Distinguishing Tobacco-Derived Nicotine from Synthetic Nicotine in Commercial Nicotine Samples

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Agenda

01 Introduction

02 Tobacco-Derived Nicotine (TDN) and Synthetic Nicotine (SN) Landscape

03 Synthesis – Kilogram Scale with Classical Resolution; Enzymatic Process

04 Published Methods for Distinguishing TDN from SN – ^{14}C Analysis and ^{1}H NMR

05 Reynolds Chromatographic Methods – Achiral and Chiral Methods

06 Results – Analysis of Commercial Nicotine Samples

07 Conclusions

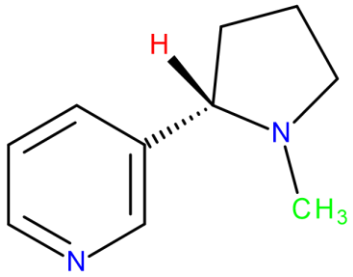
Introduction

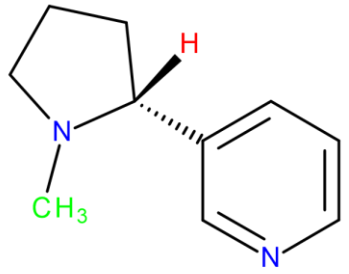
- A Premarket Tobacco Product Application (PMTA) is required for tobacco products containing Snthetic Nicotine (SN) (March 15, 2022).
- While Tobacco-Derived Nicotine (TDN) and Synthetic Nicotine (SN) are both regulated by the FDA, distinguishing between these two forms may be critical for:
  - Tobacco authentication
  - Assessing manufacturing methodology
  - Assessing nicotine chirality relative to any pharmacological concerns

TDN and SN Landscape

	TDN	SN
Manufacturing Locations	US, UK, Europe, Global	Global
Manufacturing Process	Extraction of tobacco plant material, chemical processing, and purification by high vacuum distillation	Chemical processing from chemical starting materials, enzymatic step, and purification by high vacuum distillation
Quality	cGMP, meets USP/EP Monograph testing standards; “Pharmaceutical Grade”	cGMP, meets USP/EP Monograph testing standards; “Pharmaceutical Grade”
Chiral Purity	>99% (S)-nicotine; ~0.2 – 0.6% (R)-nicotine	50:50 (R)/(S) ratio to >99% (S)-nicotine and >0.1% (R)-nicotine

