SUPPORTING INFORMATION:

Determination of (*R*)-(+)- and (*S*)-(-)-nicotine chirality in Puff Bar e-liquids by ^{1}H NMR spectroscopy, polarimetry, and gas chromatography–mass spectrometry

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Materials & Methods, Full Details:

Materials:

(*S*)-(-)-nicotine (99%) was from Alfa Aesar (Haverhill, MA). (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (≥98%; BNPPA), (±)-nicotine (≥99%), and t-butylamine (98%), were from Sigma-Aldrich (St. Louis, MO). DMSO-d_6, D 99.9%, was from Cambridge Isotope Laboratories, Inc. (Andover, MA). NMR tubes (535-PP-7) were purchased from Wilmad-LabGlass (Vineland, NJ). Ethanol (95%, USP) was from Fisher Scientific (Waltham, MA). Puff Bars labeled to contain 5% nicotine were purchased from online retailers and from local e-cigarette shops in Portland, OR. Some of the Puff Bar e-cigarettes stated that they contained "tobacco-free nicotine" (TFN; i.e. synthetic nicotine, which was anticipated to be racemic: (*R,S*)-(±)-nicotine), while the older Puff Bar e-cigarettes did not make this claim and presumably contained tobacco-derived nicotine (TDN; i.e. ~99+% (*S*)-(-)-nicotine).

NMR Method:

(*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate (BNPPA) was used as an NMR chiral complexing agent based on its use by Ravard & Crooks.[1] NMR samples were prepared by adding nicotine to the NMR solvent, DMSO-d_6, and combining aliquots of this mixture with varying quantities of BNPPA. Both pure nicotine ((*S*)-(-)-nicotine and (*R,S*)-(±)-nicotine forms) and nicotine-containing e-liquids (assumed to be (*S*)-(-)-nicotine and (*R,S*)-(±)-nicotine forms) were combined with BNPPA and tested by ^{1}H NMR. For the (*S*)-(-)-nicotine and (*R,S*)-(±)-nicotine standards, respectively, nicotine was combined with the NMR solvent, DMSO-d_6, in a ratio of 6.2 µL (~6.3 mg) nicotine:500 µL DMSO-d_6. Aliquots of this mixture (500 µL) were combined with BNPPA in ratios of 0 and 2 molar equivalents of BNPPA:nicotine. For the Puff Bar e-liquid samples, extracted e-liquid was combined with the NMR solvent, DMSO-d_6, in ratios of 26 µL e-liquid (the e-liquid is labeled as containing 5% nicotine, which is ~60 mg/mL, or ~1.6 mg nicotine in 26 µL e-liquid):500 µL DMSO-d_6. Aliquots of this mixture were combined with 0, 2, 4, and 5 molar equivalents BNPPA:nicotine based on the assumed nicotine content. A 600 MHz Bruker AVANCE III NMR spectrometer was used for data collection. Spectra were collected at 25 °C using 16 scans (NS) with a 30° observation pulse, and a relaxation delay of 3 seconds.

Polarimeter Method:

For polarimetry testing, nicotine solutions (25 mg/mL, or 25 mg/mL with 1x t-butylamine relative to mol nicotine) were diluted by a factor of 1.5, six times with ethanol to create the solutions for creation of a calibration curve. Puff Bar e-liquids with 5%, TFN and TDN (~60 mg/mL) were diluted in ethanol to ≤ 10 mg/mL nicotine. 1x t-butylamine was added to Puff Bar samples after analysis to ensure the nicotine was in the free-base (unprotonated) form, and the measurement was repeated. The optical rotations of the nicotine solutions were measured with an AUTOPOL® I (Rudolph Research Analytical, Fairfield, New jersey, U.S.A.) polarimeter at 589 nm and at 20 ± 0.1 °C in a 1.0 dm glass sample cell.

