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

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Abstract

Recently, synthetic nicotine (SN) has become commercially available and can be supplied as United States Pharmacopeia (USP) grade (S)-nicotine, as a 50/50 (S)/(R) mixture, or as a mixture in varying ratios of (S)/(R) enantiomers. Some tobacco-product manufacturers may have been turning to the use of SN as a replacement for tobacco-derived nicotine (TDN) in an effort to circumvent the FDA Center for Tobacco Products (CTP) Premarket Tobacco Product Application (PMTA) process for a new tobacco product or as a result of the receipt of a PMTA Marketing Denial Order. Because of recent Congressional action, the FDA now requires a PMTA for tobacco products containing SN. As tobacco product development continues, the need for robust analytical methods to differentiate TDN from SN will be of significant importance for tobacco authentication. The main purpose of this study was to demonstrate the utility of analytical chromatographic methods to distinguish TDN from SN. Nicotine samples were analysed by GC/MS and SPME/GC/MS and by either chiral GC-MS or chiral HPLC-UV. Several TDN samples were found to contain 2,3'-bipyridine, characteristic of TDN. Some SN samples were found to contain the synthetic starting material, ethyl nicotinate and the synthetic impurity, 1-methyl-2-pyrrolidinone. By chiral chromatography, SN was found to contain either a 50:50 mixture of (R)- and (S)-nicotine or a low level of (R)-nicotine (0.1-0.2%) compared to higher amounts in TDN (0.8%-0.9%). A low level of (R)-nicotine indicated that the nicotine was likely synthetic. The use of these chromatographic methods provides guidance for distinguishing TDN from SN in commercial nicotine samples.

Introduction

According to US federal regulations, any product containing tobacco-derived materials is deemed a tobacco product.[1] To obtain marketing authorization for any new tobacco product, manufacturers must first submit a PMTA[2] to the FDA CTP. However, tobacco-product manufacturers, including those that had received a PMTA Marketing Denial Order, may have been circumventing the PMTA process for a new tobacco product by using synthetic nicotine (SN) in place of tobacco-derived nicotine (TDN) in their electronic cigarette and modern oral tobacco products (pouch, lozenges). This regulatory loophole may have been exploited as products containing TDN, primarily composed of (S)-nicotine, were regulated by the FDA CTP whereas products containing SN, containing higher (R)-nicotine ratios, were outside of the FDA regulation. Because of recent Congressional action granting FDA authority over SN, tobacco-product manufacturers must now submit a PMTA for tobacco products containing SN.

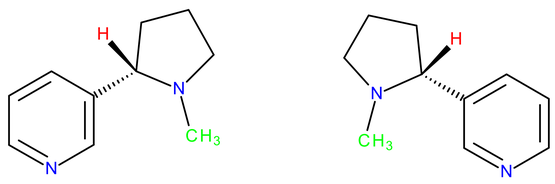
In past studies with e-liquids, SN was found as a 50:50 racemic mixture of (S)-nicotine and (R)-nicotine.[3] Even though tobacco consumers have been exposed to small amounts of the less toxic (R)-nicotine over the years, concern has been raised about the quantity of (R)-nicotine in marketed products and the pharmacology of (R)-nicotine. Numerous studies have indicated that the pharmacological effects of (R)-nicotine are qualitatively similar to but quantitatively less potent than those of (S)-nicotine.[4] As current tobacco product development continues, the need for robust analytical methods to differentiate TDN from SN will be of significant business and regulatory importance for tobacco authentication.

Nicotine is typically obtained from the tobacco plant (*Nicotiana tabacum*, *Nicotiana rustica*, etc.) by extraction and purification by vacuum distillation. The enantiomeric form is (S)-3-[1-methylpyrollidin-2-yl]-pyridine or (S)-(-)-nicotine, with a small amount of ~0.2-0.6% of (R)-(+)-nicotine.[5] Samples containing more than about 0.6% (R)-nicotine are assumed to be synthetic. The structures of (S)-nicotine and (R)-nicotine are shown in **Figure 1**.

Synthetic (S)-nicotine can be obtained by a variety of asymmetric synthetic methods.[6] For example, synthetic (S)-nicotine can also be obtained by racemic synthesis[7] starting from methyl or ethyl nicotinate and 1-methyl-2-pyrrolidinone or an N-vinyl-protected 2-pyrrolidinone, followed by subsequent synthetic steps and classical resolution using diastereomeric salts[8] to afford (S)-nicotine or alternatively by an enzymatic process involving a stereoselective reduction of the synthetic intermediate, myosmine and by further processing steps.[9]

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.[3] The differentiation between TDN and SN has been described as possibly based on the level of radiocarbon ^{14}C, which is higher in the tobacco-derived (S)-nicotine than in the synthetic petroleum-derived (S)-nicotine.[10] However, this method requires a sample size of >50 mg of nicotine and specialized ^{14}C instrumentation. A site-specific peak intensity ratio from 1D 2H/1H NMR spectroscopy method has also been described, but it has the limitation of detecting TDN adulteration with SN but only as low as 20% SN.[11]

An alternative analytical approach has been developed using GC/MS, SPME GC/MS, and chiral chromatography methods (GC/MS and HPLC-UV). The methods compare the specific impurities present in the TDN and those absent in SN and vice versa. In some examples, the amount of (R)-nicotine present as determined by chiral chromatography provides supporting evidence to characterize the nicotine source.