(S)-Nicotine



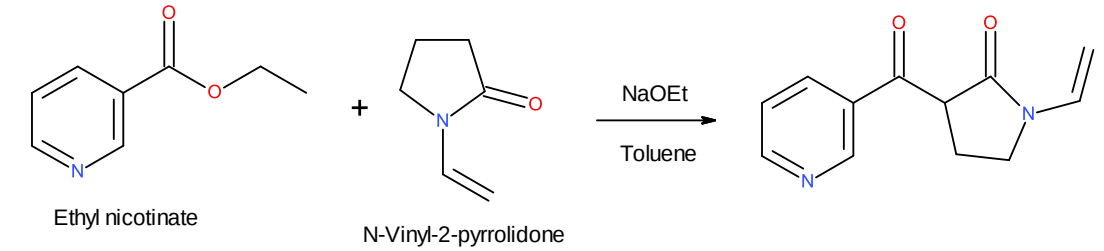


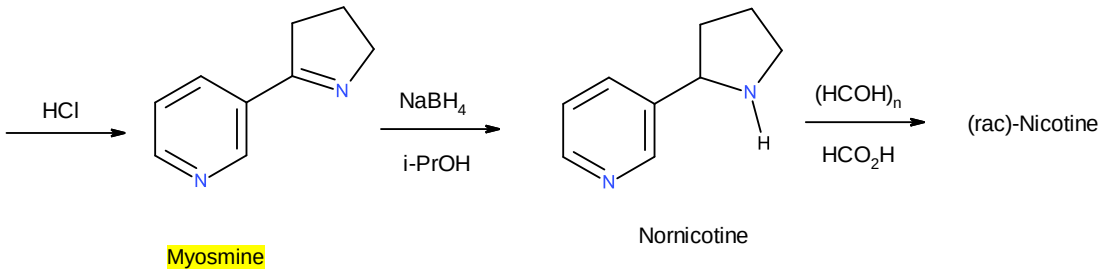
(R)-Nicotine

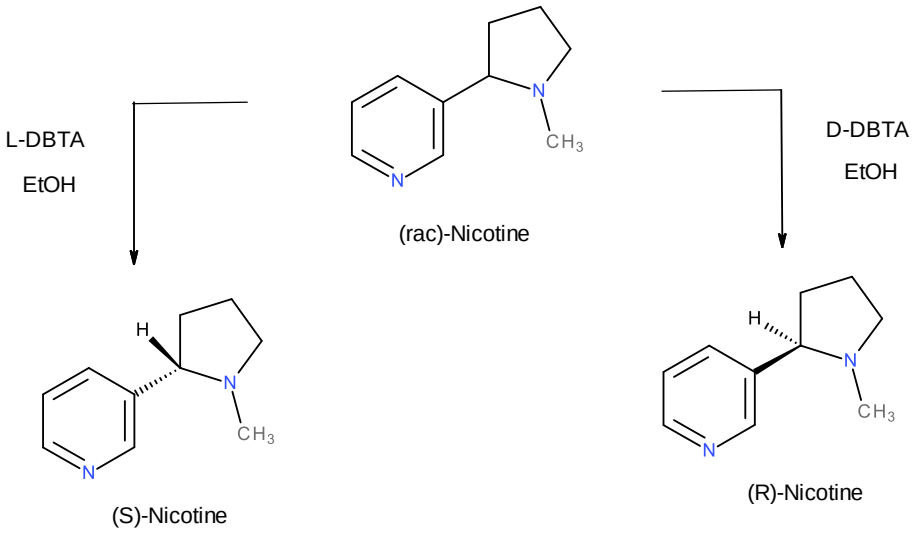
Nicotine – US Pharmacopeia (USP Monograph) for Nicotine Testing (“Pharmaceutical Grade”)

- Identification
  - FT-IR
  - HPLC-UV retention time
- Assay (Potentiometric Titration): 99.0-101.0% (anhydrous basis)
- Heavy Metals: Not More Than 20 ppm
- HPLC Method for Organic Impurities: Anatabine, Nicotyrine, Cotinine, Myosmine, Nicotine N-oxide, Nornicotine, Anabasine: Not More Than 0.3% Each; Any Unspecified Impurity, Not More Than 0.1%; Total Impurities, Not More Than 0.8%
- Optical rotation: -130° to -143° (20 mg/mL in alcohol)--(S)-Nicotine
- Water: Not More Than 0.5%

Kilogram-Scale Synthesis – Racemic Synthesis and Classical Resolution[1,2]