Gas Chromatography-Mass Spectrometry Method:

Evaluation of the nicotine stereoisomers was also performed using a Solid Phase Microextraction (SPME) GC-MS method. The GC-MS system was a Leco Pegasus 4D (Saint Joseph, MI). The secondary oven and modulator of the GC-MS were disabled. The GC column was connected directly from the GC main oven to the MS source. The 65 µm PDMS/DVB SPME fiber was from Supelco (Bellefonte, PA). For nicotine standards, 10 µL of nicotine was added to a 40 mL VOA vial and the SPME

fiber exposure time was 30 sec in the headspace. For e-liquid samples, 50 µL of e-liquid was added to a 40 mL VOA vial, then 0.4 mL of NH_3 gas was added. The SPME fiber exposure time was 3 min. The exposed SPME fiber was then inserted into the GC injector at 235 °C for desorption. The desorption time was 4.5 min and split ratio was 100 to 1. The separation was achieved using a Beta DEX 120 GC column (30 m, 0.25 mm i.d., 0.25 µm film thickness, Supleco). The GC oven temperature program was 80 °C for 2 min, 1.5 °C/min to 140 °C, then 15 °C/min to 200 °C, and held at 200 °C for 1 min. The MS source temperature was 225 °C. Electron energy was -70 eV. Detector voltage was 1600 V. The acquired mass range was 40 to 400 amu, with a data acquisition rate of 50 spectra/s. The retention times for (*S*)-(-)-nicotine and (*R*)-(+)-nicotine were 2369 and 2380 s, respectively.

Additional NMR data:

Table S1. Free-base nicotine fractions (α_{fb}), benzoic acid/nicotine ratios (by mols), and nicotine concentrations (mg/mL) for tobacco-free nicotine (TFN) and tobacco-derived nicotine (TDN) Puff Bar e-liquids labeled as containing 5% nicotine. Composition and free-base samples were prepared and analyzed by ^{1}H NMR spectroscopy following methods previously reported by Duell et al.[2,3] Monoprotonated and free-base nicotine standards were generated using Puff Bar e-liquid in flavor Cool Mint labeled as containing 5% nicotine (TDN); these references were used to calculate all α_{fb} values below.

Puff Bar Flavor (5% nicotine)	Free-base Nicotine Fraction (α_{fb})	Benzoic Acid/Nicotine Ratio (by mols)	Nicotine Concentration (mg/mL)
TFN Cool Mint	0.19	1.0	50
TDN Cool Mint	0.14	1.2	43
TFN Straw Watermelon	0.15	1.0	54
TDN Strawberry Watermelon	0.07	1.3	50
TDN Grape	0.18*	1.2	51
TDN Banana Ice	0.14	1.0	49

*Only one aromatic nicotine proton was used to calculate α_{fb} due to peak broadening

