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Effect of *O*-acetylation on ketene and carbonyl yields from conventional and emerging cannabinoids

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The pulmonary toxicant, ketene, has been observed from electronic (e–) cigarette vaping and dabbing (flash vaporisation) of acetylated compounds such as Vitamin E Acetate (VEA) and the *O*-acetates of select few cannabinoids. Yet for the majority of commercially-available cannabinoids with structural similarities to VEA, toxicant yields from vaping have not been quantified. Methodology optimisation for ketene trapping and quantification or characterisation of the co-production of other potentially harmful products have not been reported. In this work, an optimised impinger collection method was used to quantify the yield of ketene and carbonyl products from several conventional and emerging cannabinoid distillates using a commercially available cannabis e-cigarette. Ketene and carbonyls were analysed after chemical derivitisation by high performance liquid chromatography high-resolution mass spectrometry (HPLC–HRMS) and cannabinoids were analysed using gas chromatography mass spectrometry (GC-MS). The cannabinoids under study are: delta-8 tetrahydrocannbinol *O*-acetate (Δ^8-THCO), delta-9 tetrahydrocannbinol *O*-acetate (Δ^9-THCO), cannabidiol di-O-acetate (CBD-di-O), cannabigerol di-*O*-acetate (CBG-di-O), 9(*R*)-hexahydrocannabinol *O*-acetate (HHCO) and their paired non-acetylated analogues (Δ^8-THC, Δ^9-THC, CBD, CBG, and HHC). The acetyl group decomposed into ketene during vaping with nearly quantitative efficiency (>99%). No ketene was observed in the unvaped distillates or during vaping of the the non-acetylated cannabinoids. The highest summed production of ketene and carbonyl was observed from CBD-di-O which was attributed to the presence of two *O*-acetate groups on the phenyl moeity and the reactive *exo*-cyclic double bond of the terpene side chain. Higher airflow through the device due to opening the vent reduced ketene yields but increased carbonyl yields, showcasing that temperature and oxygen influence the distribution of these toxicants. Cannabis vape users can oscillate between open and closed air vents, which modify their toxicant exposure. Overall, ketene and carbonyl production yields from *O*-acetylated cannabinoids ranged between 1–4% and 0.1–1.5%, respectively, by cannabinoid mass. The carbonyl production yields for non-acetylated cannabinoids ranged between 0.1–7% by cannabinoid mass. These optimised measurements show that vape users can be exposed to yields of toxicants that far exceed safety limits for pulmonary effects, and therefore, further stringent product regulation is required to avoid health implications from vaping.

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Introduction

In the United States the 2018 Farm Bill that legalised hemp has led to the industrial production of widely accessible cannabinoid concentrates (distillate, wax, resin, rosin) containing synthetic isomers of delta-9 tetrahydrocannabinol (Δ^9-THC), such as delta-8 tetrahydrocannabinol (Δ^8-THC) or hexahydrocannabinol (HHC), and their *O*-acetylated derivatives, all of which remain psychoactive but have federally legal status.[1–3] Furthermore, in some anecdotal reports, the acetylated cannabinoids have shown increased psychoactivity and potency compared to the parent compound. Cannabinoid delivery methods for recreational and medicinal purposes are evolving. Vaping or aerosolising cannabinoid concentrates *via* an e-cigarette device is an increasingly popular delivery method amongst cannabis users, and particularly for younger generations.[4–6] Greater than 56% of adolescent and 35% of adult cannabis users now report past-month cannabis vaping.[7,8] The acetylated form of some cannabinoids has been shown to form the pulmonary toxicant, ketene,[9–11] mirroring concerns surrounding the illicit additive Vitamin E Acetate (VEA) in the e-cigarette, or vaping, product use associated to lung injury (EVALI)

