

Analysis of (S)- and (R)-nicotine in various nicotine samples and in e-liquids

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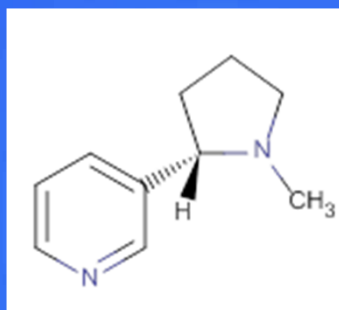
**RAI Innovation Co.

Background

- Nicotine is a common constituent of the liquids used in electronic smoking devices (e-liquids).
- The purpose of this work was to:
 - Measure the level of nicotine in 38 commercially available e-liquids used in commercial electronic smoking devices.
 - Determine if the nicotine in these e-liquids is predominantly (S)-nicotine (obtained from tobacco).
 - Measure the ratio of (S)-nicotine and (R)-nicotine in four different nicotine samples of USP purity (United States Pharmacopeia).

Background (cont.)

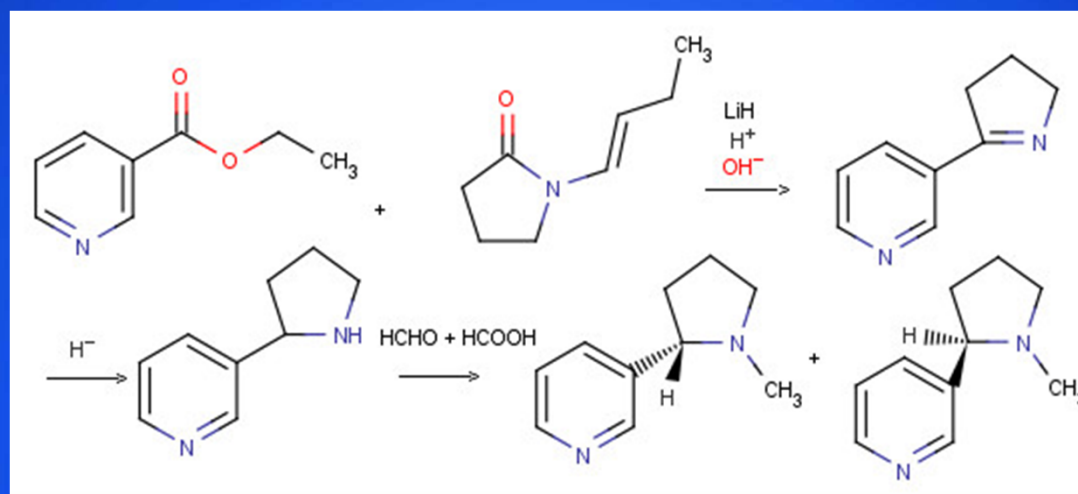
- Nicotine can be obtained from tobacco (*Nicotiana tabacum*, *Nicotiana rustica*) by extraction followed by distillation.
- Nicotine from natural sources (tobacco) is mainly (S)-nicotine:



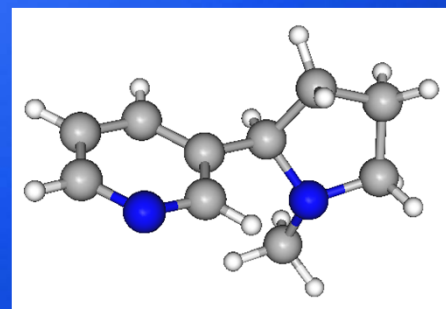
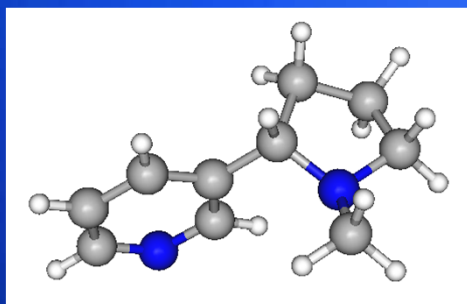
- Synthetic nicotine is also available on the market (e.g. produced by Next Generation Labs LLC).
- Synthetic nicotine can be obtained by different procedures and generates a racemic mixture of (S)- and (R)-nicotine.
- There are certain difficult additional procedures for separating the enantiomers for preparative purposes.

Background (cont.)

- A typical scheme for nicotine synthesis is the following:



- The spatial arrangements of the atoms in (S)-nicotine and in (R)-nicotine are shown below:



Previous publications related to the analysis of (R)- and (S)-nicotine

- D. W. Armstrong, X. Wang, A. Ercal, Enantiomeric composition of nicotine in smokeless tobacco, medicinal products, and commercial reagents, *Chirality*, 10 (1998) 587-591
- G. Hellinghausen, J. T. Lee, C. A. Weatherly, D. A. Lopez, D. W. Armstrong, Evaluation of nicotine in tobacco-free-nicotine commercial products, *Drug Testing and Analysis*, (www.drugtestinganalysis.com) DOI 10.1002/dta.2145
- D. Pogocki, T. Ruman, Magd. Danilczuk, Mar. Danilczuk, .M Celuch, E. Wałajtys-Rode, Application of nicotine enantiomers, derivatives and analogues in therapy of neurodegenerative disorders, *Europ. J. Pharmacology*, 563 (2007) 18-39.

Instrumentation

- Three different instruments were used in this work.
 - A GC-FID 7890A instrument (Agilent Technologies Inc.) equipped with a DB-Waxetr column, 30 m x 0.32 mm i.d., with 0.5 μm film (Agilent).
 - A GC-MS 7890B-5977B with High Efficiency Source (Agilent) equipped with two columns RT-GammaDEXsa 30 m x 0.25 mm i.d., with 0.25 μm film (Restek) in series.
 - An Agilent 1200 HPLC binary system that consisted of pump, autosampler with cooling capability, and a column thermostatted compartment. The separation was achieved on a Chiracel OJ-3 column 250 mm x 4.6 mm with 3 μm size particle (Daicel Corp.).