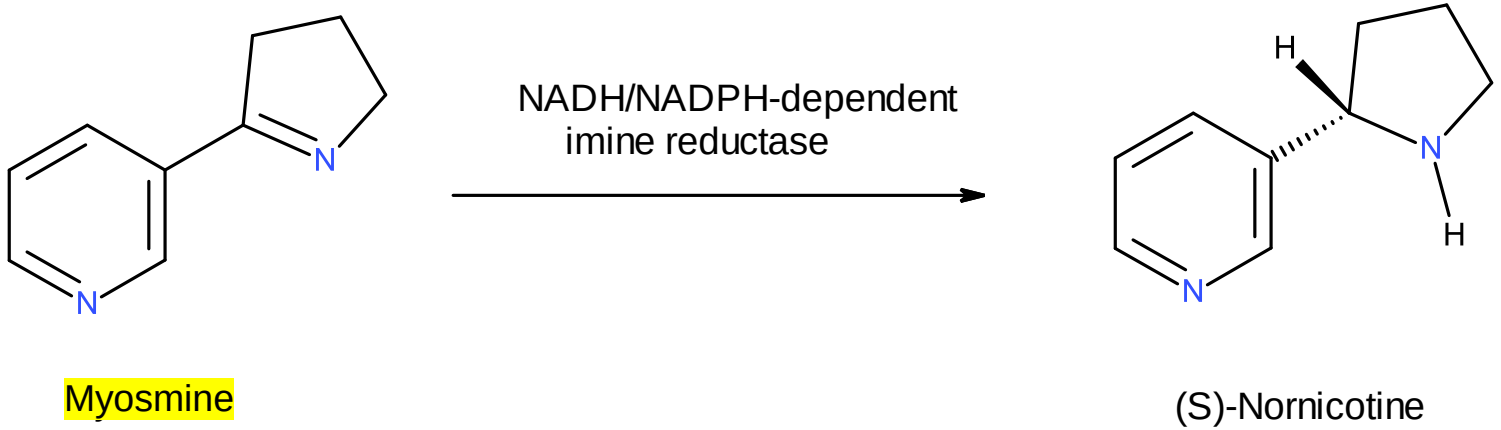




Synthetic methodology has improved such that synthetic (S)-nicotine with a very low level of (R)-nicotine (0.1-0.2%) is now commercially available.

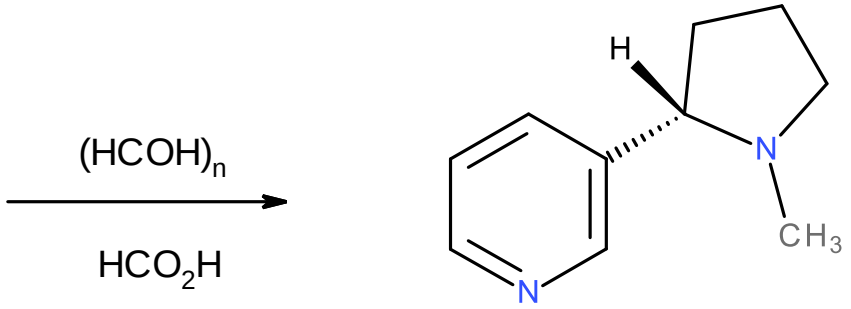
1. Weber B, Pan B, Siegfried AG. Enantiomeric separation of racemic nicotine by addition of an o,o'-disubstituted tartaric acid enantiomer. WO 2019/121649 A1, June 27, 2019. Available: https://patents.google.com/patent/WO2019121649A1/en
2. Weber BT, Lothschütz C, Pan B. Siegfried AG Contraf-Nicotex-Tobacco GmbH assignee. Preparation of racemic nicotine by reaction of ethyl nicotinate with N-vinylpyrrolidone in the presence of an alcoholate base and subsequent process steps. US2020/0331884 A1, Oct. 22, 2020. Available: https://patents.google.com/patent/US20200331884A1

Enzymatic Process – Stereoselective Reduction of Myosmine[3]



Myosmine

(S)-Nornicotine



(S)-Nicotine

3. McCague R, Narasimhan AS. Zanoprima Lifesciences Limited (London, GB), assignee. Process of making (S)-nicotine. USA patent 10,913,962 B2, Feb. 9, 2021. Available: https://patents.google.com/patent/US10913962B2/en

Published Methods for Distinguishing TDN from SN -- ^{14}C Analysis and ^{1}H NMR

- ^{14}C Analysis
  - Radiocarbon ^{14}C is higher in TDN than SN[4]
  - Sample size > 50 mg; specialized ^{14}C instrumentation

