Comparison of CNT Synthetic and Tobacco Derived Nicotine

Ed Carmines, Bryan Burd, Kevin Burd
Chemular Inc, Hudson MI, USA
Poster #76

Abstract

Most commercially available nicotine is the S isomer derived from tobacco. All tobacco derived nicotine (TDN) products are regulated by the FDA as drugs or tobacco products. Synthetic nicotine (SynNic) products were not previously regulated but are now regulated by the FDA as a tobacco product. Depending on the manufacturing process, SynNic can be produced as a racemic mixture of R and S nicotine or predominantly S nicotine. TDN can be differentiated from SynNic by radiocarbon dating. TDN is >99% S-isomer. The amount of S-isomer in SynNic can be used to determine if the synthetic version is equivalent to the tobacco derived version. Not all SynNic is >99% S-isomer. The SynNic from CNT is equivalent to the TDN, also being composed of >99% S-isomer and also highly exceeding all USP requirements as well as the European Pharmacopoeia (Ph. Eur.) requirements.

Background

Commercially available nicotine is available as tobacco derived nicotine (TDN) where the nicotine is extracted out of tobacco and purified or the nicotine is produced by various synthetic routes (SynNic). TDN is predominately the S isomer of nicotine (>99%). Depending on the synthetic route, SynNic can be predominantly S isomer or a racemic mixture of R and S isomers. Contraf-Nicotex-Tobacco GmbH (CNT) is the world's largest supplier of highly purified TDN to the pharmaceutical industry. CNT is also a significant supplier of pharmaceutical grade Nicotine to the tobacco industry. CNT's starting material for the TDN production derives from the company's extraction facility in India. The final processing is then done in Switzerland by CNT's exclusive contract manufacturer Siegfried AG under full pharmaceutical cGMP. CNT owns a patented process for producing SynNic. CNT's TDN meets or exceeds United States Pharmacopeia and European Pharmacopeia standards. There are no published standards for synthetic (SYN) nicotine. Synthetic nicotine is synthesized using either a chemical or an enzymatic process. This is then often followed by a chemical or enantiomeric purification. There are multiple forms of synthetic nicotine. Synthetic nicotine can be identical to tobacco derived nicotine and be >99% S nicotine or it can be a racemic mix of R- and S- (50/50 with the CAS # 22083-74-5) or vary in the ratios of R- and S-nicotine which have different CAS numbers.

Differentiating Synthetic from Tobacco Derived Nicotine

There are multiple proposed ways to distinguish synthetic nicotine from tobacco derived nicotine. These range from evaluating the impurities that are common markers for nicotine analogues to radiocarbon dating to determine the age of the carbon source which indicates if the carbon came from plant-based materials or petrochemical based materials. Recently, Campbell et al., (2021) and Cheetham et al. (2022) presented the results of a study analyzing various sources of synthetic and tobacco derived nicotine They used different approaches to evaluate synthetic and tobacco derived nicotine samples. The tests they performed included:

1. Quantitation of Nicotine Enantiomers by HPLC-UV Chiral Chromatography
2. Quantitation of Nornicotine Enantiomers by LC-MS/MS Chiral Chromatography
3. Nicotine degradants, metals, and TSNAs analyses
4. Non-Targeted Analysis by GC-MS
5. Radiocarbon Analysis

