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

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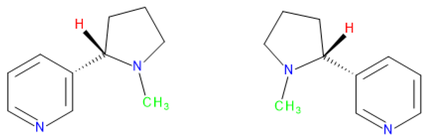
Abstract

Nicotine has been studied extensively because of its presence in tobacco products. Due to its chirality, nicotine can exist in two enantiomeric forms; however, the tobacco plant produces predominantly (S)-nicotine with only a trace of (R)-nicotine. Consequently, consumers of tobacco products have been exposed to rather small amounts of (R)-nicotine over the years. Recently, synthetic nicotine 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. Synthetic (S)-nicotine can be obtained by asymmetric synthesis or by racemic synthesis followed by classical resolution using diastereomeric salts. The primary objective of this study was to determine the amount of (R)-nicotine in commercial sources of tobacco-derived USP nicotine, synthetic nicotine, and in e-liquids used in Electronic Nicotine Delivery Systems (ENDS). A secondary objective was to examine the literature on the pharmacology of (R)-nicotine considering the potential exposure to tobacco consumers. Nicotine samples were analysed by either chiral GC-MS and/or chiral HPLC-UV. The analysis of four lots of tobacco-derived, USP nicotine revealed only a small amount of (R)-nicotine, whereas one sample of synthetic nicotine was found to contain a 50/50 mixture of enantiomers. Analysis of two lots of e-liquids used in ENDS indicated that each contained a 50/50 mixture of (R)- and (S)-nicotine and was synthetic. In this study, large amounts (50%) of (R)-nicotine were found in some commercial nicotine sources. Consequently, tobacco consumers may be subjected to higher (R)-nicotine exposure levels than in the past. A literature review indicated that with some exceptions (R)- and (S)-nicotine were found to exhibit a similar pharmacological profile, with (R)-nicotine being often, but not always, less pharmacologically active than (S)-nicotine.[1]

Introduction

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.[2] 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.[3] Synthetic (S)-nicotine can also be obtained by racemic synthesis[4] followed by classical resolution using diastereomeric salts.[5] However, synthetic nicotine may contain traces of related substances such as starting materials and residual solvents.

Nicotine is a common component of the e-liquids used in ENDS. The source of nicotine that has been found in the e-liquids can be tobacco-derived nicotine (>99% (S)-nicotine)[6,7] or synthetic (50/50 mixture of enantiomers).[8] An enantiomeric analysis of the nicotine found in commercially available nicotine samples and e-liquids was performed. Historically, the human exposure to (R)-nicotine in tobacco products has been rather minimal. Because of the presence of larger amounts of (R)-nicotine in some commercial nicotine samples, consumers may be exposed to more (R)-nicotine than in the past. Consequently, a literature search on (R)-nicotine pharmacology was performed to inform the topic.



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

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

Analytical Methods

There are several methods for the detection of nicotine enantiomers reported in the literature. Examples include chiral normal phase HPLC-UV,[6] chiral HPLC-MS,[8] and chiral HPLC-UV.[9] In this study, two chiral analytical methods were used; a chiral GC-MS method which had a slightly unresolved baseline separation of (S)- and (R)-nicotine and a relatively long run time (>73 min) (**Figure 2**), and a chiral HPLC-UV method with excellent enantiomeric separation and a short run time (>10 min) (**Figure 3**).[7]

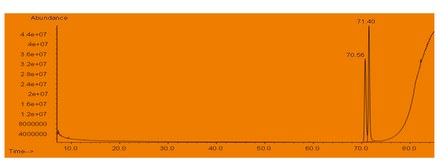


Figure 2: Chiral GC-MS chromatogram of a racemic, synthetic nicotine solution (250 µg/mL in methanol). The method involved a GC-MS 7890B/5977B with a high efficiency source from Agilent (Wilmington, DE) equipped with two columns of Rt-GammaDEXsa 30 m x 0.25 mm i.d., with 0.25 µm film from Restek in series, monitoring from 33-250 amu.