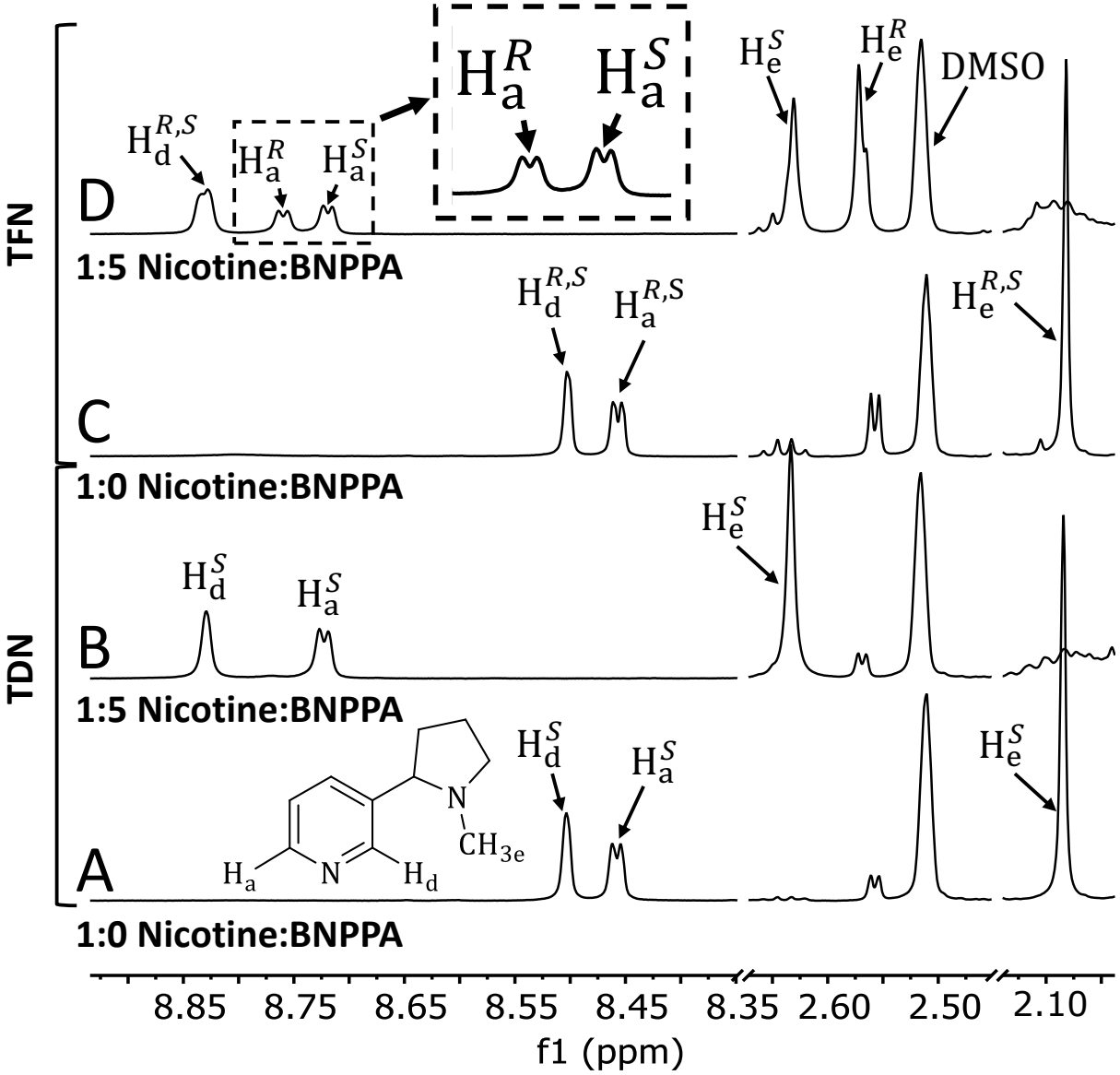

Figure S1. ^{1}H NMR spectra for Puff Bar e-liquid in flavor "Strawberry Watermelon"/"Straw Watermelon" without and with (*R*)-(−)-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate (BNPPA). Samples were prepared in DMSO-d_6. E-liquid presumed to be tobacco-derived nicotine (TDN) is shown in spectra A and B without and with BNPPA, respectively. E-liquid advertised as containing tobacco-free nicotine (TFN) is shown in spectra C and D without and with BNPPA, respectively. For TDN, no splitting of protons H_a or H_e is observed confirming that only one form of nicotine, (*S*)-(-)-nicotine, is present. For TFN, the addition of BNPPA results in distinct peaks appearing for the (*R*)-(+) and (*S*)-(-) forms of nicotine for protons H_a and H_e. H_a^R and H_a^S integrate ~1:1.2 ± 0.1, which represents molar equivalents.