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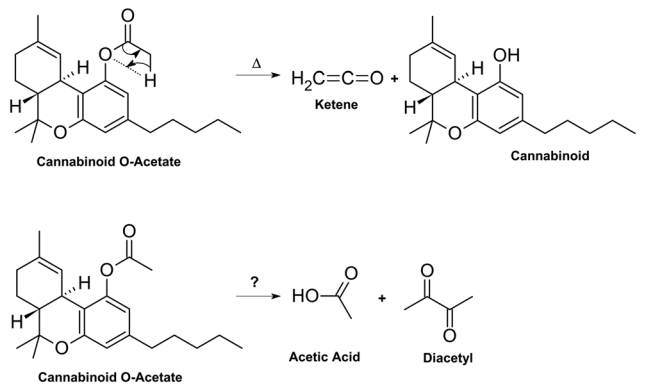
outbreak in the United States during 2020.[12–16] It is hypothesised that during vaping, the acetyl group can undergo heat-induced hydrogen transfer to release ketene and the non-acetylated cannabinoid, amongst other potential products such as acetic acid and diacetyl (Scheme 1).[14,17]

Ketene is a colourless gas that can be fatal when inhaled at high concentrations as well as induce delayed toxicity when exposed at lower concentrations.[18] It is hypothesised that ketene exhibits a mechanism of action analogous to the poisonous gas phosgene, in which it acetylates cellular enzymes and proteins.[18] Ketene can also react with water in the airways to form acetic acid, which can cause respiratory harm.[19,20] The National Institute for Occupational Safety and Health have set the immediately dangerous to life or health limit from exposure to ketene as 5 ppm,[21] however, short term exposure can still cause lasting toxicological effects.[18] Previous studies have estimated the ketene exposure from vaping pure VEA and VEA mixtures with THC to range from 0.77–12.9 μg per puff.[12,22] Whilst flash vaporisation ("dabbing") of cannabinol *O*-acetate (CBNO), caused an estimated ketene exposure as high as 0.078 mg over typical "dabbing" usage.[9] Munger *et al.*,[9] also observed ketene was produced through "dabbing" other synthetic cannabinoid acetates such as delta-8 tetrahydrocannabinol *O*-acetate (Δ^8-THCO) and cannabidiol di-*O*-acetate (CBD-di-O). Additionally, Munger *et al.*,[9] showed that ketene formation was possible from vaping Δ^8-THCO, however, did not provide a quantification. Therefore, considerable knowledge gaps persist for quantification of ketene formation from realistic vaping conditions for several conventional and emerging cannabinoids and in the understanding of their yield relative to cannabinoid dosage.

Studies also show that other features of the cannabinoid structures degrade during vaping, predominantly stemming from the reactive terpene moiety, producing harmful and potentially harmful carbonyls.[17,23–26] Acetaldehyde, acetone, methacrolein and formaldehyde, amongst other toxic or potentially harmful carbonyls, have been widely reported from cannabis vaping with reported yields in the range of 100–1000 ng per mg of vape aerosol.[14,17,23,25,27–32] Furthermore, studies show carbonyl production are significantly affected by the addition of terpenes to the distillate, coil temperature, and device power.[14,23,28,30,33] However, at the time of writing there are no studies directly comparing carbonyl product yields from different popular cannabinoid *O*-acetate distillates, nor reporting carbonyls alongside ketene, both of which are needed to improve product safety labelling.

Lastly, it is not clear how previous studies of ketene formation compare to one another, as sample optimisations or validations have not been discussed. As ketene is a highly volatile gas, most previous studies aiming to quantify ketene production have adopted impinger collection methods, using benzylamine, to trap it as a more stable compound that can be detected by Nuclear Magnetic Resonance, Liquid Chromatography–Mass Spectrometry or Gas Chromatography–Mass Spectrometry.[9,12,15] Although a lack of a ketene analytical standard hinders quantitative method development and validation, a qualitative optimisation of impinger trapping efficiency is still needed. Although impingers are widely used for collecting aerosol in many fields,[29,34–39] there are wide ranging differences in impinger apparatus and trapping reagent characteristics, which results in difficulty creating a standard operating procedure that can be compared between laboratories.[40] An alternative method was adopted in one study using a pipette based collection technique to directly condense the vape aerosol and quantify ketene using a co-product, duroquinone, that forms upon vaping.[22,41] However, due to the thicker nature of THC distillates this method required centrifuging to obtain the sample which could reduce throughput compared to direct analysis of the impinger contents.[22,42] This work aims to optimise an impinger method for ketene collection from cannabinoid vaping, quantify ketene and carbonyls from a range of conventional and emerging cannabinoid distillates and report minimum product yields with respect to cannabinoid dose using relevant metrics for improved regulation.