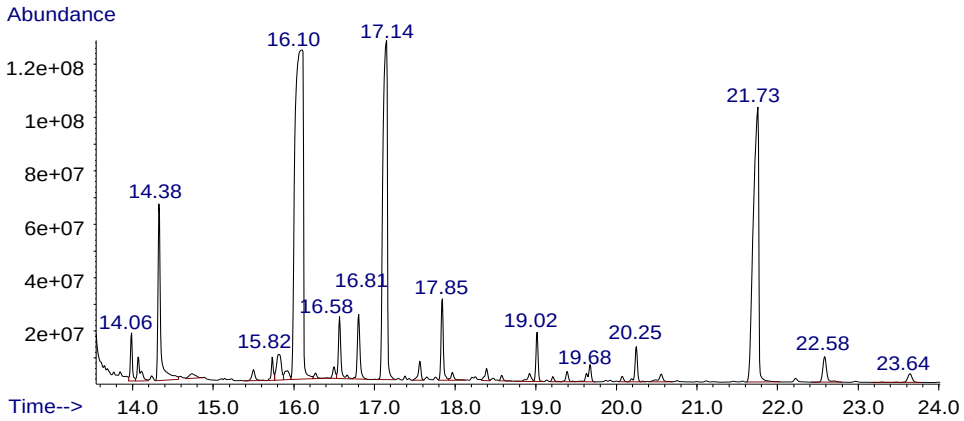
- ^{1}H NMR
  - Site-specific peak intensity ratio from 1D $^{2}H/^{1}H$ NMR spectroscopy method [5]
  - Can only detect TDN adulteration with SN as low as 20% SN

4. Cheetham AG, et al. Analysis and differentiation of tobacco-derived and synthetic nicotine samples: Addressing an urgent regulatory issue. *PLOS ONE.* 2022;17(4):1-17 (Enthalpy Analytical, LLC).
5. Liu B, et al. Site-specific peak intensity ratio (SPIR) from 1D $^{2}H/^{1}H$ NMR spectra for rapid distinction between natural and synthetic nicotine and detection of possible adulteration. *Anal. Bioanal. Chem.* 2019;411:6427-6434 (Innovative Institute of Chinese Medicine and Pharmacy, Chengdu University of Traditional Chinese Medicine).

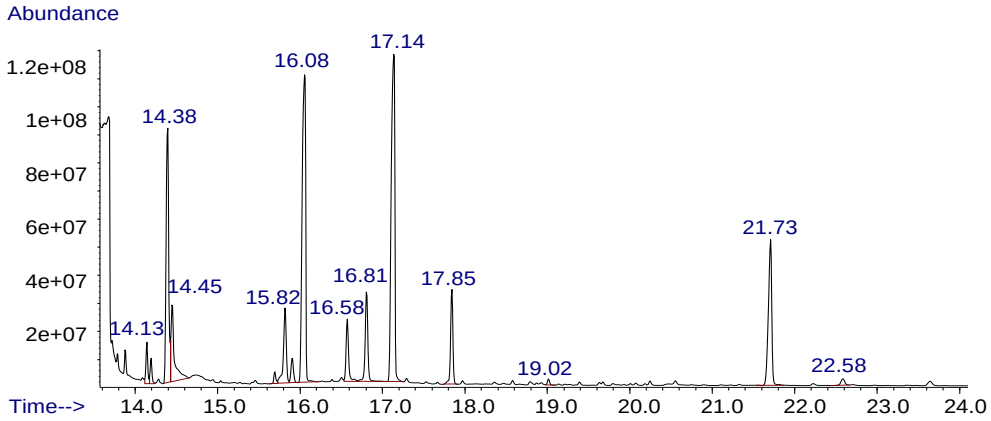
Reynolds Chromatographic Methods – GC/MS and SPME GC/MS

- **Method 1. GC/MS and SPME/GC/MS**
  - **GC/MS Method:**
    - Agilent 6890 with 5973 MSD; DB-Waxeter column, 30 m x 0.25 mm i.d., with 0.25 µm film
    - Monitoring at 33-300 amu
    - 50 mg in 1 mL methanol
  - **SPME/GC/MS Method:**
    - Agilent 7890A/5975C; Gerstel MPS Multipurpose Analyzer; SPME capability
    - SPME fiber: 50/30 mm DVB/CAR/PDMS Stableflex 23 Ga (Gray)
    - DB-WaxEtr column, 30 m x 0.25 mm i.d., with 0.25 µm film
    - 20 mg in a SPME vial
    - NIST mass spectral library; quality match of ≥80%
- **Method 2. GC/MS**
  - **GC/MS Method:**
    - Agilent 6890/5973 system; DB-Waxeter column, 30 m x 0.25 mm i.d., with 0.25 µm film
    - Monitoring at 33-550 amu
    - 10 mg in 1 mL tert-butyl methyl ether
    - NIST, Wiley mass spectral libraries