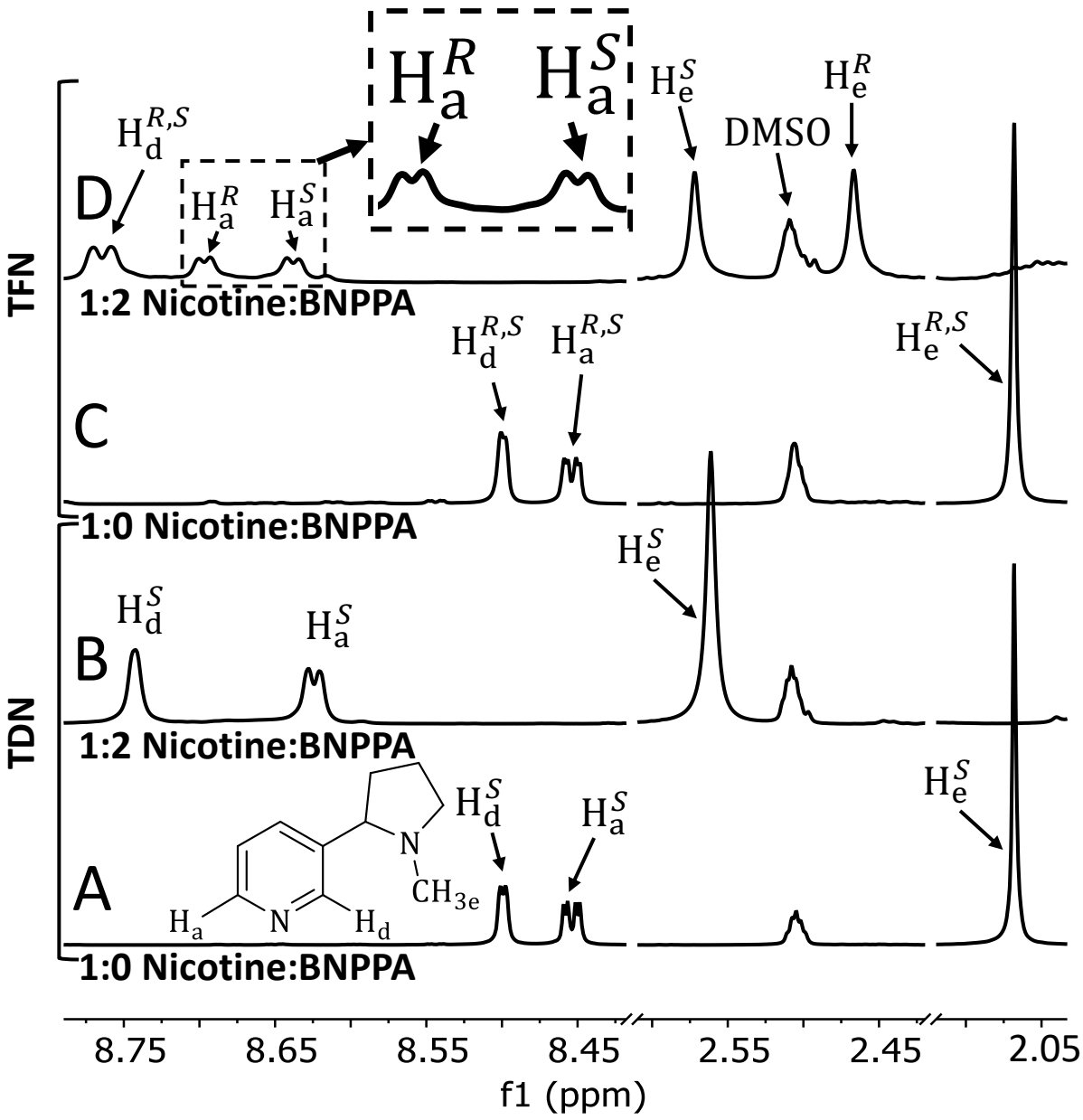

Figure S2. [1]H NMR spectra for pure nicotine standards ((*S*)-(-)-nicotine and (*R,S*)-(±)-nicotine) without and with (*R*)-(+)-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate (BNPPA). Samples were prepared in DMSO-d_6. (*S*)-(-)-nicotine is presumed to be tobacco-derived nicotine (TDN) and is shown in spectra A and B without and with BNPPA, respectively. Synthetic (*R,S*)-(±)-nicotine (TFN) is shown in spectra C and D without and with BNPPA, respectively. For TDN, no splitting of protons H_a or H_e is observed confirming that only one form of nicotine, (*S*)-(-)-nicotine, is present. For TFN, the addition of BNPPA results in distinct peaks appearing for the (*R*)-(+) and (*S*)-(-) forms of nicotine for protons H_a and H_e. H_a^R and H_a^S integrate ~1.0:1.0, which represents molar equivalents.