The samples tested are shown in **Table 1**. The results of the HPLC / UV chiral chromatography are shown in **Figure 1**. All of the tobacco derived nicotine (TDN) samples were greater than 99% S-isomer. All of the synthetic nicotine (SynNic) samples tested except the Next Generation Labs tobacco free nicotine (TFN) were also > 99% S-isomer. The TFN sample was a racemic mixture or R- and S-nicotine. The quantification of the nornicotine enantiomers is shown in **Figure 2**. Nornicotine in the tobacco plant is predominantly formed by enzymatic demethylation of nicotine, a process that appears biased toward the R-nicotine enantiomer and leads to an observed wide variation in the R/S-ratio of tobacco-derived nornicotine (4 to 75%) which would not match the R/S-ratio of the nicotine from the same plant (0.1 to 1.2%). SynNic can be formed via methylation of nornicotine, which will give the same R/S-ratio in the nicotine as in the nornicotine. The results of the chiral analysis of the nornicotine indicated differences between the TDN and SynNic. Nicotine degradants were analyzed in the samples (**Figure 3**). There were no discernable patterns that could be used to definitively distinguish TDN from synthetic nicotine. Non-target analysis was performed to evaluate if there were unique markers that could be used to differentiate the two nicotine sources. This failed to yield any analyte that was specific to a particular nicotine origin. Plants incorporate Carbon-14 via photosynthesis of CO2. Radiocarbon (C-14) is present in all living and recently expired matter. Anything that is more than 50,000 years old, such as fossil-derived petrochemicals, no longer contains C-14. A radiocarbon analysis was performed on the samples (**Figure 4**). As expected, all of the tobacco derived nicotine samples showed 100% incorporation of C-14. The synthetic samples contained 35-36% C-14 indicating that they were partially produced from a petrochemical source of carbon. This work demonstrated that it was possible to differentiate TDN from SynNic by using chiral analysis and radiocarbon dating.

Data

Table 1. Nicotine Samples Evaluated by Campbell et al., 2021

Tobacco Derived Nicotine (TDN)	Synthetic Nicotine (SynNic)
TCI America (lab reference)	Next Generation Labs TFN® (R/S-Nicotine) **NextGen** (R/S Nicotine)
Alchem International (NicSelect)	Tobacco Technology Inc. (**TTI**) (S-Nicotine)
Siegrfried TDN (S-Nicotine)	**Siegrfried** NTN (S-Nicotine)
North American Nicotine	**Hangsen Group**

Figure 1. Enantiomeric Purity of Nicotine Samples (Campbell et al., 2021)

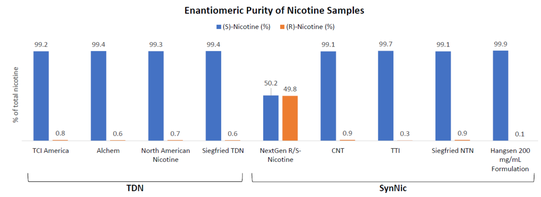


Figure 2. Comparison of Nornicotine Enantiomers (Campbell et al., 2021)

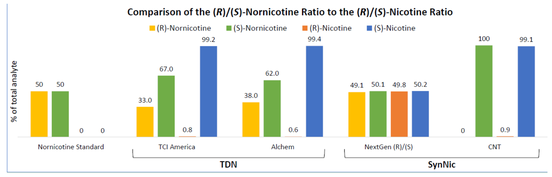


Figure 3. Nicotine Degradants (Campbell et al., 2021)

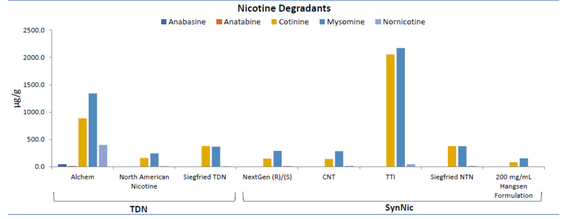
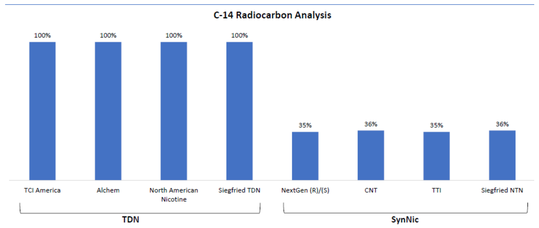


Figure 4. Radiocarbon Analysis of the Nicotine Samples (Campbell et al., 2021)



CNT SynNic samples were analyzed by radiocarbon dating. **Figure 5** shows the distribution of the radiocarbon. The percent of C-14 was 35.9%. This is consistent with the results above from Campbell et al., (2021) and Cheetham et al., (2022) indicating that the nicotine was partially made with synthetic chemicals, that is, a synthetic route was used to make the material. CNT (Siegfried) provides a certificate indicating that its synthetic nicotine is indeed synthetic (**Figure 6**).

