzymatic demethylation of nicotine in tobacco plants, tends to be biased towards the (R)-nicotine enantiomer. This bias results in a wide variation in the (R)/(S)-ratio of tobacco-derived nornicotine (ranging from 4–75 percent), which does not match the (R)/(S)-ratio of nicotine from the same plant (0.1–1.2 percent). Despite the potential of this method, it is challenged by sensitivity and matrix effects, which can complicate the analysis.

The most significant application of 14C analysis lies in its ability to identify the biological or synthetic origin of a molecule. Entirely biological substances, such as TDN, result in a 100 percent Bio-carbon value, whereas purely petrochemical synthetic compounds, like some TFN, return a 0 percent Bio-carbon value. Materials that combine biological and petrochemical synthetic elements, or those that are adulterated, fall between these two extremes. In practical terms, biobased TDN samples will yield a 100% bio-carbon value, whereas TFN samples typically exhibit lower Bio-carbon values, around 35% or 36%.

In a study cited by Cheetham et al. [6], nicotine analyses were categorised based on their percent modern carbon (pMC) values: pMC less than 40 percent indicated Synthetic Nicotine (SyN), pMC of 100 percent confirmed TDN, and pMC values falling between these extremes necessitated further investigation, as they could indicate a combination of SyN and TDN. This categorisation highlights the effectiveness of radiocarbon analysis in the regulatory evaluation of nicotine products. However, nicotine must be isolated from various components used in product formation to ensure accurate analysis. Despite this requirement, radiocarbon analysis remains a definitive method for assessing nicotine products, particularly when combined with other analytical techniques, such as liquid and gas chromatography, for comprehensive analysis.

Each of these has unique strengths and faces distinct challenges. The choice of method for distinguishing between TDN and TFN depends on the specific analytical requirements, including sensitivity, specificity, available resources, and the complexity of the sample matrix.

III. MATERIALS AND METHODS

This study's methodologies adhere to the standards set by the US Pharmacopeia [1]. The primary material of interest is Synthetic Nicotine, which has been resolved to the S-isomer, identical to Tobacco-Derived Nicotine (TDN), using chiral resolution methods.

A. Polarimetry Procedure

i. Materials Used:

The samples tested were synthetic nicotine. The test solution consisted of synthetic nicotine in water at a concentration of 1 mg/mL. Other reagents included the following:

Nicotine Bitartrate Dihydrate: Nicotine bitartrate dihydrate, also known as nicotine tartrate, is a stable salt form of nicotine, a well-known stimulant compound found in tobacco. This chemical appears as a white to off-white crystalline powder and is highly soluble in water, which makes it suitable for various aqueous formulations. It's primarily used in medical and research settings. In the medical field, nicotine bitartrate dihydrate is utilized in nicotine replacement therapies (NRTs) such as gums, lozenges, and patches. These products are designed to help smokers quit by providing a controlled dose of nicotine, thereby reducing withdrawal symptoms and craving associated with smoking cessation. In research, it's used for studying nicotine's effects on the body and for developing new treatments for nicotine addiction.

Ammonium Hydroxide: Ammonium Hydroxide in commercial form is formed by mixing ammonia in gaseous form with water. It is a colourless, pungent-smelling liquid. On an industrial level, it can be used as a precursor to certain alkyl amines. Moreover, it can be used in household cleaners, fertilisers, etc.

Sodium Hydroxide: Sodium Hydroxide, a white crystalline solid in its pure form, is also known as caustic soda. It is white in colour and odourless. Commercially, it can be mixed with water to form a solution. It is used to manufacture soaps, paper, dyes, and other products.

N hexane: N hexane is a strong solvent; it is easily combustible and has a slight, unpleasant odour. It can be used to formulate shoes and roofing glues. They can also be used to extract vegetable oils from crops.

Nitrogen gas: Nitrogen gas is a non-flammable, colourless, odourless gas. Commercially, it is used for purging due to its lack of chemical reactivity and the elimination of unwanted volatile organic Compounds (VOCs) from industrial waste materials.

ii. Procedure:

1.0 mL of the Synthetic Nicotine solution is first transferred to a 50-mL volumetric flask and diluted with 0.1 N hydrochloric acid. Then, an equivalent amount of Nicotine Bitartrate Dihydrate RS is added to 50 mg of synthetic nicotine in a 25-mL glass stoppered tube. 5 ml of 6 N ammonium hydroxide was added to the tube, 2 ml of 1 N sodium hydroxide, and 20 ml of n-hexane. This mixture was shaken for 5 minutes and allowed to phase separate. The upper n-hexane layer was then transferred to a vial and evaporated with Nitrogen gas. The residue is then dissolved in water to obtain a concentrated solution, which is subsequently diluted with 0.1 N hydrochloric acid to 50.0 mL to prepare the Standard solution. The specific rotation is generally measured between -130° and -143°.
The result obtained was -138.62°.