Scheme 1 Products proposed to be associated with the decomposition of the *O*-acetyl group upon vaping cannabinoid *O*-acetates, including ketene, acetic acid and diacetyl.

Experimental

Ketene and carbonyl trapping experiments

Fig. 1 shows the workflow of the sample collection to sample analysis. An impinger style trapping method was used for the quantification of ketene and carbonyls from *O*-acetylated cannabinoid distillates, and consisted of a 13 mm outer diameter glass trap (Kimble KIMAX, DWK Life Sciences), with 15 mL maximum volume, containing 4 mL of the trapping solution connected *via* 1/8″ tubing to a vape device which was controlled by a time relay controller (PTR4-SP, Changzhou Xuchuang Info. Tech. Co., China). The trapping solution consisted of methanol (LC-MS Optima Grade), excess recrystallised 2,4-dinitrophenylhydrazine (DNPH, 97%, Sigma Aldrich), 6 M hydrochloric acid (HCl, Sigma Aldrich), and benzylamine (>99%, Sigma-Aldrich). The ketene was trapped *via* benzylamine forming *N*-benzylacetamide upon reaction whilst any car-

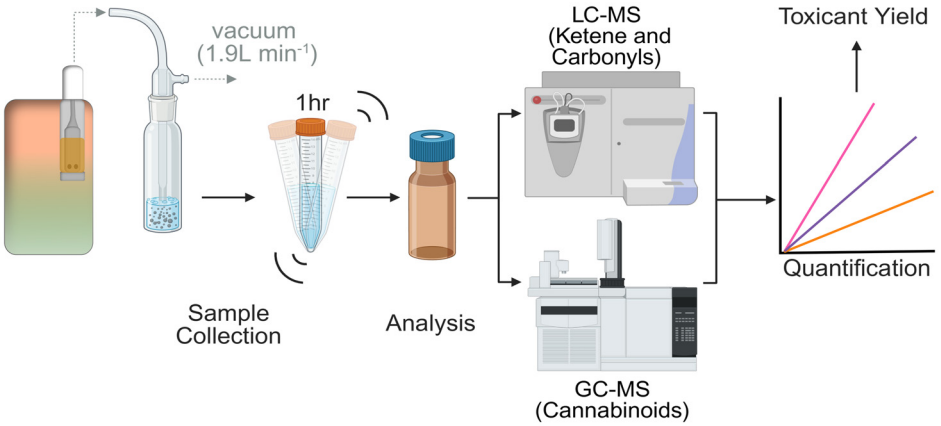
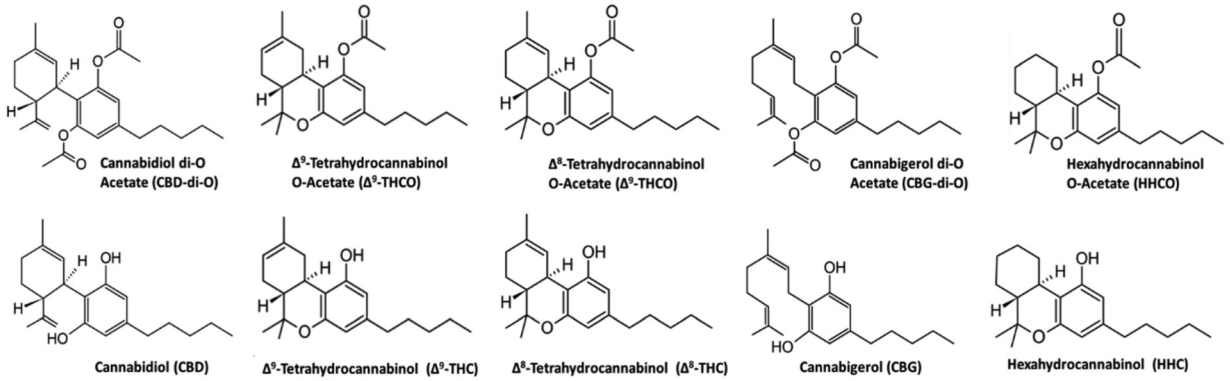