GC-FID separation and analysis

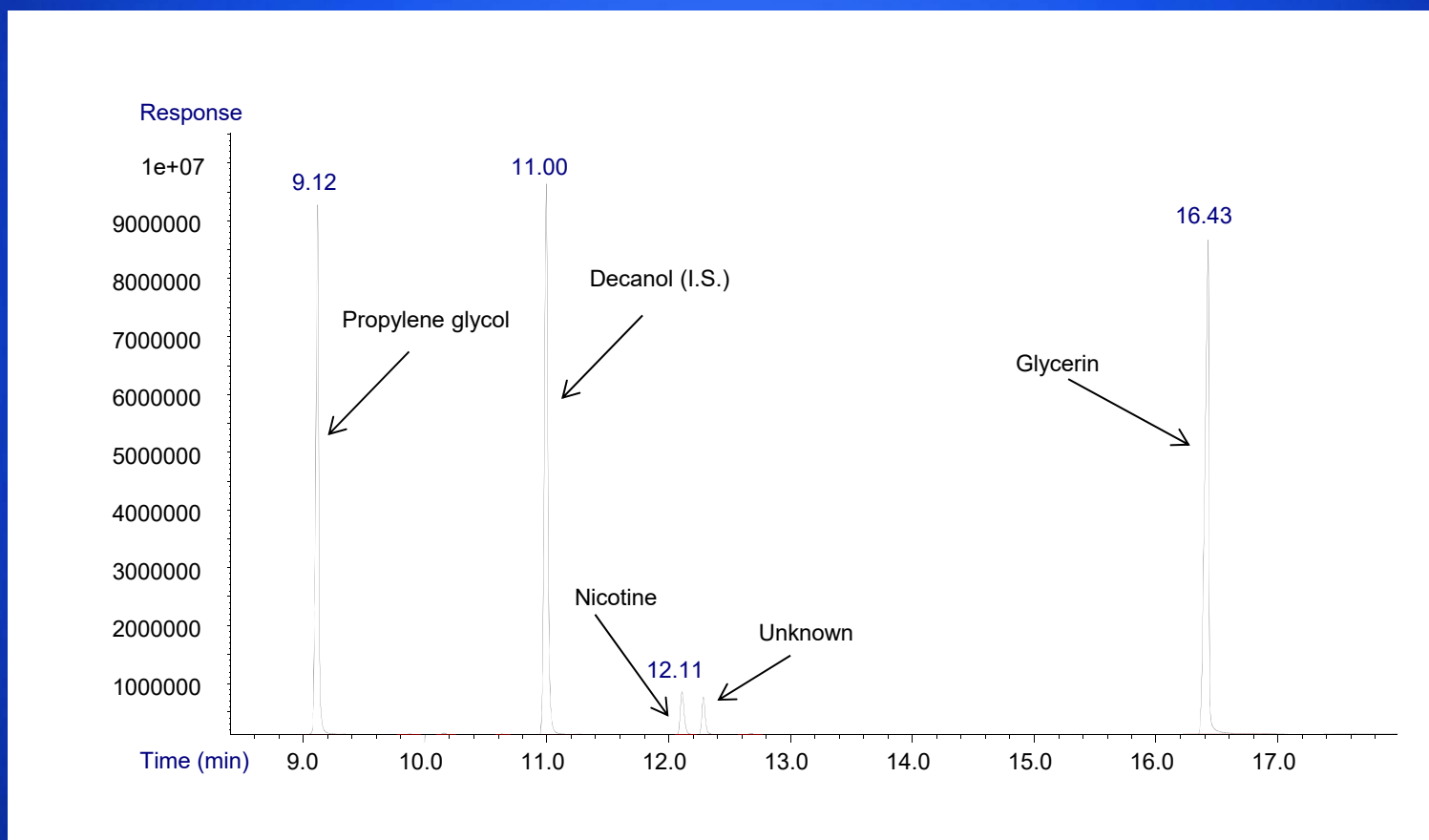
- This analysis was used to analyze total nicotine, as well as propylene glycol, glycerin, and menthol in e-liquids. The GC-FID conditions are described in the following table (DB-Waxetr column).

| Parameter | Description | Parameter | Description |
|--------------------------|-------------|---------------------------|---------------|
| Carrier gas | Hydrogen | Flow mode | Constant flow |
| Initial oven temperature | 50°C | Flow rate | 1.0 mL/min |
| Initial time | 1.0 minutes | Nominal initial pressure | 12.0 psi |
| Oven ramp rate | 10°C/min | Average velocity | 25 m/s |
| Oven temperature | 240°C | GC outlet | FID |
| Final time | 10 min | Temperature | 250 °C |
| Total run time | 30.0 min | Outlet pressure | Ambient |
| Inlet temperature | 290°C | Hydrogen flow in detector | 30 mL/min |
| Inlet mode | Split | Air flow | 400 mL/min |
| Split ratio | 50:1 | Make up gas | Nitrogen |
| Injection volume | 1.0 mL | Make up gas flow | 40 mL/min |

- The quantitation used calibration curves with $Y = \text{mg/mL}$ and $X = \text{normalized area by the area of I.S. (1 mg/mL decanol)}$.

| No. | Compound | Equation | R ² |
|-----|----------|-------------------|----------------|
| 1 | PG | $Y = 2.1816e+3 X$ | 0.9976 |
| 2 | Menthol | $Y = 9.8532e+2 X$ | 0.9976 |
| 3 | Nicotine | $Y = 1.0377e+3 X$ | 0.9976 |
| 4 | Glycerin | $Y = 2.9661e+3 X$ | 0.9993 |

