Analysis of (S)- and (R)-Nicotine in Commercial Nicotine Samples and E-liquids

Gary M. Dull, Ph.D. and Serban C. Moldoveanu, Ph.D. (RAI Services Company and R.J. Reynolds Tobacco Co., Winston-Salem, NC, USA 27102)

PURPOSE

Chiral analysis of nicotine in tobacco and commercial samples

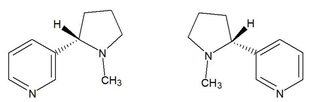
Nicotine has been studied extensively because of its presence in tobacco products. Because of its chirality, nicotine can exist in two enantiomeric forms; however, the tobacco plant produces predominantly (S)-nicotine with only a trace of (R)-nicotine.[1,2] Consequently, consumers of tobacco products have been exposed to rather small amounts of (R)-nicotine over the years. It should be mentioned that with some exceptions, (R)-nicotine typically has a qualitatively similar but quantitatively less potent pharmacological profile than (S)-nicotine.[3] Recently, synthetic nicotine has become commercially available and can be supplied as a 50/50 racemic mixture or as USP grade (S)-nicotine. The purpose of this study was to determine the amount of (R)-nicotine in commercially available nicotine and in liquids (e-liquids) used in Electronic Nicotine Delivery Systems (ENDS). The structures of (S)-nicotine and (R)-nicotine are shown in **Figure 1**.

METHODS

Chiral GC-MS and HPLC-UV

Nicotine sources included tobacco-derived, US Pharmacopeia (USP) nicotine from two commercial suppliers, synthetic nicotine from four suppliers, and two e-liquids from one supplier. Samples were analyzed for (R)- and (S)-nicotine using either chiral GC-MS or chiral HPLC-UV.[4] The chiral GC-MS method had a slightly unresolved baseline separation of (S)- and (R)-nicotine and a relatively long run time (>73 min) (**Figure 2**). The chiral HPLC-UV method provided an excellent enantiomeric separation and a shorter run time (>10 min) (**Figure 3**).

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



(S)-Nicotine

(R)-Nicotine

Figure 2: Chiral GC-MS chromatogram of a solution of racemic, synthetic nicotine (250 µg/mL in methanol). The 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.

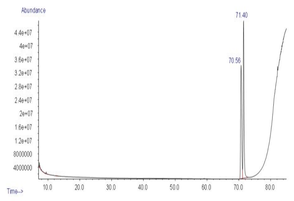
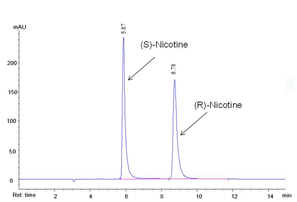
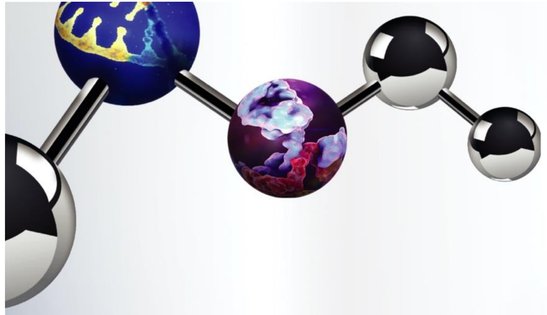


Figure 3: Chiral HPLC-UV chromatogram of a solution of (R)/(S)-nicotine standard (500 µg/mL in methanol). The method involved an Agilent 1200 HPLC binary system that consisted of a pump, autosampler with cooling capability, and a thermostatted column compartment. The separation was achieved on a Chiracel OJ-3 column 250 mm x 4.6 mm with 3 µm particle size from 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.

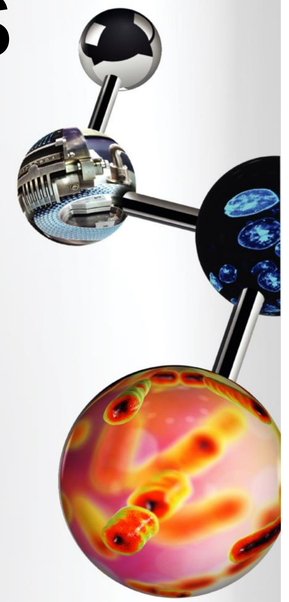


aaps PharmSci 360

Advancing Pharmaceutical Sciences, Careers, and Community



Chiral analysis of commercial nicotine, synthetic nicotine, and ENDS e-liquids identifies mixtures of (S)- and (R)-nicotine



Poster Number M1030-01-03

REYNOLDS
A BETTER TOMORROW

CONTACT INFORMATION: dullg2@rjrt.com

RESULTS

Tobacco-derived nicotine compared to synthetic nicotine

The results for the enantiomeric analysis of nicotine are shown in **Table 1.** The chiral GC-MS method was initially developed and was replaced by the more effective chiral HPLC-UV method that provided better enantiomer separation. Consequently, samples were analyzed by two different chiral methods as shown in **Table 1**.