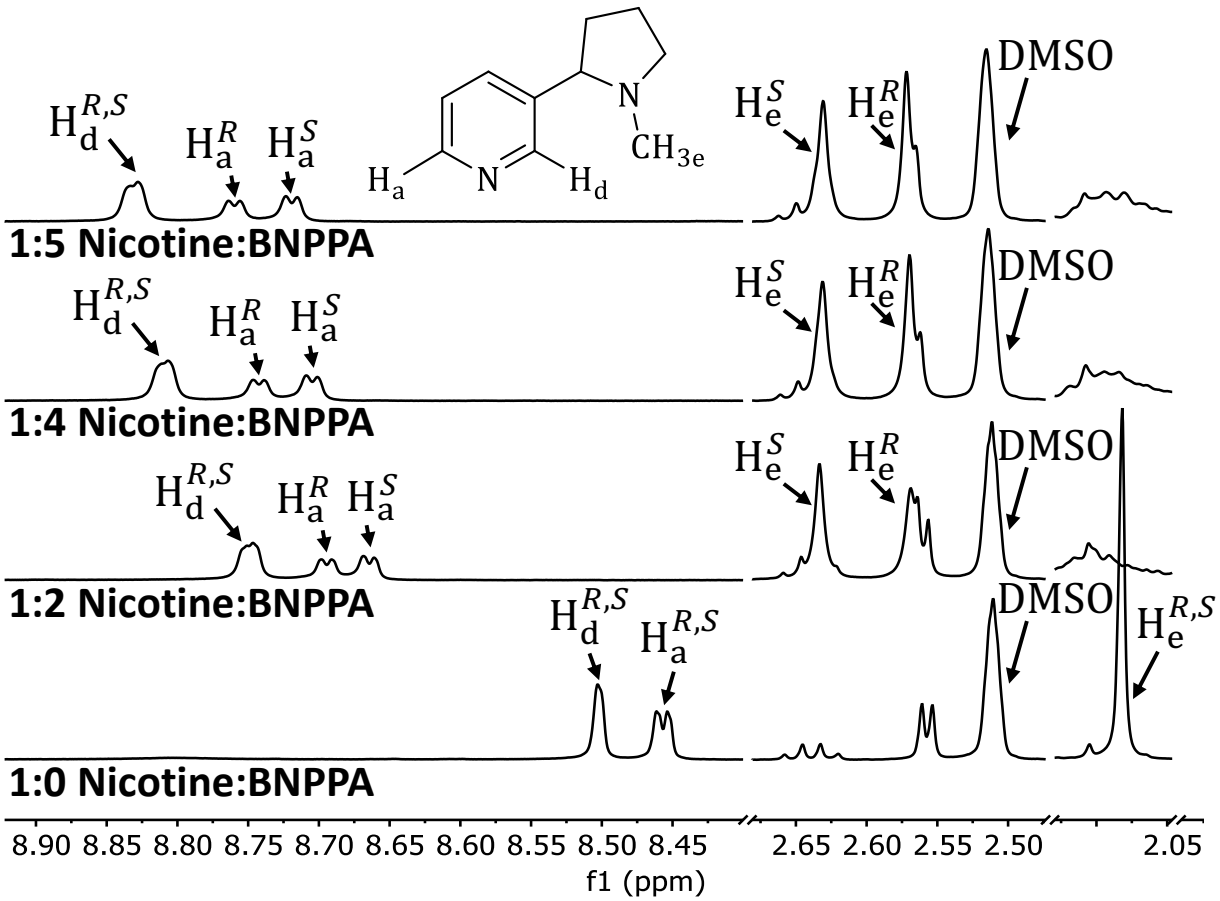

Figure S3. [1]H NMR spectra showing the addition of increasing amounts of (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (BNPPA) to Strawberry Watermelon Puff Bar e-liquid labeled as containing tobacco-free nicotine (TFN). Samples were prepared in DMSO-d_6. One set of nicotine peaks was observed without BNPPA, and as BNPPA is added to the mixture, nicotine protons H_a^R and H_a^S separate. Separation between nicotine protons H_e^R and H_e^S was also observed.

Additional information about the polarimeter method results:

Standard Curves

The TDN standards in ethanol ranged from 3.3 – 25.0 mg/mL, and had a negative linear slope at m = -0.109 (Figure S4). As the concentration of nicotine increased the optical rotation decreased linearly with an R^2 value of 0.99 (Figure S4). To ensure that the nicotine was completely in the free-base form, we created a curve with the same concentrations of nicotine in ethanol + 1x t-butylamine (Figure S4). The linear equations and R^2 values were nearly identical when comparing the nicotine standard curve with and without added t-butylamine. The linear equation generated from the curve with nicotine dissolved in ethanol was used to determine known concentrations of nicotine in the Puff Bar (5%, TDN) e-liquids.