B. Gas Chromatography (GC) Procedure

i. Chromatographic Conditions:

- Chromatographic System: Agilent 8890 GC system equipped with a flame-ionisation detector.
- Column: 0.53-mm × 30-m fused silica column bonded with a 1.5-um layer of phase G1.
- Carrier Gas: Helium at a flow rate of 20 mL per minute.
- Temperature Program: Column temperature maintained at 50° for 6 seconds, programmed to rise from 50° to 250° at 6° per minute, and held isothermally at 250° for 3 minutes.
- Maximum Temperature Ramp Rate: 120 °C/min.
- Operating Temperature Range: +4 above ambient to 450 °C.
- Oven Cool Down: 450 °C to 50 °C in 4.0 minutes.

ii. Instrument Specifications:

- Peak Area Repeatability: <0.5% RSD.
- Retention Time Repeatability: <0.008%.

iii. Reference Solutions:

- Solutions A and B are prepared by diluting the Test solution with dichloromethane to concentrations of about 26 µg per mL and 52 µg per mL, espectively.

iv. Test Solution:

- Approximately 0.13 g of Synthetic Nicotine is dissolved in dichloromethane and diluted to 25.0 mL.

v. Testing Procedure:

Equal volumes (about 1 µL) of the Test solution, Reference solution A, and Reference solution B are injected into the chromatograph. The chromatograms were recorded, and peak responses were measured. The sum of peak responses from the Test solution, excluding nicotine, should not exceed the nicotine response from Reference solution B (1.0%), and no single peak response should be greater than the nicotine response from Reference solution A (0.5%).

vi. Environmental Conditions

- Temperature: Below 25°C (storage), protected from light and moisture.

IV. FINDINGS

The findings from the experiments conducted using a polarimeter and gas chromatography provide insightful data regarding the enantiomeric composition and purity of synthetic nicotine samples. These tests were crucial in establishing the enantiomeric ratios and confirming the expected outcomes by US Pharmacopoeia standards.

Table I: Shows the Findings from the Experiments Conducted using the Polarimeter and Gas Chromatography

Instrument	Experiment	Sample	Expected Outcome	Result
Polarimeter	Determination of the enantiomeric ratio of nicotine in standard nicotine samples	i) Synthetic Nicotine (resolved to S-isomer)	Specific rotation (781S): between -130° and -143°	- 138.62 °
Polarimeter	Determination of the enantiomeric ratio of nicotine in standard nicotine samples	ii) Synthetic Nicotine (Racemic Mixture of R and S isomers)	Racemic nicotine mixtures are expected to yield an α value of 0 °.	0.00 °
Gas Chromatography	Determining the enantiomeric purity of nicotine	i) Synthetic Nicotine (resolved to S-isomer)	Peaks by USP Nicotine	Result as per USP Nicotine achieved

In terms of Polarimeter findings for the experiment with synthetic nicotine resolved to S-isomer, the specific rotation (781S) was expected to be between -130° and -143°, as per the standard for S-isomer nicotine. The result of the experiment showed the specific rotation to be -138.62°. This result falls within the expected range,

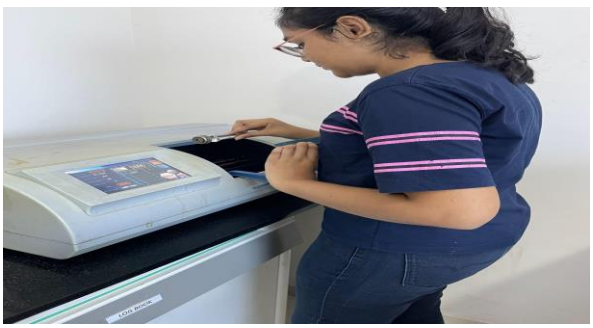
confirming that the synthetic nicotine was indeed resolved to the S-isomer. This finding is consistent with the properties of the S-isomer of nicotine, which is identical to Tobacco-Derived Nicotine (TDN).

For the experiment with synthetic nicotine (Racemic Mixture of R and S Isomers), the expected α value was 0.0°, indicating equal proportions of both enantiomers. The result obtained was exactly 0.00°, affirming the racemic nature of the synthetic nicotine sample. This outcome corroborates the theoretical understanding that a racemic mixture should have no net optical rotation, as the rotations of the R and S isomers cancel each other out.