SPME GC/MS Chromatogram -- Time Window 14 – 24 Min

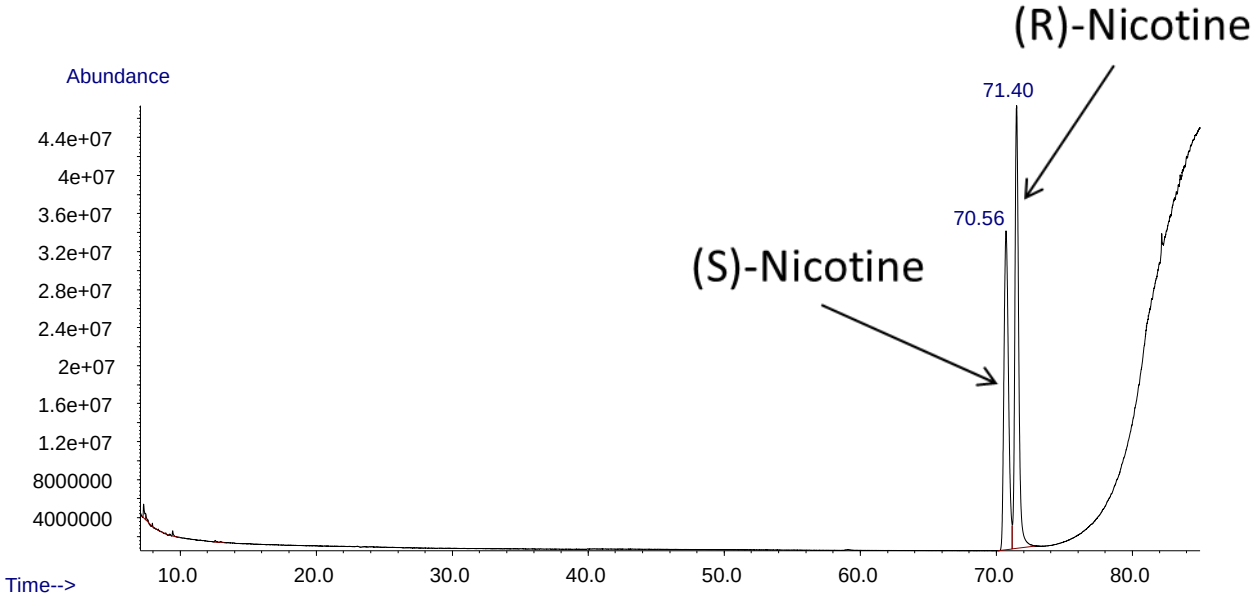


Tobacco-Derived Nicotine (AmeriNic)