GC-FID chromatogram of an e-liquid sample

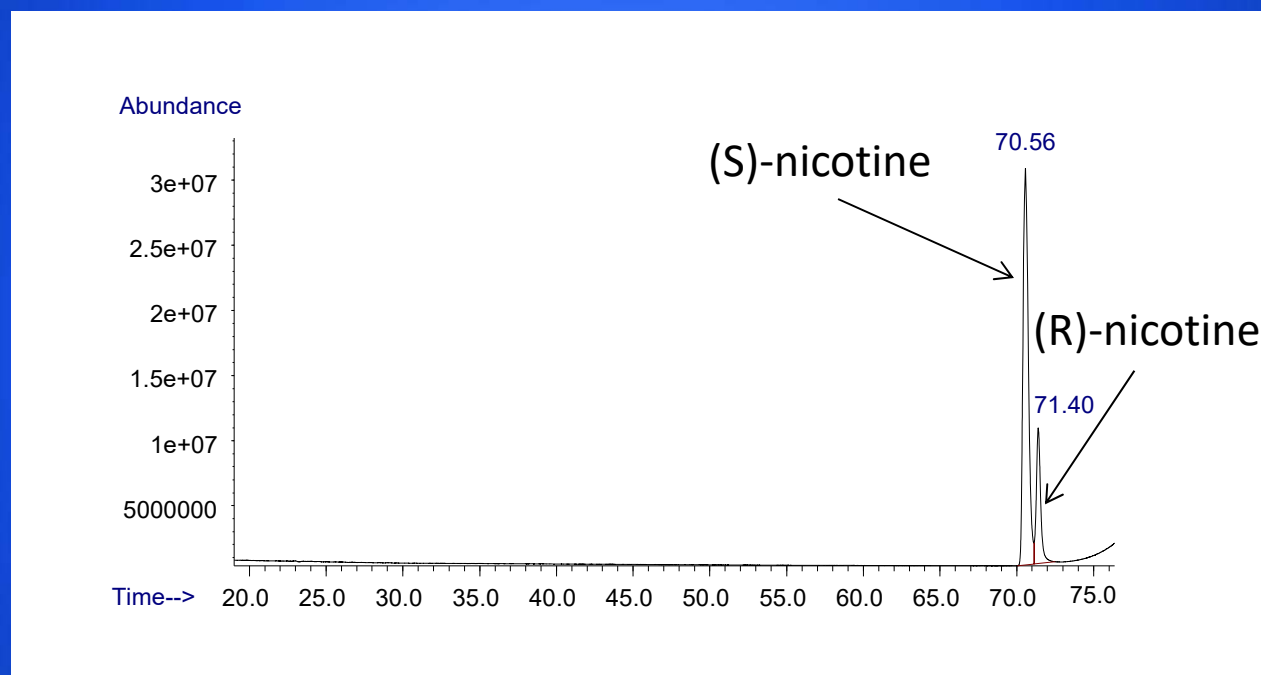


GC-MS separation and analysis

- This analysis was used to analyze (S)- and (R)-nicotine in e-liquids and USP nicotine samples. The GC-MS conditions are described in the following table (two columns RT-GammaDEXsa in series).

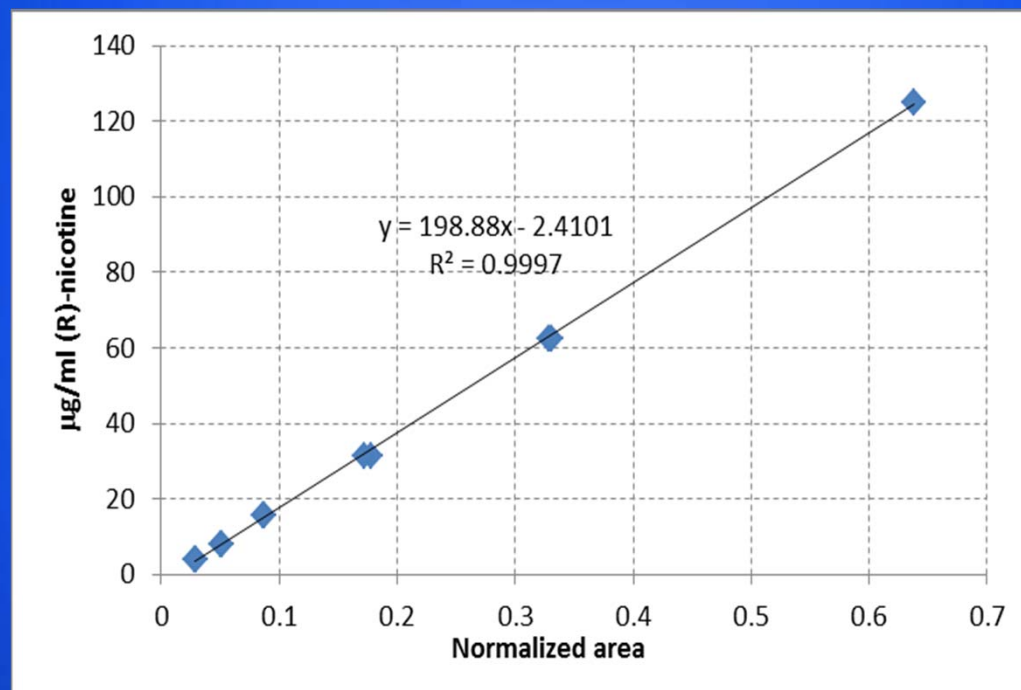
| Parameter | Description | Parameter | Description |
|--------------------------|-------------|--------------------------|---------------|
| Carrier gas | Helium | Split flow | 20.531 mL/min |
| Initial oven temperature | 37°C | Injection volume | 1.0 mL |
| Initial time | 2.0 min | Flow mode | Constant flow |
| Oven ramp rate | 35°C/min | Flow rate | 1.0265 mL/min |
| Oven temperature | 125°C | Nominal initial pressure | 19.553 psi |
| Hold time | 65.986 min | Average velocity | 20.756 cm/s |
| Oven ramp rate | 10°C/min | GC outlet | MSD |
| Oven temperature | 230°C | Aux. temperature | 250 °C |
| Final time | 4 min | Mass range (scan) | 33 - 250 amu |
| Total run time | 85.0 min | Solvent delay | 7 min |
| Inlet temperature | 250°C | Gain factor | 4.0 |
| Inlet mode | Split | MS source temperature | 230°C |
| Split ratio | 20:1 | MS quad temperature | 150°C |

GC-MS chromatogram of a sample containing 250 $\mu\text{g}/\text{mL}$ (S)-nicotine and 62.5 $\mu\text{g}/\text{mL}$ (R)-nicotine



(S)-nicotine retention time 70.56 min,
(R)-nicotine retention time 71.40 min.

Calibration of (R)-nicotine versus peak area ratio of (R)-nicotine/(S)-nicotine at (S)-nicotine constant level of 250 $\mu\text{g}/\text{mL}$



LC-UV analysis with chiral separation

- This procedure has been utilized only for the evaluation of the ratio (S)-nicotine/(R)-nicotine in nicotine samples.
- Solutions containing 500 $\mu\text{g}/\text{mL}$ nicotine in methanol were prepared.
- The separation was performed on the Chiracel OJ-3 column 250 mm x 4.6 mm with 3 μm size particle.
- The mobile phase consisted of 85% hexane and 15 % ethanol with the addition of 7.5 mL trifluoroacetic acid and 7.5 mL triethylamine for 1 L mobile phase.
- The flow rate in the column was 1 mL/min, the injection volume 10 μL , and the detection was performed at 254 nm.
- The procedure was a modification of “D. W. Armstrong, X. Wang, A. Ercal, Enantiomeric composition of nicotine in smokeless tobacco, medicinal products, and commercial reagents, *Chirality*, 10 (1998) 587-591.”