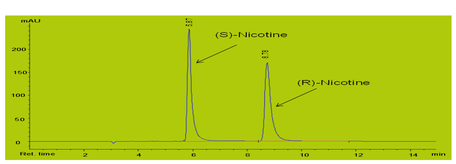


Figure 3: Chiral HPLC-UV chromatogram of a solution of (R)/(S)-nicotine (500 µg/mL in methanol) using an Agilent 1200 HPLC binary system consisting of a pump, autosampler with cooling capability, and a thermostatted column compartment. Separation was achieved on a Chiracel OJ-3 column 250 mm x 4.6 mm 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.

Conclusions

- Two chiral chromatography methods were developed for the analysis of (S)- and (R)-nicotine.
- Tobacco-derived nicotine was found to contain the naturally occurring (S)-enantiomer as is typically found in most commercial ENDS products. 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, consumers using ENDS with e-liquids prepared with synthetic nicotine may possibly be exposed to higher levels of (R)-nicotine compared to combustible cigarettes and oral tobacco products which contain the naturally occurring (S)-enantiomer.
- With some exceptions, (R)- and (S)-nicotine exhibit a similar pharmacological profile, with (R)-nicotine being often but not always less pharmacologically active than (S)-nicotine.
- Synthetic nicotine composed of large proportions of (R)-nicotine may not, in and of itself, pose a pharmacological safety issue,[1] though impurities resulting from the synthetic process may complicate the issue.

Results

The results for the enantiomeric analysis of nicotine are shown in **Table 1.** The chiral GC-MS method was initially developed but subsequently 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 USP nicotine, synthetic nicotine and nicotine in e-liquids.

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	HPLC-UV	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 proportion 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 purification. 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 ENDs indicated that each lot contained a 50/50 mixture of (R)- and (S)-nicotine and therefore was synthetic nicotine.

(R)-Nicotine Pharmacology

The pharmacology of (R)- and (S)-nicotine has been discussed and reviewed by Zhang,[6] Nordberg,[10] Pogocki[1] and Aceto,[11] and supported by references therein. A summary of important pharmacological results for (R)-nicotine is given below:

- The binding affinity (K_i) of (R)-nicotine to the $\alpha_4\beta_2$ nicotinic acetylcholine receptor (nAChR) is about 10 times lower than that of (S)-nicotine
- In several animal species, the LD_{50} for (R)-nicotine is 7-18 times higher than (S)-nicotine
- Both nicotine enantiomers release dopamine, with (R)-nicotine being less potent and efficacious than (S)-nicotine; (R)-nicotine has an EC_{50} of 1000 nM and an E_{max} of 76 nM, whereas (S)-nicotine has an EC_{50} of 125 nM and an E_{max} of 100 nM.[12a]
- The maximum potential dopamine release for R-nicotine was 120% that of S-nicotine[12b]
- (R)-nicotine does not release the neurotransmitter, norepinephrine, as does (S)-nicotine
- (R)-nicotine does not affect body weight in rats, as does (S)-nicotine, which causes a weight loss
- Both enantiomers share a similar metabolic pathway; however, the metabolic rate is 1.4-fold faster for (R)-nicotine than for (S)-nicotine
- (R)-nicotine is approximately 80 times less cytotoxic than (S)-nicotine
- The effects of (R)-nicotine on elevated blood pressure and increased heart rate in the anesthetized rat is reported to have a potency of approximately 1/8th that of (S)-nicotine
- Both enantiomers have antinociceptive effects; however, (S)-nicotine is 2-29 times more potent than (R)-nicotine
- Rat brain studies suggest that (R)-nicotine may preferably bind to different nAChR subtypes compared to (S)-nicotine. Thus, the mechanistic effects of (R)- and (S)-nicotine may be slightly different
- Some behavioral studies have shown that the subjective hedonistic effects among the smokers caused by (R)-nicotine are of an intensity comparable to that caused by (S)-nicotine

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