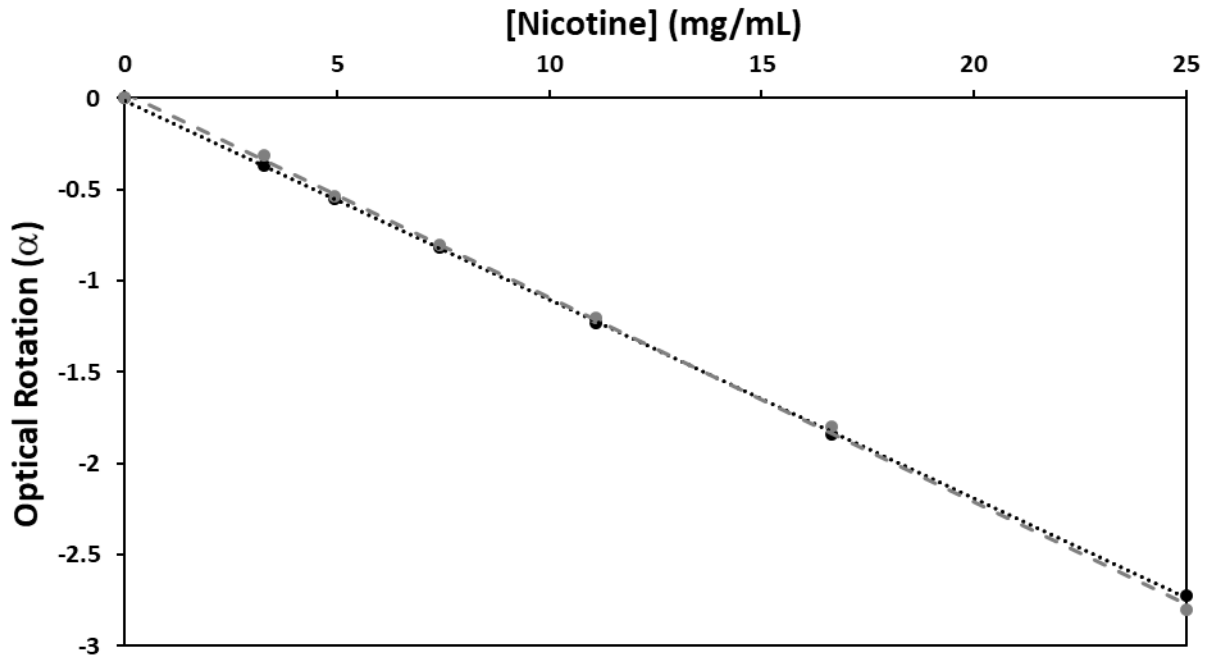

Figure S4. The standard curves for the optical rotation of nicotine in ethanol (black line, *y = -0.109x – 0.012*, *R^2 = 0.99*) and nicotine + 1x t-butylamine (relative to mols nicotine) in ethanol (grey line, *y = -0.112x +0.030*, *R^2 = 0.99*). The nicotine was likely in the free-base form in the solution without t-butylamine since the linear equations are nearly identical for both conditions.

Puff Bar E-liquids (5% TFN & TDN)

The average optical rotations (α) for the diluted Straw Watermelon (5%, TFN) was 0 with a specific rotation ($[\alpha]_D^{20}$) of 0 (Table S2). The 1 and 5 mg/mL TFN Straw Watermelon samples had an α of -0.10 and -0.44 with a $[\alpha]_D^{20}$ of -100.0 and -52.0, respectively (Table S2). Lastly, when 1x t-butylamine was added to the previous samples there was a decrease in the α values to -0.12 and -0.44 with a $[\alpha]_D^{20}$ of -120.0 and -88.0 (Table S2).

The α for the Banana Ice (5%, TFN) samples at 1 and 5 mg/mL were -0.01 and -0.44 with $[\alpha]_D^{20}$ of -10.0 and -12.0, respectively (Table S2). The Banana Ice (5%, TDN) samples at concentrations of 1.1, 3.4, and 10 mg/mL had α of -0.10, -0.27, and -0.43 with $[\alpha]_D^{20}$ of -90.9, -80.1, and -43.0, respectively (Table S2). When 1x t-butylamine was added to the previous samples the α values decreased to -0.14, -0.42, and -1.13 with $[\alpha]_D^{20}$ of -90.9, -80.1, and -43.0 for the previous concentrations (Table S2).

The Cool Mint (5%, TFN) samples at 1.4 and 5.0 mg/mL had an α of -0.02 and -0.07 with $[\alpha]_D^{20}$ of -14.3 and -14.0, respectively (Table S2). However, the Cool Mint (5%, TDN) samples at 1.1, 3.4, and 10.0 mg/mL had an α of -0.12, -0.3, and -0.58 with $[\alpha]_D^{20}$ or -109.1, -89.0, and -58.0, respectively (Table S2). When 1x t-butylamine was added to the previous Cool Mint (5%, TDN) samples, α decreased along with the $[\alpha]_D^{20}$, similarly to the Straw Watermelon (5%, TDN) and Banana Ice (5%, TDN) samples.

The linear equation in Figure S4 was then used to determine the nicotine concentration in the Straw Watermelon (5%, TDN), Banana Ice (5%, TDN), and Cool Mint (5%, TDN) e-liquids from their measured α. The calculated nicotine concentrations were about half the actual nicotine concentrations (Table S2). The average percent errors were 36.7%, 38.9%, and 25.9% for Straw Watermelon, Banana Ice, and Cool Mint, respectively. However, when using the α values from the Puff Bar (5%, TDN) e-liquids + 1x t-butylamine, the calculated nicotine concentrations were more accurate than using the α values from the e-liquids without t-butylamine (Table S2). The average percent errors decreased to 10.9%, 7.5%, and 14.5% for Straw Watermelon, Banana Ice, and Cool Mint samples, respectively.