Results for nicotine (mg/g), propylene glycol %, glycerin %, and menthol % in e-liquids

| No. | Brand | Flavor | Nicotine reported mg/mL | | Nicotine mg/g | Glycerin % (w/w) | PG % (w/w) | Menthol % (w/w) |
|-----|-------------------|------------------|-------------------------|------|---------------|------------------|------------|-----------------|
| 1 | Artist Collection | Para Mour | 0 | avg | - | 54.25 | 38.58 | - |
| | | | | %rsd | - | 1.61 | 1.02 | - |
| 2 | Artist Collection | Sambra Sun | 3 | avg | 2.49 | 64.80 | 31.37 | - |
| | | | | %rsd | 0.18 | 0.80 | 0.60 | - |
| 3 | X2O | Tobacco Menthol | 18 | avg | 17.22 | 57.62 | 24.18 | 4.12 |
| | | | | %rsd | 0.09 | 0.18 | 1.52 | 0.40 |
| 4 | X2O | Natural Tobacco | 18 | avg | 17.77 | 60.93 | 35.39 | - |
| | | | | %rsd | 0.01 | 0.60 | 0.68 | - |
| 5 | Haus | Washington Red | - | avg | 22.33 | 27.79 | 55.43 | - |
| | | | | %rsd | 0.15 | 0.05 | 0.36 | - |
| 6 | Haus | Java | - | avg | 22.87 | 28.49 | 62.02 | - |
| | | | | %rsd | 0.13 | 0.75 | 0.38 | - |
| 7 | Haus | Ocean Mist | - | avg | 22.55 | 23.20 | 59.56 | - |
| | | | | %rsd | 0.01 | 0.69 | 1.14 | - |
| 8 | Haus | Berry | - | avg | 22.06 | 25.33 | 59.93 | - |
| | | | | %rsd | 0.08 | 0.86 | 0.10 | - |
| 9 | Haus | Dark Ice Menthol | 4 | avg | 3.23 | 60.82 | 38.90 | 0.05 |
| | | | | %rsd | 0.15 | 1.54 | 0.40 | 0.13 |
| 10 | Taffy Man | TR4 BLU | 0 | avg | 0.17 | 77.93 | 18.06 | - |
| | | | | %rsd | - | 0.52 | 0.81 | - |
| 11 | Five Pawns | Mixology Edition | - | avg | 2.45 | 53.97 | 36.63 | - |
| | | | | %rsd | 0.23 | 0.87 | 0.03 | - |
| 12 | Black Note | Prelude | 0.6% | avg | 5.51 | 46.14 | 49.21 | - |
| | | | | %rsd | 0.14 | 0.79 | - | - |
| 13 | Oakwood Vapor | Eden | 12 | avg | 9.52 | 71.18 | 21.81 | - |
| | | | | %rsd | 0.08 | 1.45 | 0.89 | - |

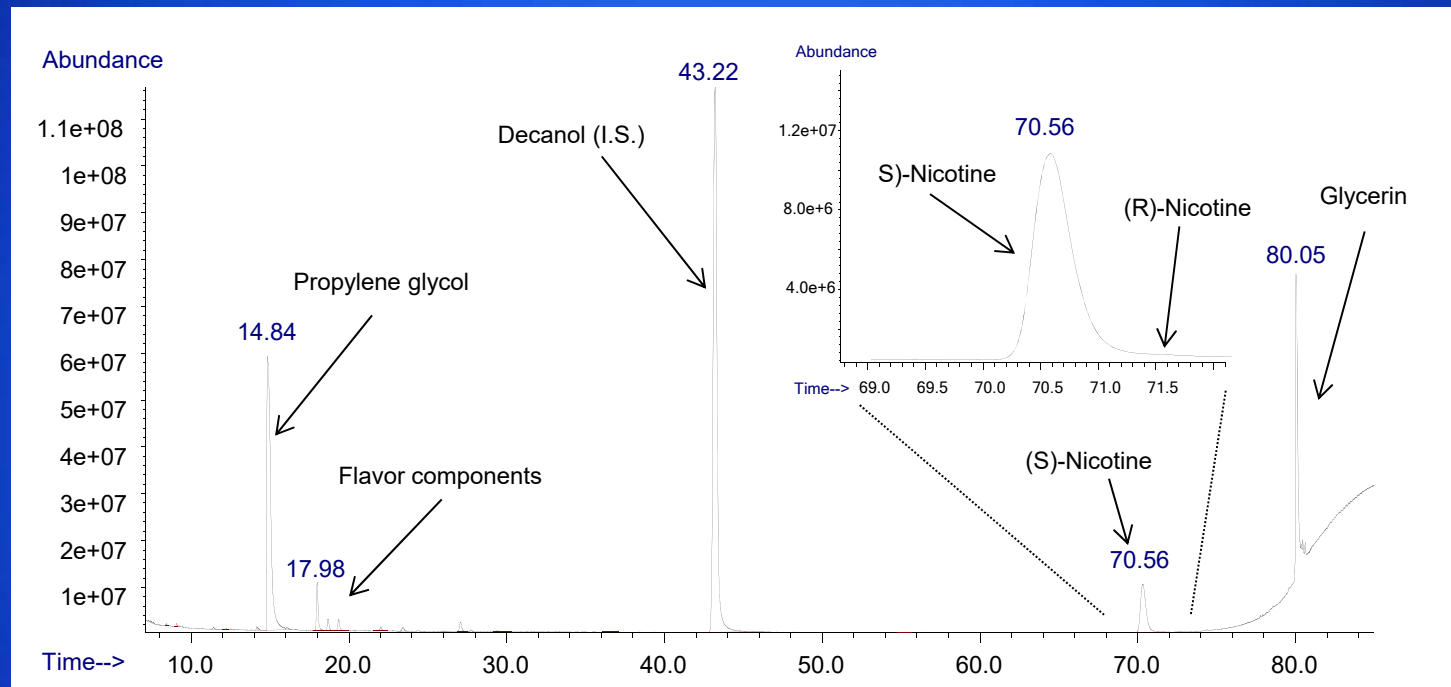
Results for nicotine (mg/g), propylene glycol % glycerin %, and menthol % (Cont.)

