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Supporting Information 2

Supplementary materials

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Appendix to: Larcombe E, Allard S, Pringle P, et al. Chemical analysis of fresh and aged Australian e-cigarette liquids. *Med J Aust* 2021; doi: 10.5694/mja2.51280.

Methodology development

One challenge with the analysis of e-liquids is that of the sample matrix (glycerol/propylene glycol). Direct injection of pure standards in a simple solvent (either methanol or pyridine) is too 'ideal' and not a true representation of the sample matrix representative in commercial e-liquids. The analytical standards were therefore analysed in a 50/50 mixture of propylene glycol and glycerol.

Solid-phase microextraction (SPME) is a technique that enables solvent-less extraction on a fused silica or stainless steel fibre coated with a thin film polymer. It works by placing the extracting phase (fibre) in contact with the sample matrix for a pre-determined period, and if that time is sufficient, a concentration equilibrium is established between the sample matrix and the extraction phase. Heating and agitation of the sample matrix can also facilitate adsorption. We developed a method for optimal outcomes, concentrating on extraction time, agitation, and extraction temperature. By using SPME we removed a large fraction of matrix interference. For all experiments, the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre from Supleco was used. The compounds detected with this technique matched those found using direct injection.

Method details

Analysis of polar compounds

1) Sample preparation

0.25g of sample was weighed accurately and placed in amber vials with 4.75mL ultrapure water. Then 10µL 1g/L 4-bromophenol-2,3,5,6-d4 stock solution was added as internal standard. Prior to the analysis, 1.6g NaCl was added to the samples to increase volatilization and the SPME vials were capped tightly.

2) Gas chromatography (GC) oven temperature program

Initial temperature: 37°C, maximum temperature: 260°C; initial time: 2 minutes, equilibration time: 0.50 minute. The temperature was then raised by 5°C/min to 260°C and then left for 10 minutes. The total run time was 56 minutes. Experiments were carried out with a Agilent 6890N GC interfaced with an Agilent 5973 Network Mass Selective Detector. The separation was carried out on a 30m x 0.2mm ID HP-INNOWax polyethylene glycol analytical column with a film thickness of 0.25µm. The carrier gas was helium and the initial flow rate 1.2mL/min. Optimal GC–MS conditions were determined, as measured by maximum sensitivity, baseline separation of analytes, and Gaussian peak shapes.

3) SPME procedure

The samples were incubated at 90°C for 15 minutes before the SPME was introduced in the headspace of the vial for 13.6 minutes, allowing adsorption of the analytes onto the fibre. The fibre was then desorbed in the injector at 250°C in splitless mode for 5 minutes, followed by 15 minutes in split mode. A Gerstel MPS2 multifunction autosampler performed automated SPME injections.

4) Mass spectrometry (MS) parameter

Analytes were detected using a mass spectrometer in electron impact (EI) ionisation mode at 70eV. The mass spectrometer quadrupole temperature was set to 150°C and the mass spectrometer source to 230°C. For improved sensitivity, the compounds were quantified in selected ion monitoring (SIM) mode. Mass-to-charge ratios (m/z) are used in mass spectroscopy for identifying compounds and their fragmentation. The SIM mode, in contrast to the SCAN mode (which measures every possible fragmentation pattern and GC eluent), specifically targets defined m/z values, greatly increasing sensitivity.

The compounds were identified using a combination of retention time (RT), comparison of the mass spectra data for pure compounds with the Wiley275 and NIST2005 databases, and the specific diagnostic ion fragments of each component. For each compound, several monitoring ions were selected, one for quantification and the others for confirmation. The ratio between the peak area corresponding to the fragment with the highest signal-to-noise ratio (and not the highest peak area value)

and the peak area of the internal standard 4-bromophenol-2,3,5,6-d4 were used for quantification. Fibre conditioning was carried out after each sample to avoid carryover contamination.

Analysis of poly-cyclic aromatic hydrocarbons

1) Sample preparation

0.5g of sample was weighed accurately and dissolved in 4.75mL ultrapure water. Then 10µL 1g/L biphenyl-d10 stock solution was added as internal standard. Prior to the analysis, 1.6g NaCl was added to the samples to increase volatilisation and the SPME vials were capped tightly.

2) GC oven temperature program

Initial temperature: 40°C, maximum temperature: 320°C; initial time: 3 minutes, equilibration time: 0.25 minutes. The temperature was raised by 20°C/min to 160°C, then raised by 10°C/min to 320°C and left for 10 minutes. The total run time was 35 minutes. We used an Agilent 6890N GC interfaced with an Agilent 5975 Network Mass Selective Detector. The separation was carried out on a 30m x 0.25mm ID Zebron ZB5-MS analytical column with a film thickness of 1µm. The carrier gas was helium and the initial flow rate 1.3mL/min.