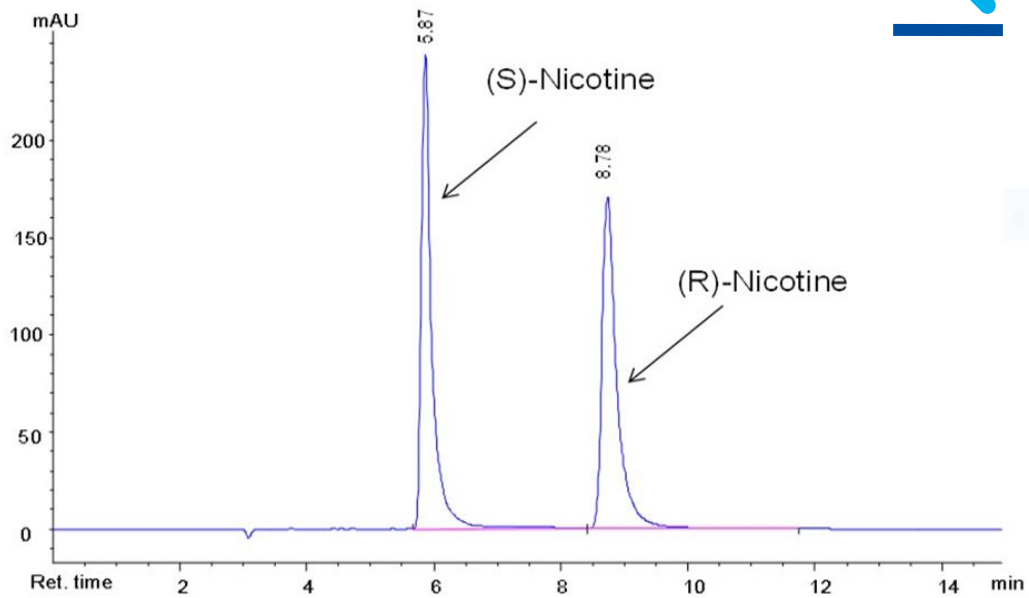


Synthetic Nicotine (Next Generation Labs)

Reynolds Chiral GC/MS and Chiral HPLC/UV Chromatograms



Chiral GC-MS chromatogram of a solution of 250 mg/mL nicotine in methanol (Synthetic). (S)-Nicotine retention time 70.56 min, and (R)-Nicotine retention time 71.40 min.



Chiral HPLC-UV chromatogram of a solution of (R)/(S)-nicotine standard (500 µg/mL in methanol). (S)-Nicotine retention time 5.87 min, and (R)-Nicotine retention time 8.78 min.

The chiral GC/MS method that was initially developed had a slightly unresolved baseline separation of (S)- and (R)-nicotine and a relatively long run time (>73 min). The chiral HPLC/UV method improved upon the GC/MS method by providing an excellent enantiomeric separation and a shorter run time (<10 min).

Table 1. Tobacco-Derived Nicotine (TDN) Results

Entry	Supplier	Nicotine Source	Analytical Method	Characteristic Impurity Results	Distinguishing Impurity Results for TDN	%(R)-Nicotine by Chiral GC/MS[a] or HPLC-UV[b]	Nicotine Source Conclusion
1	AmeriNic	TDN	GC/MS	Myosmine, β-nicotyrine, cotinine, 2,3'-bipyridine	2,3'-Bipyridine	Not analyzed	TDN
2	AmeriNic	TDN	SPME/GC/MS	Cotinine, nornicotine, myosmine, β-nicotyrine	Lack of 1-methyl-2-pyrrolidinone	Not analyzed	TDN
3	AmeriNic	TDN (Philippines)	GC/MS	3-Vinylpyridine, pyridinecarboxaldehyde, anatabine, myosmine, β-nicotyrine, cotinine, 2,3'-bipyridine	2,3'-Bipyridine	0.8-0.9[a]	TDN
4	AmeriNic	TDN (India)	GC/MS	3-Vinylpyridine, pyridinecarboxaldehyde, anatabine, myosmine, β-nicotyrine, cotinine, 2,3'-bipyridine	2,3'-Bipyridine	0.8-0.9[a]	TDN
5	Siegfried AG	TDN (India)	GC/MS	3-Vinylpyridine, pyridinecarboxaldehyde, anatabine, myosmine, β-nicotyrine, cotinine, 2,3'-bipyridine	2,3'-Bipyridine	0.8-0.9[a]	TDN
6	Siegfried AG	TDN (India)	GC/MS	Low levels of oxidation products-myosmine, β-nicotyrine, cotinine, and low levels of anatabine	No distinguishing	0.6[b]	TDN

TDN = Tobacco-Derived Nicotine; SN = Synthetic Nicotine; NGL = Next Generation Labs TFN®; eLT (TTI) = e-LiquiTech (Tobacco Technology, Inc.)