| No. | Brand | Flavor | Nicotine reported mg/mL | | Nicotine mg/g | Glycerin % (w/w) | PG % (w/w) | Menthol % (w/w) |
|-----|--------------------|-------------------|-------------------------|------|---------------|------------------|------------|-----------------|
| 14 | VaporFi (1) | ? | - | avg | 10.91 | 52.79 | 41.88 | 0.37 |
| | | | | %rsd | 0.67 | 0.70 | 0.64 | 0.15 |
| 15 | VaporFi (2) | ? | - | avg | 11.14 | 53.74 | 41.74 | 0.34 |
| | | | | %rsd | 0.05 | 0.33 | 0.15 | 0.30 |
| 16 | Charlie Chalk Dust | CA X OH | 6 | avg | 3.04 | 79.74 | 16.89 | - |
| | | | | %rsd | 0.13 | 0.56 | 0.60 | - |
| 17 | Series 8 | Strawnilla | 0 | avg | - | 48.40 | 43.65 | - |
| | | | | %rsd | - | 0.17 | 0.67 | - |
| 18 | Dewwy Bobba | | 6 | avg | 4.60 | 72.24 | 20.72 | - |
| | | | | %rsd | 0.03 | 1.91 | 0.13 | - |
| 19 | Henley | Brighton Peach | 3 | avg | 1.86 | 57.14 | 36.46 | - |
| | | | | %rsd | - | 1.24 | 0.65 | - |
| 20 | MG Consortium | Mindfold | 12 | avg | 7.97 | 66.20 | 24.64 | - |
| | | | | %rsd | 0.90 | 1.85 | 0.41 | - |
| 21 | Vype | Aniseed | 12 | avg | 10.83 | 50.78 | 30.88 | - |
| | | | | %rsd | 0.06 | 3.47 | 1.00 | - |
| 22 | Bad Drip | Bad Blood | 3 | avg | 2.24 | 69.47 | 20.69 | - |
| | | | | %rsd | - | 1.38 | 0.96 | - |
| 23 | BRV | Colonel's Custard | 0.3% | avg | 2.43 | 49.83 | 42.07 | - |
| | | | | %rsd | 0.17 | 0.45 | - | - |
| 24 | Cuttwood | Boss Reserve | 0.3% | avg | 2.11 | 65.14 | 24.27 | - |
| | | | | %rsd | - | - | 0.32 | - |
| 25 | Ludicrous Speed | The Schwartz | 12 | avg | 7.54 | 51.82 | 23.00 | - |
| | | | | %rsd | 0.14 | 3.14 | 0.80 | - |
| 26 | OKVMI | Haute Mocha | 12 | avg | 5.64 | 48.09 | 23.42 | - |
| | | | | %rsd | 0.24 | 2.85 | 0.19 | - |
| 27 | Clancy's | Riptide | 12 | avg | 9.28 | 69.97 | 21.96 | - |
| | | | | %rsd | 0.39 | 0.34 | 0.76 | - |

Results for nicotine (mg/g), propylene glycol % glycerin %, and menthol % in e-liquids

| No. | Brand | Flavor | Nicotine reported mg/mL | | Nicotine mg/g | Glycerin % (w/w) | PG % (w/w) | Menthol % (w/w) |
|-----|----------------------|-------------------|-------------------------|------|---------------|------------------|------------|-----------------|
| 28 | AlfaLiquid | Noble Leaf | 3 | avg | 2.56 | 49.80 | 47.54 | - |
| | | | | %rsd | 0.52 | 2.38 | 0.34 | - |
| 29 | Virgin Vapor | Best Damn Tobacco | 12 | avg | 11.76 | 86.90 | - | - |
| | | | | %rsd | 0.11 | 0.33 | - | - |
| 30 | Cosmic Fog | The Shocker | 3 | avg | 2.25 | 73.55 | 15.50 | - |
| | | | | %rsd | 0.13 | 0.86 | 0.32 | - |
| 31 | CRFT | Gravel Pit | 6 | avg | 3.22 | 53.10 | 40.18 | - |
| | | | | %rsd | 0.17 | 1.73 | 1.32 | - |
| 32 | Kings Crest | Duchess | 12 | avg | 8.97 | 77.00 | 11.30 | - |
| | | | | %rsd | 0.55 | 1.56 | 1.13 | - |
| 33 | Kilo | Dewberry Cream | 12 | avg | 8.57 | 61.72 | 29.53 | - |
| | | | | %rsd | 0.13 | 1.21 | 0.17 | - |
| 34 | Noca Elixirs | Bezoar | 3 | avg | 2.28 | 78.72 | 13.49 | - |
| | | | | %rsd | 0.14 | 0.16 | 0.65 | - |
| 35 | Classified e-Liquids | Crisp | 6 | avg | 4.31 | 62.55 | 29.50 | - |
| | | | | %rsd | 0.14 | 0.01 | 0.28 | - |
| 36 | ZVR | Lemon Cheesecake | 6 | avg | 5.25 | 54.70 | 32.42 | - |
| | | | | %rsd | 0.34 | 1.23 | 0.80 | - |
| 37 | Frequency Liquid | Boom Box | 3 | avg | 2.35 | 73.27 | 21.75 | - |
| | | | | %rsd | 0.07 | 0.40 | 0.45 | - |
| 38 | A031 | ? | 18 | avg | 15.26 | 57.44 | 32.34 | - |
| | | | | %rsd | 0.34 | 1.32 | 0.84 | - |

Chiral GC-MS chromatogram for sample Haus - Washington Red indicating natural nicotine



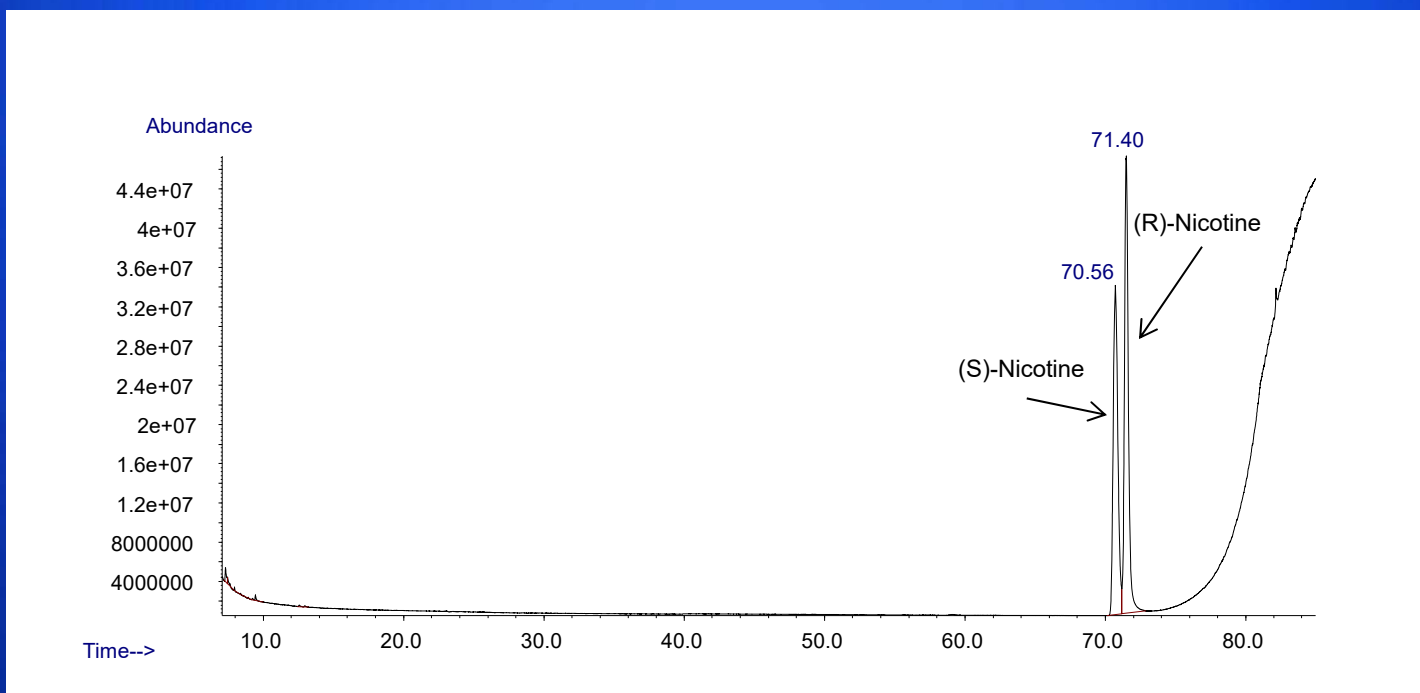
The time range from 69 min to 72 min is expanded to show the area where (R)-nicotine elutes.