(S)-Nicotine **(R)-Nicotine**

Figure 1. The structure of (S)-Nicotine and (R)-Nicotine.

Conclusions

- The chromatographic methods provided data to successfully distinguish TDN from SN and provided additional chemical characteristics of several commercial nicotine samples.
- 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 the synthetic starting material, ethyl nicotinate and the synthetic impurity, 1-methyl-2-pyrrolidinone or other synthetic impurities such as 1,3-dichloro-2-propanol and the residual solvent, methylene chloride.
- Chiral chromatography results provided supporting evidence that nicotine containing low levels (0.1-0.2%) of (R)-nicotine were likely synthetic.
- The use of these chromatographic methods provides guidance for distinguishing TDN from SN in commercial nicotine samples.

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Materials and Analytical Methods

Materials

TDN and SN samples were received from commercial suppliers and were used as is for sample preparation.

Analytical Methods

1. GC/MS and SPME/GC/MS

The GC/MS method involved a GC/MS Agilent 6890 Series GC (Wilmington, DE) with 5973 MSD equipped with a DB-Waxeter 30 m x 0.25 mm i.d., with 0.25 µm film column, monitoring from 33-300 amu. SPME/GC/MS samples were analyzed with Gerstel MPS Multipurpose Analyzer with SPME capability. GC/MS samples were prepared by dissolving 50 mg in 1 mL methanol. SPME/GC/MS samples used 20 mg in a SPME vial. Peak identification in the chromatograms was done by a library search using the NIST library of mass spectral data with a quality match of ≥80%.

A separate GC/MS analysis was done using a 6890/5973 system equipped with a DB-Waxeter 30 m x 0.25 mm i.d, with 0.25 µm film column, monitoring from 33-550 amu. GC/MS samples were prepared by dissolving 10 mg in 1 mL tert-butyl methyl ether. Peak identification in the chromatograms was done by a library search in the common mass spectral libraries (NIST, Wiley).

2. Chiral GC/MS and Chiral HPLC-UV

The chiral GC/MS method involved a GC-MS 7890B/5977B with a high efficiency source from Agilent (Wilmington, DE) equipped with two columns of Rt-GammaDEXsa 30 m x 0.25 mm i.d., with 0.25 µm film from Restek in series, monitoring from 33-250 amu. Samples were prepared as a 250 µg/mL solution in methanol.

The chiral HPLC-UV method involved an Agilent 1200 HPLC binary system consisting of a pump, autosampler with cooling capability, and a thermostatted column compartment. Separation was achieved on a Chiracel OJ-3 column 250 mm x 4.6 mm with 3 µm particle size (Daicel Corp.), using a mobile phase consisting of hexane and 15% ethanol with the addition of 7.5 mL trifluoroacetic acid and 7.5 mL triethylamine for 1 L mobile phase, with detection at 254 nM. Samples were prepared as a 500 µg/mL solution in methanol.

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 and provided an excellent enantiomeric separation and a shorter run time (<10 min).

Results

Using GC/MS, TDN was found to contain 2.3'-bipyridine as a characteristic tobacco compound not found in SN samples, whereas SN was found to contain the synthetic starting material, ethyl nicotinate and the synthetic impurity, 1-methyl-2-pyrrolidinone (**Table 1**). An additional impurity in one sample of SN included 1,3-dichloro-2-propanol, along with the residual solvent, methylene chloride. In some instances, SN was found to contain lower levels of the nicotine oxidation products, myosmine and β-nicotyrine. The analytical results provide valuable information to distinguish TDN from SN.

The samples of TDN were found to contain 0.8-0.9% (R)-nicotine by chiral GC/MS and 0.6% by chiral HPLC-UV. SN was found to contain either a 50:50 mixture of (R)- and (S)-nicotine in one sample or and 99% (S)-nicotine and a very small amount of (R)-nicotine in three other samples. This amount is much lower than what is typically found in TDN and provides supporting evidence for the determination of SN.

Table 1: Analytical results for commercially available TDN and SN.

Entry	Supplier	Nicotine Source	Analytical Method	Characteristic Impurity Results	Distinguishing Impurity Results for TDN	Distinguishing Impurity Results for SynNic	% (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	Cotinine, lack of 1-methyl-2-pyrrolidine		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	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	TDN (India)	GC/MS	Low levels of oxidation products--myosmine, β-nicotyrine, cotinine, and low levels of anatabine	No distinguishing		0.6[b]	TDN
7	NGL	SynNic	GC/MS	Myosmine, β-nicotyrine, cotinine, 1,3-dichloro-2-propanol, ethyl nicotinate		1,3-Dichloro-2-propanol, ethyl nicotinate	Not analyzed	SN
8	NGL	SynNic	SPME/GC/MS	Cotinine, nornicotine, myosmine, β-nicotyrine, 1-methyl-2-pyrrolidinone, methylene chloride		1-Methyl-2-pyrrolidinone, methylene chloride	Not analyzed	SN
9	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
10	Siegfried	SN	GC/MS	β-nicotyrine (higher level than in tobacco-derived nicotine)		No distinguishing	0.2[b]	SN
11	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
12	Zanoprima	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.)

Acknowledgments

The authors wish to acknowledge the contributions of Ching-En Chou to this poster.

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