The TFN e-liquids likely contain a racemic mixture of nicotine because the synthesis would be cheaper and less selective than synthesizing either (*R*)-(+)- or (*S*)-(-)-nicotine. The samples with 5%, TFN e-liquid rotated the plane of light slightly, possibly from some chiral flavorant(s), or contamination with TDN. The polarimetry data supports the conclusions from the ^{1}H NMR data (i.e. that the e-liquids contain TFN).

(*S*)-(-)-nicotine, or TDN, is levorotary and rotates the plane of light counterclockwise, but is dextrorotary in the protonated form, similar to other alkaloids.[4] The α values from the Puff Bar (5%, TDN) e-liquids were from nicotine salts, and contained acid(s) that could protonate the nicotine to make the aerosol more palatable and inhalable.[3] Nicotine was likely in the protonated form which would give reduced α values from the samples. However, when t-butylamine was added to the e-liquids the nicotine was deprotonated (the free-base form), and the measured α values were more accurate compared to the α values from e-liquids without t-butylamine.

The polarimetry method works well for mixtures containing only nicotine but there are some limitations of this method when applied to e-liquids. E-liquids commonly contain many compounds including flavorants and often contain acids used to alter the protonation state of nicotine. If these other compounds are chiral, they can alter the specific rotation and result in inaccuracies in determining the ratio of (*S*)-(-)- to (*R*)-(+)-nicotine. In the presence of other chiral compounds, we therefore recommend using another technique described herein such as NMR spectroscopy or GC-MS.

Table S2. The optical rotations (α) for various concentrations of commercial e-liquids with tobacco-free nicotine (TFN), tobacco-derived nicotine (TDN), and TDN + t-butylamine. The specific rotations ($[\alpha]_D^{20}$) were calculated using Biot's law ($[\alpha]_D^{20} = \alpha/(lc)$), and nicotine concentrations (mg/mL) were calculated using the linear equation from Figure S4 (*y = -0.109x – 0.012*). Optical rotation measurements were collected at 20.0 °C, at a wavelength of 589 nm, and using a 1.0 dm sample tube.

		Undiluted Nicotine (mg/mL)	Diluted Nicotine (mg/mL)	Optical Rotation (α)	Specific Rotation ($[\alpha]_D^{20}$)	Calculated Nicotine (mg/mL), Error (%)
Straw Watermelon*	5%, TFN	52.2	1.0	0.00	0.0	N/A
			5.0	-0.01	0.0	N/A
	5%, TFN + t-butylamine	52.2	1.0	-0.01	-10.0	N/A
			5.0	-0.04	-8.0	N/A
	5%, TDN	48.6	1.0	-0.10	-100.0	0.8, 18.9
			5.0	-0.26	-52.0	2.3, 54.4
	5%, TDN + t-butylamine	48.6	1.0	-0.12	-120.0	1.0, 0.6
			5.0	-0.44	-88.0	3.9, 21.3
Banana Ice	5%, TFN	50.8	1.0	-0.01	-10.0	N/A
			5.0	-0.06	-12.0	N/A
	5%, TFN + t-butylamine	50.8	1.0	0.00	0.0	N/A
			5.0	-0.02	-4.0	N/A
	5%, TDN	57.3	1.1	-0.10	-90.9	0.8, 26.3
			3.4	-0.27	-80.1	2.4, 28.8
			10.0	-0.43	-43.0	3.8, 61.6
	5%, TDN + t-butylamine	57.3	1.1	-0.14	-127.3	1.2, 7.1
			3.4	-0.42	-124.6	3.7, 12.6
			10.0	-1.13	-113.0	10.3, 2.7
Cool Mint	5%, TFN	49.6	1.4	-0.02	-14.3	N/A
			5.0	-0.07	-14.0	N/A
	5%, TFN + t-butylamine	49.6	1.4	-0.01	-7.1	N/A
			5.0	-0.03	-6.0	N/A
	5%, TDN	48.5	1.1	-0.12	-109.1	1.0, 9.6
			3.4	-0.30	-89.0	2.6, 20.5
			10.0	-0.58	-58.0	5.2, 47.8
	5%, TDN + t-butylamine	48.5	1.1	-0.15	-136.4	1.3, 15.5
			3.4	-0.42	-124.6	3.7, 12.6
			10.0	-1.27	-127.0	11.6, 15.5