Table 2. Synthetic Nicotine (SN) Results

Entry	Supplier	Nicotine Source	Analytical Method	Characteristic Impurity Results	Distinguishing Impurity Results for SN	%(R)-Nicotine by Chiral GC/MS[a] or HPLC-UV[b]	Nicotine Source Conclusion
1	NGL	SN	GC/MS	Myosmine, β-nicotyrine, cotinine, 1,3-dichloro-2-propanol, ethyl nicotinate	1,3-Dichloro-2-propanol, ethyl nicotinate	Not analyzed	SN
2	NGL	SN	SPME/GC/MS	Cotinine, nornicotine, myosmine, β-nicotyrine, 1-methyl-2-pyrrolidinone, methylene chloride	1-Methyl-2-pyrrolidinone, methylene chloride	Not analyzed	SN
3	NGL	SN	GC/MS	3-Vinylpyridine, pyridinecarboxaldehyde, anatabine, myosmine, β-nicotyrine, cotinine, ethyl nicotinate, 1-methyl-2-pyrrolidinone, N-ethyl nornicotine	Ethyl nicotinate, 1-methyl-2-pyrrolidinone, N-ethyl nornicotine, lower levels of myosmine, β-nicotyrine	50.0[b]	SN
4	Siegfried AG	SN	GC/MS	β-nicotyrine (higher level than in tobacco-derived nicotine)	No distinguishing	0.2[b]	SN
5	eLT (TTI)	SN	GC/MS	Low levels of oxidation products--myosmine, β-nicotyrine, cotinine, and low levels of anatabine	No distinguishing	0.2[b]	SN
6	Zanoprima Lifesciences	SN	GC/MS	Low levels of oxidation products--myosmine, β-nicotyrine, cotinine, and low levels of anatabine	No distinguishing	0.1[b]	SN

TDN = Tobacco-Derived Nicotine; SN = Synthetic Nicotine; NGL = Next Generation Labs TFN®; eLT (TTI) = e-LiquiTech (Tobacco Technology, Inc.)

Analytical Findings

- Table 1 –TDN Results
  - Two suppliers of TDN included AmeriNic and Siegfried
  - The nicotine was manufactured from tobacco material from the Philippines and India
  - GC analysis provided characteristic, distinguishing TDN impurities
  - Distinguishing impurities included 2,3'-bipyridine and levels of (R)-nicotine at 0.6-0.9%, typical of (R)-nicotine found in TDN

- Table 2 – SN Results
  - Four suppliers of SN included Next Generation Labs, Siegfried, e-LiquiTech, and Zanoprima Lifesciences
  - GC analysis provided characteristic distinguishing SN impurities, including starting materials, ethyl nicotinate; synthetic impurities,1-methyl-2-pyrrolidinone, 1,3-dichloro-2-propanol; other impurities, N-ethyl nornicotine; and the residual solvent, methylene chloride
  - Chiral chromatography indicated a 50/50 mixture of (S)/(R)-nicotine in one sample (Entry 3) and was confirmation of synthetic nicotine
  - Low levels of (R)-nicotine at 0.1–0.2% were an indication that the nicotine was likely synthetic

Conclusions

- Two achiral chromatography methods and two chiral chromatography methods were used to distinguish TDN from SN.
- TDN was found to contain the characteristic tobacco compound, 2,3'-bipyridine, while in some instances, SN, was found to contain synthetic starting materials, synthetic impurities, or residual solvents.
- Chiral chromatography results provided supporting evidence that nicotine samples containing low levels of (R)-nicotine (0.1-0.2%), as compared to TDN which typically contains 0.8-0.9% (R)-nicotine, were likely synthetic.
- The chiral methods provide an approach for determining (S)/(R) nicotine ratios that may be of importance related to any pharmacological concerns and future research.
- The combination of these analytical methods provides valuable information for distinguishing TDN from SN in commercial nicotine samples.

Acknowledgments

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- Thank you for your attention!

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