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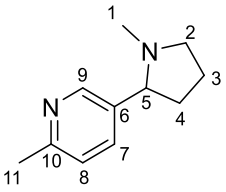
Supporting Information

Supplementary methods

Appendix to: Jenkins C, Kelso C, Morgan J. 6-Methylnicotine: a new nicotine alternative identified in e-cigarette liquids sold in Australia. *Med J Aust* 2024; doi: 10.5694/mja2.52423.

Supplementary methods

6-Methyl nicotine [2-methyl-5-(1-methylpyrrolidin-2-yl)pyridine]



6-methylnicotine was extracted (from a 100 mg/mL commercially purchased solution containing propylene glycol) by adding 1.5 mL to a glass separatory funnel followed by the addition of 20 mL 0.1M sodium hydroxide and 15 mL dichloromethane. The solution was mixed and allowed to settle; after complete phase separation, the heavier organic layer was collected in a conical flask. The remaining aqueous phase was extracted two more times with 15 mL dichloromethane, and the collected organic phases pooled. The pooled fraction (dichloromethane, 45 mL) was washed twice with 20 mL ultrapure water and dried with $MgSO_4$. The dichloromethane was removed by rotary evaporation (room temp) to yield 6-methylnicotine as a yellow oil (0.1435 g).

Compound elemental makeup was determine using a Waters Xevo TOF high resolution mass spectrometer, while the compound structure was determined using nuclear magnetic resonance (NMR) on a Bruker Avance Neo 500-MHz spectrometer.

HR-MS (ESI): $C_{11}H_{16}N_2$ Calculated $[M+H]^+$=177.1392; Measured $[M+H]^+$=177.1392 (0.0 ppm).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40, d, 1H, H_9 (*J* = 2.6 Hz); 7.59, dd, 1H, H_7 (*J* = 8.0, 2.3 Hz); 7.13, d, 1H, H_8 (*J* = 7.9 Hz); 3.24, td, 1H, H_2 (*J* = 9.7, 8.1, 2.1 Hz); 3.04, td, 1H, H_5 (*J* = 9.1, 7.7 Hz); 2.54, s, 3H, H_{11}; 2.29, td, 1H, H_2 (*J* = 9.4, 8.4 Hz); 2.23 – 2.16 m, 1H, H_4; 2.16, s, 3H, H_1; 2.00 – 1.90, m, 1H, H_3; 1.85 – 1.78, m, 1H, H_3, 1.77 – 1.67, m, 1H, H_4.

^{13}C NMR (126 MHz, $CDCl_3$): δ 157.45, C_{10}; 149.00, C_9; 135.63, C_6; 135.49, C_7; 123.47, C_8; 68.93, C_5; 57.22, C_2; 40.54, C_1; 35.26, C_4; 24.25, C_{11}; 22.70, C_3.

Calibration curve details

The quantification of 6-methyl nicotine in samples used an internal calibration curve, with quinoline (Thermofisher Scientific Australia) as the internal standard (25 µg/mL). A 10 mg/mL stock solution of 6-methylnicotine was prepared in methanol (Thermofisher Scientific Australia). This solution was further diluted to for the intermediate solution (0.5 mg/mL in methanol), from which seven working standards (8-400 µg/mL) were prepared, each containing the quinoline internal standard (25 µg/mL). Areas under the curve for 6-methyl nicotine and quinoline for each analysed working standard were used for the internal calibration curve (R^2 = 0.999) and to determine the 6-methyl nicotine concentration in commercially purchased samples.