*Depending on the package, Puff Bar e-liquids were labeled as both "Strawberry Watermelon" and "Straw Watermelon"

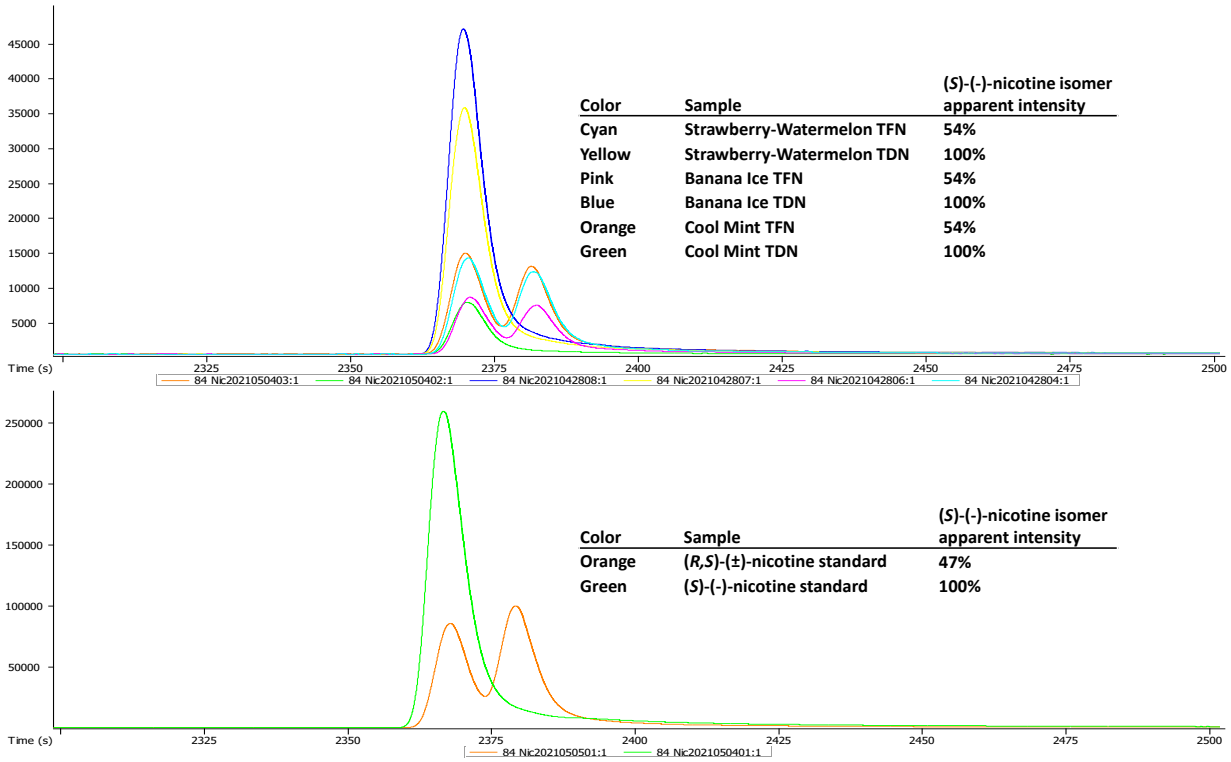

Figure S5. Gas chromatography-mass spectrometry (GC-MS) data for Puff Bar e-liquids containing tobacco-free nicotine (TFN) and tobacco-derived nicotine (TDN) with GC-MS-determined (*S*)-(-)-nicotine isomer percentage relative to (*R*)-(+)-nicotine. The (*S*)-(–)-nicotine isomer apparent intensity values are not corrected for lineshape or overlap, but demonstrate that the amounts of the (*S*)-isomer are larger from the TFN commercial samples (top panel traces) than from the (*R,S*)-(±)-nicotine standard (bottom panel red trace), which is synthetic and therefore a 1:1 (50%:50%) mixture of enantiomers.

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(2) Duell, A. K.; Pankow, J. F.; Peyton, D. H. Free-Base Nicotine Determination in Electronic Cigarette Liquids by ^{1}H NMR Spectroscopy. *Chem Res Toxicol* **2018**, 31 (6), 431.

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