In terms of the Gas Chromatography experiment with synthetic nicotine resolved to the S-isomer, the expectation was to observe peaks corresponding to the US Pharmacopoeia Nicotine standards [1], which would indicate the enantiomeric purity of the nicotine. The outcome was in alignment with the USP Nicotine standards, indicating that the synthetic nicotine resolved to the S-isomer had high enantiomeric purity. This result is crucial as it demonstrates the effectiveness of the chiral resolution methods employed in ensuring the desired enantiomeric composition of the synthetic nicotine.

These findings are significant as they validate the methodologies used in the resolution of synthetic nicotine and its analysis. The results from the Polarimeter experiments delineate the enantiomeric compositions of the two different synthetic nicotine samples. At the same time, the Gas Chromatography data confirm the enantiomeric purity of the synthetic nicotine, resolved as the S-isomer. Such precise determination of enantiomeric compositions is vital for both quality control in nicotine product manufacturing and for ensuring compliance with regulatory standards. Furthermore, the ability to accurately characterise these samples paves the way for more detailed pharmacological and toxicological studies, which are essential for understanding the implications of using synthetic nicotine in various commercial products.

[Fig.4: Result of Polarimetry Experiment]



[Fig.5: Insertion of a Glass Polarimeter Tube (Containing Sample 1) into the Polarimeter]

[Fig.6: Result of Polarimetry Experiment]

V. DISCUSSION

The findings from the two sets of experiments using Synthetic Nicotine, resolved to the S-isomer through chiral resolution, align closely with the established US pharmacopeia methods for natural nicotine (TDN). This discussion compares these findings to those of earlier studies to ascertain whether they are consistent or contradictory.

In terms of the Polarimetry experiment, for the first sample of Synthetic Nicotine (S-isomer), the result of -138.62° indicates laevorotatory rotation, akin to natural nicotine (TDN). This finding aligns with the properties of the S-isomer, as discussed in the systematic review by Salam et al. [2], and corroborates the existing understanding of the optical activity of S-isomer nicotine.

For the second sample of Synthetic Nicotine (Racemic Mixture of R and S isomers), the result of 0.00° (no optical rotation) for the racemic mixture confirms the equal presence of S and R isomers. This outcome is consistent with the observations made in the study by Salam et al. [2], who noted similar optical behaviour in TFN e-liquids with racemic mixtures.

In terms of the Gas Chromatography experiment, for the sample of Synthetic Nicotine (S-isomer), the total peak responses from the test solution are lower than those in the Reference solution B (1.0 per cent), and no individual peak exceeds the response from Reference solution A (0.5 per cent), indicating high enantiomeric purity. This purity level matches what is typically observed in Tobacco-derived natural nicotine, as suggested by [4] and [7]. The findings indicate that synthetic nicotine, when resolved to the S-isomer, closely resembles natural nicotine in its chromatographic behaviour.

The results obtained in this study are broadly consistent with those of earlier studies. The findings from the polarimetry experiments confirm the established understanding of the optical activity of nicotine enantiomers. Similarly, the gas chromatography findings validate the chromatographic behaviour of nicotine, as indicated by [6], who emphasised the importance of accurately identifying nicotine sources, particularly in the context of regulatory compliance.

However, a notable observation is that Synthetic Nicotine, when resolved to a 99 percent S-isomer, becomes indistinguishable from TDN using these methods. This finding has significant implications for the differentiation of synthetic

and natural nicotine, as highlighted by [3] and [5]. It underscores the need for precise and accurate analytical methods to ensure proper identification and regulation of nicotine products, especially considering the growing prevalence of synthetic nicotine in the market.

VI. CONCLUSION

In conclusion, the findings from this study are consistent with earlier research and add to the body of knowledge on the analytical characterization of nicotine. The close resemblance of resolved Synthetic Nicotine to TDN underscores the efficacy of current analytical techniques while also highlighting the challenges in differentiating between synthetic and natural sources in specific contexts.

A. Limitations

The study, while yielding substantial findings, encounters certain limitations. Firstly, the sample range was restricted; a broader array of Tobacco-Free Nicotine (TFN) liquids would enhance the reliability of conclusions regarding enantiomeric purity. Secondly, the testing methodology was limited, focusing predominantly on Synthetic Nicotine resolved to the S-isomer. Critical tests, such as High-Performance Liquid Chromatography (HPLC) and Radiocarbon Analysis (14C Analysis), which could have provided additional insights, were not employed.