3) SPME procedure

The samples were incubated at 90°C for 15 minutes before the SPME was introduced in the headspace of the vial for 15 minutes, allowing adsorption of the analytes onto the fibre. The fibre was then desorbed in the injector at 260°C in splitless mode for 3 minutes followed by 9 minutes in split mode. A Gerstel MPS2 multifunction autosampler performed automated SPME injections.

4) MS parameter

The MS parameters were similar to those for the polar compounds, except that m/z values relevant to the PAHs were used. After each sample, fibre conditioning was carried out to avoid carryover contamination. Calibration was based on the ratio of the peak area of the analytes to the peak area of the internal standard.

Analysis of propylene glycol and glycerol

As previously mentioned, the challenge with analysing chemical in e-liquids is the matrix, as propylene glycol and glycerol are present in large amounts. SPME was used to reduce the matrix effect and lower the quantification limits for other components of the e-liquid matrix. At lower concentrations, however, it is not possible to accurately quantify propylene glycol or glycerol, as they overload the fibre. Another method, using direct injection, was therefore developed.

1) Sample preparation

0.03g of sample was weighed accurately and dissolved in 5mL methanol. Then 50µL of the solution was diluted in 1.45mL methanol, and 10µL 1g/L 4-bromophenol-2,3,5,6-d4 stock solution was added as internal standard.

2) GC oven temperature program

Initial temperature: 40°C, maximum temperature: 260°C; initial time: 2 minutes, equilibration time: 0.30 minutes. The temperature was raised by 15°C/min to 260°C and then left for 10 minutes. The total run time was 26 minutes. We used an Agilent 7890A GC interfaced with an Agilent 5975C Network Mass Selective Detector. The separation was carried out on a 30m x 0.25mm ID HP-INNOWax polyethylene glycol analytical column with a film thickness of 0.25µm. The carrier gas was helium and the initial flow rate 1.1mL/min.

3) Injection procedure

The volume of sample injected was 1µL. The injector was set to 250°C with a split ratio of 100:1.

4) MS parameter

All analyses were run in single ion monitoring (SIM). Calibrations was based on the ratio of the peak area of the analytes to the peak area of the internal standard.

Ageing experiments

We developed a method for accelerated "ageing" of e-liquids to examine chemical changes in-e-liquids caused by vaping (evaporation/condensation aerosol generation process). This process can lead to thermal decomposition, oxidation, and polymerisation of chemicals. Our method was validated against vaping tests using a common e-cigarette setup (Innokin MVP4 with Scion 0.28Ω Kanthal coil) and common vaping protocol (50mL, 3 second puffs every minute for 2 hours). The aerosol generated was collected in an impinger for subsequent comparison with the results of the accelerated method, and the residual e-liquid in the tank was also collected for analysis.

For the final accelerated ageing method, we used a beaker with a new coil (Innokin Scion Kanthal 0.28Ω) connected to a switched power supply operating at 0.28Ω. 30mL of e-liquid was poured into the beaker and the coil fully submerged. The coil was operated for five 20-second intervals broken by 20-second pauses. This (submerged) method ensured the coil stays fed with liquid (ie, avoids a "dry" vape condition) and also acts as an impinger, as the bulk of the aerosol generated is re-absorbed into the liquid. This method produced chemical profiles that did not differ markedly from those of the vape aerosol or the residual liquid (Figure).

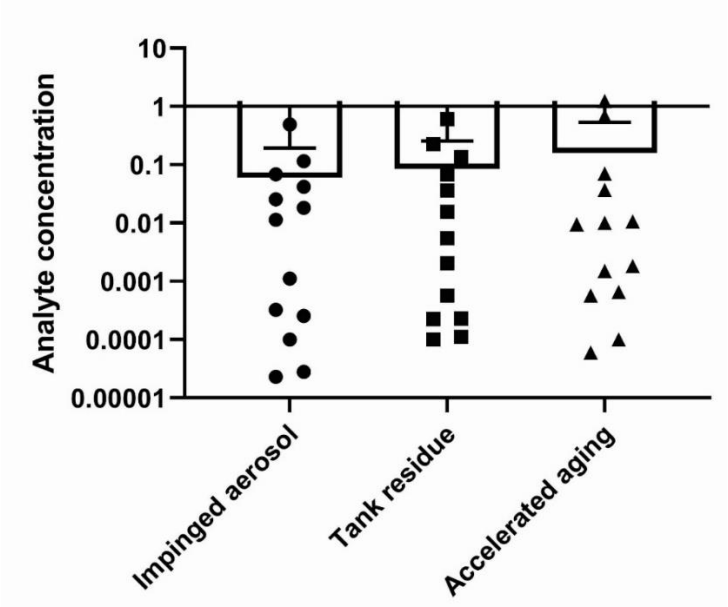

Figure. Comparison of the concentrations of 13 analytes detected after accelerated ageing of 50/50 glycerol/propylene glycol samples (no flavouring): impinged aerosol (generated by an electronic cigarette device), residual e-liquid, and sample subjected to the accelerated ageing method.