All e-liquids in the evaluated 38 samples contained predominantly (S)-nicotine indicating tobacco as a natural source of nicotine.

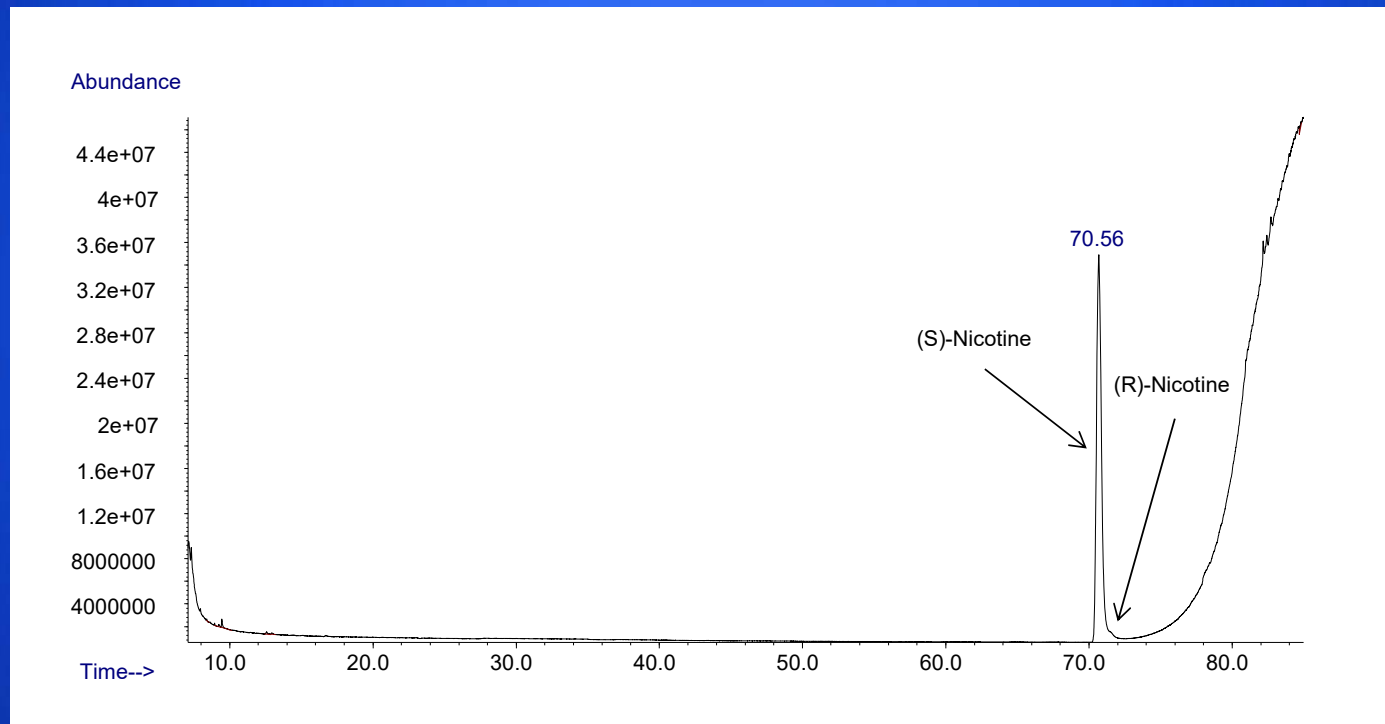
List of nicotine samples analyzed in this study

| Sample No. | Supplier | Source |
|--------------------|--------------------------|--------------------|
| Spl. 1 (Natural) | - | Philippine Tobacco |
| Spl. 2 (Natural) | - | India Tobacco |
| Spl. 3 (Natural) | - | India Tobacco |
| Spl. 4 (Synthetic) | Next Generation Labs LLC | Synthetic |

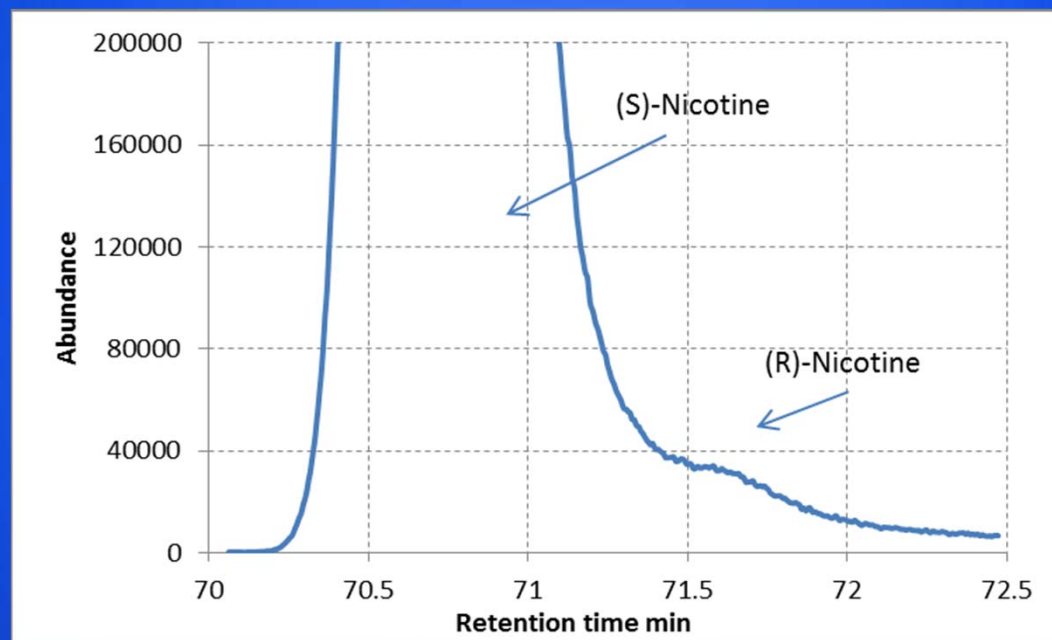
GC-MS chromatogram of a solution containing 250 $\mu\text{g}/\text{mL}$ of Spl. 4 (Synthetic) in methanol .