Fig. 1 Illustrated workflow of the vape aerosol sample generation and collection, and subsequent chromatographic analyses of toxicants: ketene and carbonyls *via* liquid chromatography Orbitrap mass spectrometry (LC-MS) and cannabinoids *via* gas chromatography mass spectrometry (GC-MS).

bonyl products were derivatised by DNPH in solution.[29] It was observed that the total volume of the impinger contents before and after vaping was constant. By comparing impinger collection results with and without DNPH, it was observed that the addition of DNPH did not affect the trapping of ketene by benzylamine. However, benzylamine was found to produce benzaldehyde as sampling artifact, potentially from reactions with reactive oxygen species produced from the vaping process,[43] which was detected by the DNPH carbonyl analysis. In the absence of benzylamine, benzaldehyde wasn't detected and the distribution of remaining carbonyls was the same, therefore, in the remaining analyses benzaldehyde is ignored. Distillates of the federally controlled Δ^9-THC and its associated derivatives were obtained from the National Institute for Drug Abuse (NIDA) drug supply program. Other distillates of acetylated and non-acetylated cannabinoids were obtained from Gilded Extracts (San Antonio, TX), GVB Biopharma (Las Vegas, NV) and The Hemp Collect (Portland, OR) and used without modification.

The distillates tested in this study were delta-8-THC (Δ^8-THC), delta-8 THC *O*-acetate (Δ^8-THCO), delta-9-THC (Δ^9-THC), delta-9 THC *O*-acetate (Δ^9-THCO), cannabidiol (CBD), cannabidiol di-*O*-acetate (CBD-di-O), cannabigerol (CBG), cannabigerol di-*O*-acetate (CBG-di-O), 9(*R*)-hexahydrocannabinol (HHC) and 9(*R*)-hexahydrocannabinol *O*-acetate (HHCO) (Scheme 2). Non-acetylated distillates were used to confirm the lack of ketene production without the acetyl group and compare the carbonyl yields. A CCELL Palm Pro cannabis vaping battery device (CCELL, Shenzhen, China) with a ceramic CCELL Kera cartridge was used for vaping the cannabinoid distillates using a vacuum flow rate of approximately 1.9 L min^{-1} to activate the device and a power setting of 3.6 V. Each distillate was puffed 15 times for a puff duration of 4 seconds and repeated in triplicate for *O*-acetate cannabinoids. Non-acetylated cannabinoids were assessed in single experiments. An inhalation time of 4 seconds was previously observed to increase cannabinoid delivery to the deeper lung and corresponded to a puff volume of 126 mL which is consistent with the range of volumes observed in previous puffing topography studies amongst vape users.[22,44]

Trapping efficiency was evaluated using two impingers in series to evaluate the relative amount of cannabinoid, ketene, and carbonyls trapped in each impinger under the same conditions as the trapping experiments. Cannabinoids were



Scheme 2 Chemical structures and abbreviated names of the cannabinoids used in this study.

trapped in the first impinger at 94% efficiency, allowing for accurate normalisation of the toxicant data. Secondly, the trapping efficiency of ketene was determined to be 75.3% which is significantly improved compared to the 22% previously reported in Munger *et al.*,[10] using benzylamine and similar to the efficiency reported in Wang *et al.*,[12] using methylaniline. In consideration of trapping efficiency, the ketene concentrations may be up to 1.25 times higher than that reported here; however, without a ketene analytical standard, the absolute quantification remains a challenge. Finally, due to the usage of excess DNPH it was observed that 100% of carbonyls were captured in the first impinger.