Table 1. Chiral GC-MS and HPLC-UV analytical results for commercially available tobacco-derived USP nicotine, synthetic nicotine, and nicotine in liquids (e-liquids) used in electronic cigarettes.

Entry	Sample	Supplier	Source	Analytical Method	% (S)-Nicotine	% (R)-Nicotine
1	Nicotine	Supplier A	Tobacco	GC-MS	99.2-99.1	0.8-0.9
2	Nicotine	Supplier A	Tobacco	GC-MS	99.2-99.1	0.8-0.9
3	Nicotine	Supplier B	Tobacco	GC-MS	99.2-99.1	0.8-0.9
4	Nicotine	Supplier B	Tobacco	HPLC-UV	99.4	0.6
5	Nicotine	Supplier C*	Synthetic	GC-MS	50.0	50.0
6	Nicotine	Supplier C*	Synthetic	HPLC-UV	50.0	50.0
7	Nicotine	Supplier B	Synthetic	HPLC-UV	99.8	0.2
8	Nicotine	Supplier D	Synthetic	HPLC-UV	99.8	0.2
9	Nicotine	Supplier E	Synthetic	HPLC-UV	99.9	0.1
10	Nicotine	Supplier C	Synthetic	HPLC-UV	50.0	50.0
11	Nicotine	Supplier C	Synthetic	HPLC-UV	93.0	7.0
12	ENDS e-liquid	Supplier F	Commercial 2.2% Nicotine	GC-MS	99.2	0.8
13	ENDS e-liquid	Supplier G	Commercial, 2.4% Nicotine	HPLC-UV	50.0	50.0
14	ENDS e-liquid	Supplier G	Commercial, 5.0% Nicotine	HPLC-UV	50.0	50.0

*Entries 5 and 6 share the same lot number.

The analysis of four commercial samples of tobacco-derived, USP nicotine (Entries 1-4) revealed only a small amount of (R)-nicotine, whereas one sample of synthetic nicotine (Entry 5) was found to contain a 50/50 mixture of enantiomers. Entries 5 and 6 for synthetic nicotine (same lot) indicated a 50/50 mixture of enantiomers as determined by both chiral methods. Three lots of synthetic nicotine (Entries 7, 8, and 9) when analyzed by chiral HPLC-UV were found to contain ≤0.2% (R)-nicotine. Two different lots of synthetic nicotine from the same supplier were found to contain a 50/50 mixture of (R)- and (S)-nicotine (Entry 6 and 10), whereas another synthetic nicotine sample from the same supplier contained 93% (S)-nicotine and 7% (R)-nicotine (Entry 11) indicating incomplete separation. Chiral analysis of an e-liquid used in a commercial electronic cigarette indicated predominantly (S)-nicotine and a similar amount of (R)-nicotine that is typically observed in tobacco-derived nicotine (Entry 12). Chiral analysis of nicotine in two lots of e-liquids (Entries 13 and 14) used in electronic cigarettes indicated that each lot contained a 50/50 mixture of (R)- and (S)-nicotine, and therefore these lots contained synthetic nicotine.

CONCLUSIONS

Two chiral chromatography methods were used for the analysis of (R)- and (S)-nicotine. Tobacco derived nicotine was found to contain the naturally occurring (S) enantiomer as is typically found in most commercial electronic cigarettes. However, large amounts (50%) of (R)-nicotine were found in some commercial synthetic nicotine sources and in the nicotine of two ENDS e-liquids. Consequently, depending upon product selection, e-cigarette consumers using ENDS with e-liquids prepared with synthetic nicotine may possibly be exposed to higher levels of (R)-nicotine as compared to combustible cigarettes and oral tobacco products which contain the naturally occurring (S)-enantiomer. Synthetic nicotine composed of large amounts of (R)-nicotine may not necessarily be a pharmacological safety issue,[5] though impurities resulting from the synthesis of the synthetic nicotine may be present.

REFERENCES

1. Armstrong DW, Wang X, Ercal N. Enantiomeric composition of nicotine in smokeless tobacco, medicinal products, and commercial reagents. *Chirality.* 1998;10(7):587-591.
2. 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.
3. Pagocki D, Ruman T, Danilczuk M, Danilczuk M, Celuch M, Walajtys-Rode E. Application of nicotine enantiomers, derivatives and analogues in therapy of neurodegenerative disorders. *Eur. J. Pharm.* 2007;563:18-39.
4. Moldoveanu SC, Dull GM, Oden RJ, Scott WA. Analysis of (S)- and (R)-nicotine in various nicotine samples and in e-liquids. *71st TSRC Tob. Sci. Res. Conf.* 2017;71: abstr.086.
5. Pagocki D et al. *Eur. J. Pharm.* 2007;563:18-39.