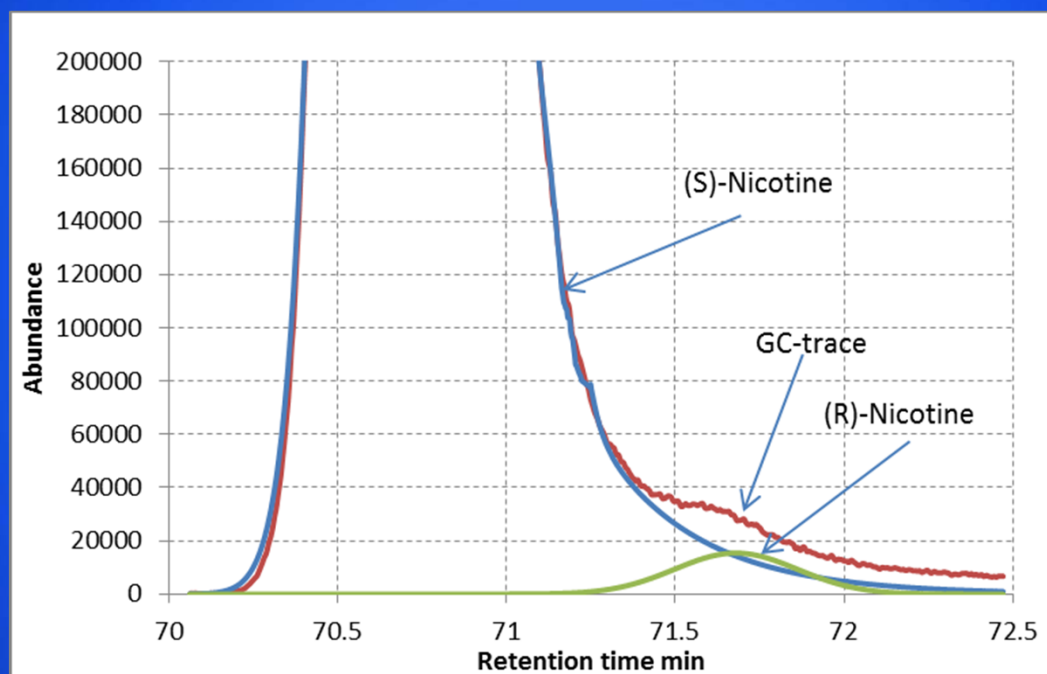
GC-MS chromatogram of a solution containing 500 $\mu\text{g}/\text{mL}$ of Spl. 2 (Natural) in methanol .



Expanded profile of the nicotine peak in Spl. 2 (Natural) with the separation of (S)-nicotine and (R)-nicotine.

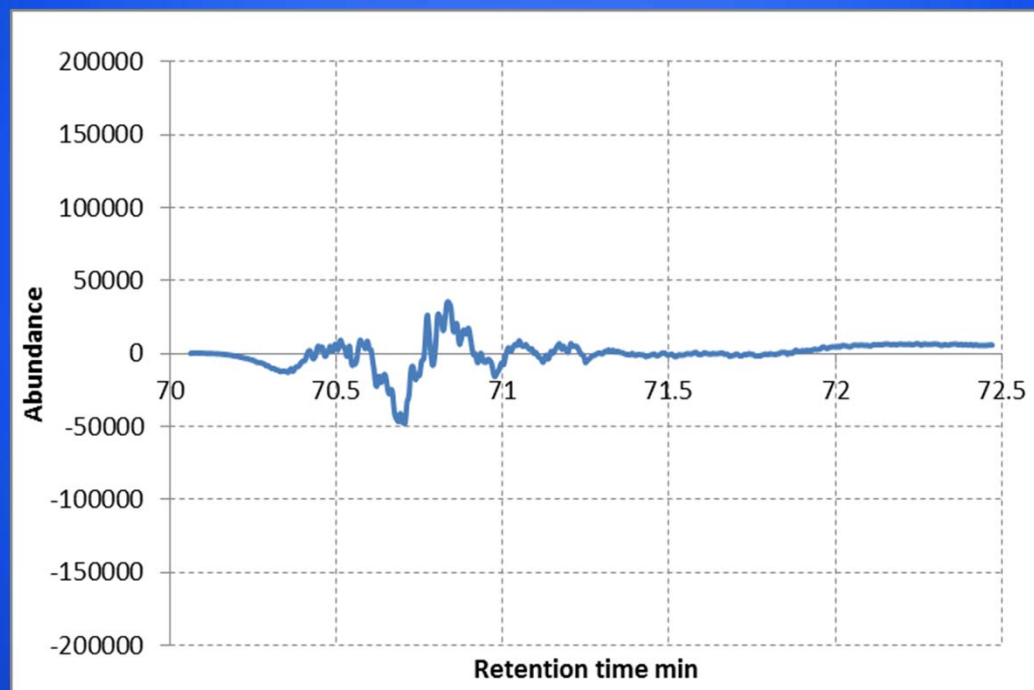


Deconvolution profile using a combination of Gaussian curves and exponential decay (for (S)-nicotine) for one Gaussian for (R)-nicotine from Spl. 2 (Natural).