B. Implications

This study plays a crucial role in bridging the gap in analytical methods for distinguishing between synthetic and Natural Nicotine. The findings are particularly significant for manufacturers, indicating the necessity of precise production techniques for Synthetic Nicotine. For users, especially in India, this study highlights the importance of being aware of the composition and potential effects of the nicotine products they consume. Policy makers are expected to have a crucial role in regulating the production and distribution of Synthetic Nicotine, ensuring that products on the market adhere to safety standards and are correctly labelled.

C. Scope for Further Research

Future research in this area could include studies to more conclusively ascertain the enantiomeric purity and composition of synthetic nicotine. Further research could focus on a wider variety of samples and employ a more diverse range of tests. Additionally, extensive research on the pharmacological and toxicological effects of R and S isomers present in TFN is warranted. This is particularly pertinent considering the rising popularity of e-cigarettes and vapes among younger age groups in India, paralleled by the development of TFN e-liquids by companies like Puffbar in the US. As the market for these products grows, there is an increasing need for well-established techniques and regulations for Synthetic Nicotine, not just globally but also in the Indian context. This further research will be instrumental in ensuring safe consumption and effective regulation of nicotine products.

DECLARATION STATEMENT

I must verify the accuracy of the following information as the article's author.

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REFERENCES

1. US Pharmacopoeia. Nicotine Monograph. Available from http://www.pharmacopeia.cn/v29240/usp29nf24s0_m56620.html
2. Salam S, El-Hajj Moussa F, El-Hage R, El-Hellani A, Aoun Saliba N. A Systematic Review of Analytical Methods for the Separation of Nicotine Enantiomers and Evaluation of Nicotine Sources. Chem Res Toxicol. 2023;36:334-341. https://doi.org/10.1021/acs.chemrestox.2c00310
3. Jordt S-E. Synthetic nicotine has arrived. Tob Control. 2023;32:113-117. https://doi.org/10.1136/tobaccocontrol-2021-056626
4. Berman ML, Zettler PJ, Jordt S-E. A Systematic Review of Analytical Methods for the Separation of Nicotine Enantiomers and Evaluation of Nicotine Sources. Chem Res Toxicol. 2023;36(1):334-341. https://doi.org/10.1021/acs.chemrestox.2c00310
5. Zhang H, Pang Y, Luo Y, Li X, Chen H, Han S, Jiang X, Zhu F, Hou H, Hu Q. Enantiomeric composition of nicotine in tobacco leaf, cigarette, smokeless tobacco, and e-liquid by normal phase high-performance liquid chromatography. Chirality 2018; 30(7): 923− 931. https://doi.org/10.1002/chir.22866
6. Cheetham AG, Plunkett S, Campbell P, Hilldrup J, Coffa BG, Gilliland S III, et al. Analysis and differentiation of tobacco-derived and synthetic nicotine products: Addressing an urgent regulatory issue. PLoS One. 2022;17(4):e0267049. https://doi.org/10.1371/journal.pone.0267049
7. Liu B, Chen Y, Ma X, Hu K. Site-specific peak intensity ratio (SPIR) from 1D 2H/1H NMR spectra for rapid distinction between natural and synthetic nicotine and detection of possible adulteration. Anal Bioanal Chem. 2019;411:6427-6434. https://doi.org/10.1007/s00216-019-02023-6
8. Agilent Technologies. 8890 GC System. Available from: https://www.agilent.com/en/product/gas-chromatography/gc-systems/8890-gc-system?gclid=CjwKCAjw15eqBhBZEiwAbDomEsme1Y-V6nusn73CH3v1D5XEsC-lKFSDxNIVj-1oGOkQ2HCQ1Ex0XxoCdZMQAvD_BwE&gclsrc=aw.ds
9. Hellinghausen G, Lee J, Weatherly C, Lopez, D, Armstrong, D. Evaluation of nicotine in tobacco-free nicotine commercial products: Nicotine enantiomers in tobacco-free nicotine. Drug Test. Ana. 2017; 9: 944-948. https://doi.org/10.1002/dta.2145
10. Duell AK, Kerber PJ, Luo W, Peyton DH. Determination of (R)-(+)- and (S)-(−)-nicotine chirality in Puff Bar e-liquids by 1H NMR spectroscopy, polarimetry, and gas chromatography-mass spectrometry. Chem. Res. Toxicol. 2021; 34(7); 1718−1720. https://doi.org/10.1021/acs.chemrestox.1c00192
11. Ji H, Wu Y, Fannin F, Bush L. Determination of tobacco alkaloid enantiomers using reversed phase UPLC/MS/MS. Heliyon 2019; 5(5):

e01719. https://doi.org/10.1016/j.heliyon.2019.e01719