Figure 5. Radiocarbon Analysis of CNT SynNic

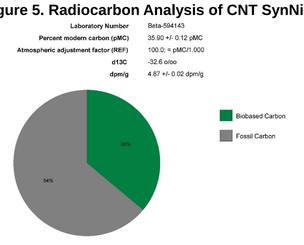


Figure 6. Synthetic Nicotine Statement from Siegfried

Siegfried

SYNTHETIC NICOTINE - STATEMENT

Siegfried Ltd
4800 Zofingen
Switzerland

+41 62 746 1650
enno.schweinberger@siegfried.ch

PRODUCT: **Nicotine (synth.)**
MATERIAL #: **101857**
MANUFACTURER: **SIEGFRIED LTD**

14-Aug-19

TO WHOM IT MAY CONCERN

Siegfried confirms that the product Nicotine (synth.) (material number: 101857) is a completely synthesized product. The production does not include tobacco leaves or any other tobacco related ingredients.
The synthesis is proprietary and protected by a patent (Number WO: 2019/121644) filed by Siegfried.

Dr. Enno Schweinberger
Qualified Person
Siegfried Ltd

Table 2 shows a comparison of Siegfried's specifications for TDN and SynNic. The specifications are identical. Representative results of analysis are also shown in **Table 2**. **Both the synthetic and tobacco derived nicotine from Siegfried have the identical specs and identical analytical results**. There is no difference between the synthetic and tobacco derived nicotine from Siegfried. The **chirality analyses by Siegfried and also Campbell et al., (2021) show that the Siegfried synthetic nicotine is >99% S-isomer just like the tobacco derived nicotine**.

Table 2. Comparison Of Specifications and Representative Results for CNT Tobacco Derived and Synthetic Nicotine