After collection, the resulting solution was shaken in the dark for 1 hour to allow the derivatisation to complete before analysis.[29] Samples were stored at −20 °C prior to analysis. Several days of monitoring revealed no observable degradation in the ketene or carbonyl samples due to storage compared to immediate analysis. However, during storage at −20 °C, it was observed that the acetylated cannabinoid would convert to its parent molecule (*i.e.* Δ^8-THC is formed from Δ^8-THCO) without impacting the ketene signal, which is discussed in greater detail in the results. The total mass of cannabinoid was conserved compared to immediate analysis.

Production yields of toxicants were normalised to total cannabinoid concentration in the vape aerosol as determined by GC-MS. Previously studies have adopted metrics such number of puffs or puff volume and mass difference in the vape cartridge as a proxy for exposure and vape aerosol mass produced. However, these metrics ignore the small reductions in vacuum flow rate during a trapping experiment and fluctuations in vape behaviour despite following a standard operating procedure. Across previous studies following similar puffing regimes the vape aerosol mass varied between 78–138.2 mg.[28,29] In our preliminary testing, we found using the mass difference of the cartridge as a proxy for aerosol mass did not reliably correlate with ketene production and distillate mass change was inconsistent (50–213 mg) for the same number of puffs in the operating procedure. Whereas using the total cannabinoid mass improved the correlation (R^2) to ketene yield by +0.3. Various control experiments were conducted to validate the method and are discussed in the results.

High performance liquid chromatography high resolution mass spectrometry

Ketene and carbonyls were quantified using High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (HPLC–HRMS). Ketene in the form of *N*-benzylacetamide from derivatisation *via* benzylamine and carbonyls in the form of hydrazones as derivatised by DNPH were quantified using an Agilent 1100 HPLC (Santa Clara, CA, USA) coupled to a Linear Ion Trap Quadrupole Orbitrap Elite MS (Thermo Scientific, USA) with heated electrospray ionisation (HESI). Standard stock solutions of *N*-benzylacetamide (98%, AmBeed) were prepared in solutions of 80 : 20 (v/v%) H_2O : MeOH (LC-MS Optima Grade) across the concentration range: 0.01–10 μg mL^{-1} and analysed in triplicate to yield a linear calibration curve (R^2 = 0.99, RSD < 2%) shown in Fig. S1. 10 μL aliquots from the impinger were taken for the ketene analysis samples and prepared into a 1 mL solution of 80 : 20 (v/v%) H_2O : MeOH (LC-MS Optima Grade Fisher Chemical) prior to HPLC–HRMS analysis. The mobile phase consisted of LC-MS Optima Grade H_2O with 10 mM ammonium formate (A) and LC-MS Optima Grade MeOH with 10 mM ammonium formate (B). Compound separation was achieved using a reversed phase Agilent InfinityLab Poroshell 120 EC-C_{18} column (2.1 mm × 100 mm, 2.7 μm). An injection volume of 5 μL was used for both the standards and the ketene samples and a flow rate of 0.25 mL min^{-1}. A gradient elution was used, starting at 80% (A) with a 3 minutes post-injection hold before decreasing to 20% (A) at 23 minutes before returning to the starting conditions at 26 minutes with a 2 minutes further hold for the column to re-equilibrate. The HESI was operated using a source temperature of 275 °C, a capillary temperature of 255 °C, a sheath gas flow of 20, auxiliary gas flow of 7 and sweep gas flow of 3. The ketene concentration was determined using the moles of *N*-benzylacetamide detected in the HPLC–HRMS method assuming a 1 : 1 mole ratio. The analysis of carbonyl-DNPH hydrazones using negative ion mode HPLC–HRMS has previously been described.[29] In summary, an unmodified 1 mL aliquot was taken directly from the impinger for HPLC–HRMS analysis and separation was achieved using a reversed phase Agilent InfinityLab Poroshell 120 EC-C_{18} column (2.1 mm × 100 mm, 2.7 μm). The separation was a gradient elution using an initial mobile phase composition of 60 : 40 (A : B) which consisted of 0.1% (v/v%) formic acid in water (A) and pure acetonitrile (ACN) (B) and a flow rate of 0.27 mL min^{-1}. After a 5 minutes post-injection hold, A was decreased to 20% over 37 minutes before returning to the starting conditions at 45 minutes with a 9 minutes hold for column re-equilibration. The HESI was operated using a source temperature of 300 °C, a capillary temperature of 275 °C, a sheath gas flow of 25 and auxiliary gas flow of 10. An authentic standard mixture of 13 DNPH hydrazones was procured from Accustandard enabling quantification of acetaldehyde, acetone, acrolein, benzaldehyde, 2-butanone, *n*-butyraldehyde, crotonaldehyde, formaldehyde, hexanal, methacrolein, propionaldehyde, *m*-tolualdehyde and valeraldehyde. Whilst an acetic acid DNPH hydrazone was synthesised from commercially available acetic acid (99.7%, Alfa Asaer) with recrystallised DNPH. Stock solutions were prepared across the concentration range 0.01–0.1 μg mL^{-1} for acetic acid, and 0–20 μg ml^{-1} for the commercial mixture. All carbonyls achieved linear calibration (R^2 = 0.94–0.99, RSD < 4%) and were analysed in triplicate (Fig. S2).