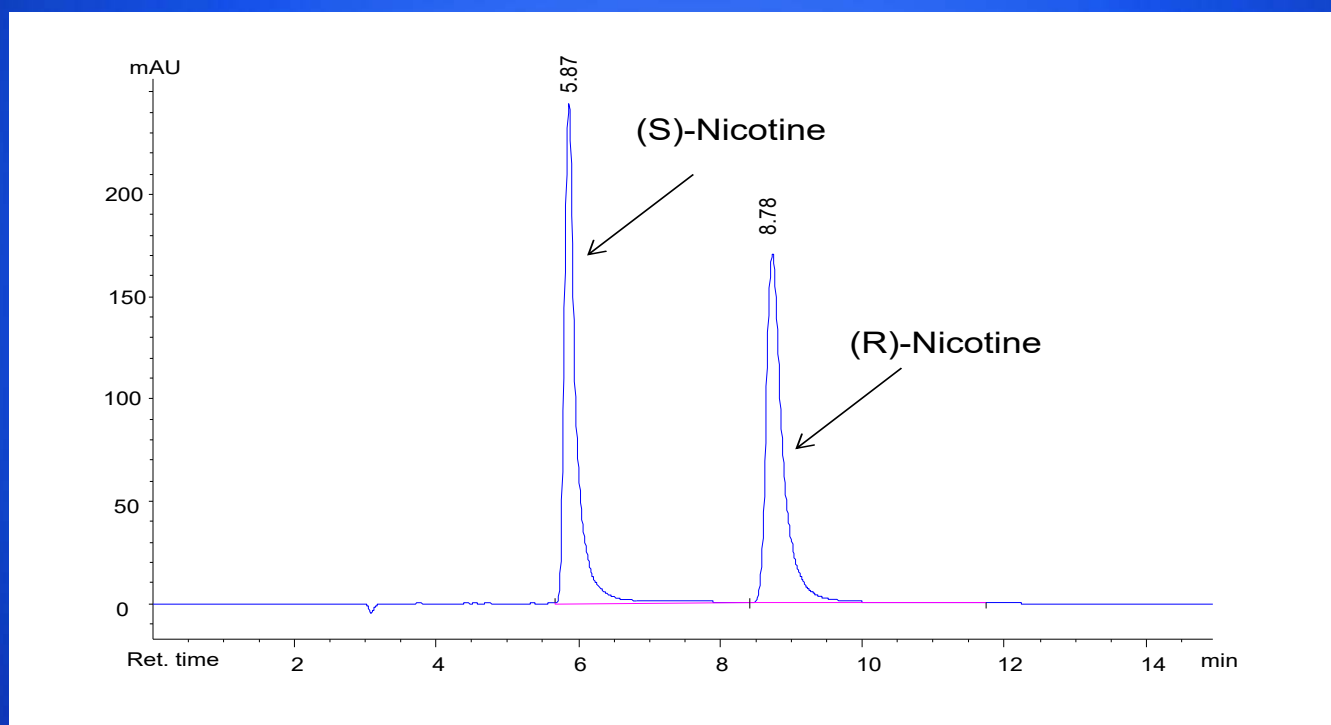


The ratio of the peak area simulated for (S)-nicotine and (R)-nicotine indicated that the level of (R)-nicotine in the natural nicotine is about 0.83% (areas under blue vs. green curves).

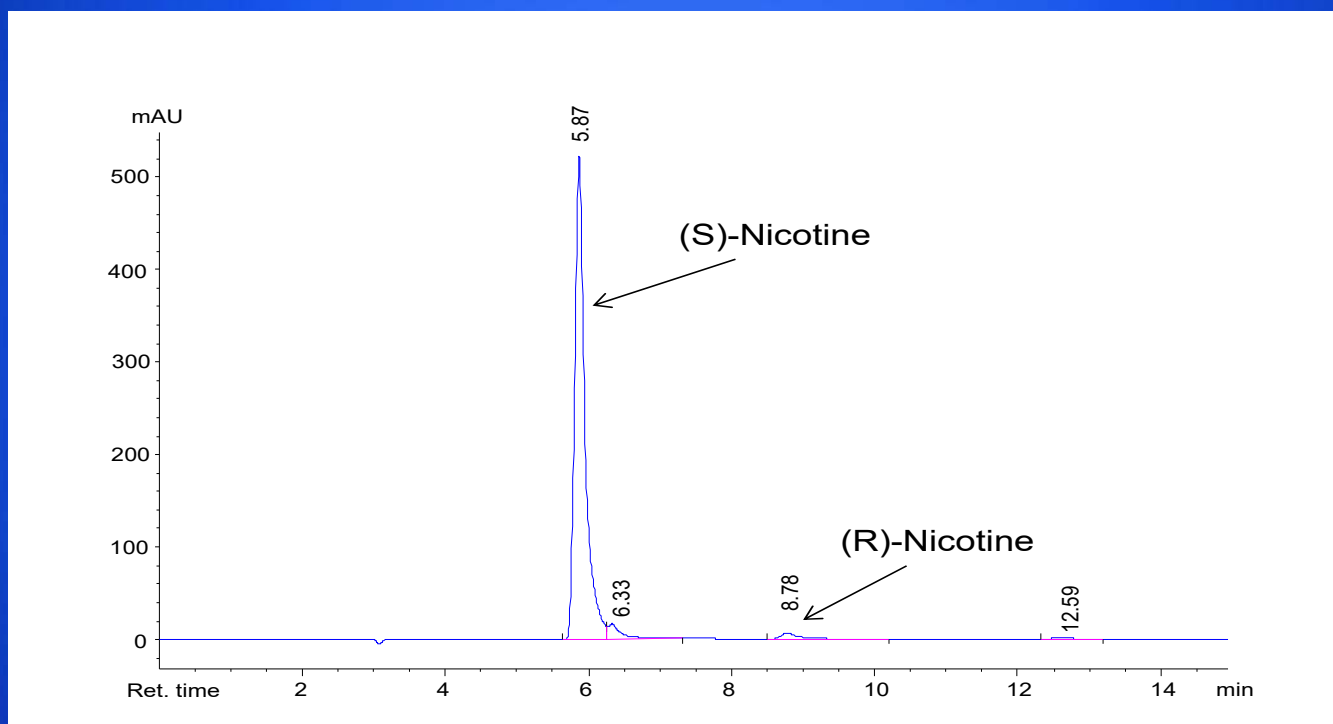
The difference between the experimental chromatogram for Spl. 2 and the deconvoluted profiles for (S)- and (R)-nicotine.



HPLC chromatogram of a solution containing 500 $\mu\text{g/mL}$ of a standard of (R)/(S)-nicotine in methanol (detection at 254 nm).



HPLC chromatogram of a solution containing 500 $\mu\text{g}/\text{mL}$ of Spl. 2 (Natural) nicotine in methanol (detection at 254 nm).



The ratio of the peak area in the HPLC chromatogram indicated that the level of (R)-nicotine in the natural nicotine is 0.67% and the (S)-nicotine accounts for 99.33%.

Conclusions

- Several analytical methods were used for the measurement of nicotine levels in different liquids used in electronic smoking devices (e-liquids).
- The level of nicotine without a separation of (S)- and (R)-nicotine were measured, and this level was found to be frequently different from the values indicated by the manufacturer of the e-liquid.
- Besides the level, the ratio of (S)- and (R)-nicotine was evaluated for all the e-liquids, and all were found to contain predominantly (S)-nicotine leading to the conclusion that all e-liquids contained natural nicotine.

Conclusions (cont.)

- The ratio of (S)- and (R)-nicotine was evaluated in four nicotine samples of USP purity.
- Three of these samples were nicotine obtained from tobacco, while the fourth was a synthetic nicotine sample.
- The first procedure to evaluate the ratio of (S)- versus (R)-nicotine in the nicotine samples was based on the deconvolution of the enantiomer peaks in a GC-MS analysis.
- The GC-MS analysis indicated about 0.83% (R)-nicotine in the natural nicotine samples.
- An HPLC procedure allowed for a better measurement of the (S)-/(R)- ratio and it was found that natural nicotine contained 0.67% (R)-nicotine and 99.33% (S)-nicotine.
- The synthetic sample of nicotine was 50/50 (S)-/(R)-nicotine.