Material Name:	**Nicotine**			**Nicotine**		
Source:	**Tobacco Derived**			**Synthetic Nicotine**		
Quality:	USP/Ph.Eur.			**NLT 99% area S-Nicotine**		
Material No:	183785			**101857**		
Lot No:	2147I003			2007L016		
Manufacture Date	11/27/2021			2/14/2020		
CAS No.	54-11-5			54-11-5		
Formula:	C10H14N2			C10H14N2		
Molecular Weight:	162.2			162.2		
Test	**Method**	**Specification**	**Result**	**Method**	**Specification**	**Result**
Description	BE-183785	Colourless to yellow or brownish liquid	Complies	BE-183785	Colourless to yellow or brownish liquid	Complies
IR-Spectrum	IR-183785	in accordance with the standard spectrum	Complies	IR-183785	in accordance with the standard spectrum	Complies
Clarity of solution	KLB-183785	NMT 3 FTU ; clear (10 % m/V in water)	0 FTU	KLB-183785	NMT 3 FTU ; clear (10 % m/V in water)	0 FTU
Colour of solution	FAB-183785	NLT 5 on scale BY, Y or R (10 % m/V in water)	7	FAB-183785	NLT 5 on scale BY, Y or R (10 % m/V in water)	7
Specific rotation	SDB-183785	-143 - -130 ° at 25°C in ethanol 96 % (calculated on the anhydrous basis)	-137 °	SDB-183785	-	-
	SDC-183785	-152 - -140 ° in ethanol abs. (calculated on the anhydrous basis)	-147 °	SDC-183785	-152 - -140 ° in ethanol abs. (calculated on the anhydrous basis)	-146 °
Arsenic	GPAS-183785	NMT 2 ppm	<1 ppm	-	-	-
Cadmium	PLCD-183785	NMT 0.5 ppm	<0.20 ppm	-	-	-
Heavy metals (Pb)	SM-183785	NMT 20 ppm	<10 ppm	-	-	-
Water (KF)	KF-183785	NMT 0.5 %	0.0 %	KF-183785	NMT 0.5 %	0.0%
Assay (Non-aqu. titr.)	TIG-183785	99.0 - 101.0 % (calculated on the anhydrous basis)	100.1 %	TIG-183785	99.0 - 101.0 % (calculated on the anhydrous basis)	99.5%
Identity (HPLC)	LCV-183785	the retention time of the major peak corresponds to that of the reference	Complies	LCV-183787	the retention time of the major peak corresponds to that of the reference	Complies
Related substances (HPLC)	LCV-183785	NMT 0.15 % cotinine	<0.05 %	LCV-183787	NMT 0.15 % cotinine	<0.06 %
	LCV-183785	NMT 0.15 % myosmine	<0.05 %	LCV-183787	NMT 0.15 % myosmine	<0.06 %
	LCV-183785	NMT 0.15 % nicotine N-oxide	<0.05 %	LCV-183787	NMT 0.15 % nicotine N-oxide	<0.06 %
	LCV-183785	NMT 0.15 % nornicotine	<0.05 %	LCV-183787	NMT 0.15 % nornicotine	<0.06 %
	LCV-183785	NMT 0.15 % anatabine	<0.05 %	LCV-183787	NMT 0.15 % anatabine	<0.06 %
	LCV-183785	NMT 0.10 % ß-nicotyrine	<0.05 %	LCV-183787	NMT 0.10 % ß-nicotyrine	<0.06 %
	LCV-183785	NMT 0.10 % anabasine	<0.05 %	LCV-183787	NMT 0.10 % anabasine	<0.06 %
	LCV-183785	NMT 0.10 % greatest unknown impurity (no unknown impurity greater than 0.10%)	<0.05 %	LCV-183787	NMT 0.10 % greatest unknown impurity (no unknown impurity greater than 0.10%)	0.10%
	LCV-183785	NMT 0.8 % sum of impurities	<0.05 %	LCV-183787	NMT 0.8 % sum of impurities	0.1
Residual solvent	GCH-183785	NMT 500 ppm cyclohexane	20 ppm	GCH-101857	Toluol (only for information)	<10 ppm

Conclusions

The synthetic nicotine produced by CNT (Siegfried AG) is identical to the nicotine derived from tobacco plants:

- Nicotine derived from plants is > 99% S-isomer
- Synthetic nicotine from Siegfried is >99% S-isomer
- The CAS numbers for the two nicotines are the same – 54-11-5
- The Siegfried tobacco derived nicotine and synthetic nicotine meet USP and Ph. Eur standards
- The Siegfried release standards for the tobacco derived nicotine and synthetic nicotine are the same
- Siegfried analysis of the synthetic and tobacco derived nicotine show they are identical

References

Cheetham, Andrew G., Susan Plunkett, Preston Campbell, Jacob Hilldrup, Bonnie G. Coffa, Stan Gilliland, and Steve Eckard. 2022. "Analysis and Differentiation of Tobacco-Derived and Synthetic Nicotine Products: Addressing an Urgent Regulatory Issue." PloS One 17 (4): e0267049. https://doi.org/10.1371/journal.pone.0267049.

Preston Campbell, Andrew Cheetham, Bonnie Coffa, Jason Hildrup, Stan Gilliland, and Susan Pluncket. 2021. "Analysis and Differentiation of Tobacco-Derived and Synthetic Nicotine Samples | CORESTA." Boston. https://www.coresta.org/abstracts/analysis-and-differentiation-tobacco-derived-and-synthetic-nicotine-samples-35263.html .

75th TSRC Conference 2022, New Orleans, USA