Cannabinoid quantification using gas chromatography mass spectrometry (GC-MS)

Cannabinoid detection by GC-MS showed improved retention, separation and repeatability compared to LC-MS. 1 mL samples were prepared by diluting the contents from the impinger with MeOH (LC-MS Optima Grade, Fisher Chemical) using a dilution factor of 100. GC-MS detection was on an

Agilent 6890N GC equipped with an Agilent 7683B Autosampler coupled to an Agilent 5973 Network Mass Selective Detector (MSD) operating in scan mode. Separation was achieved using an Agilent HP-5MS column (30 m × 0.25 mm × 0.25 μm). An injection volume of 1 μL was used in splitless mode at a temperature of 290 °C. Ultra Purity Grade Helium was used as a carrier gas at a flow rate of 1 mL min^{-1} throughout the analysis. The oven was initially programmed at 50 °C for 0.5 min and then ramped at 20 °C min^{-1} until 130 °C with a 2 minute hold before ramping again at 20 °C min^{-1} in until 300 °C with a 5 minute hold, resulting in a total run time of 20 minutes. Calibration curves of the studied cannabinoids were prepared in MeOH using commercially available certified reference standards (Cayman Chemicals) across the concentration range 0–200 μg ml^{-1}, to yield linear calibration curves (R^2 = 0.96–0.99, RSD < 3%) shown in Fig. S3.

Results and discussion

Impinger optimisation for ketene quantification

Previous studies using an impinger experimental set up to quantify ketene have used benzylamine volumes of 150 μL in a solution of chloroform or MeOH ranging between 1.5–2 mL, which is used as the base case in this work.[9,12,15] In this study a wide range of benzylamine reagent concentrations and total impinger volumes were tested to ensure optimal ketene trapping was achieved. This was assessed against both the relative amount of ketene trapped in the form of *N*-benzylacetamide and the repeatability of the replicates. The addition of glass beads was also tested as previous research suggested this can improve collection efficiency[45,46] but in this study yielded similar concentrations of *N*-benzylacetamide, therefore, further optimisations was conducted in the absence of beads. Optimisation was conducted using Δ^8-THCO due to both the high purity and availability of the distillate.

Due to the size of the impinger used in this work (15 mL), impinger volumes were tested across the range 2–5 mL to allow for bubble height. Generally, increasing the solution volume increased ketene trapping in the form of *N*-benzylacetamide compared to the base case 2 mL due to greater interaction between the sample being bubbled through and the trapping solution.[46] It was determined that 4 mL trap volume provided the optimum residence time of ketene in solution and best repeatability (RSD < 15%, (n = 3)), in addition to approximately 3 times enhancement in *N*-benzylacetamide at equimolar concentrations of benzylamine compared to the base case. The concentration of benzylamine was then varied from 0.33 M to 2.37 M at 4 mL volume to ensure the trapping reagent was in excess. Fig. 2 shows increasing the concentration of the trapping reagent increased the detected concentration of *N*-benzylacetamide, suggesting further ketene was being trapped compared to the original protocol. However, benzylamine concentrations in excess of 2 M did not further increase yields and the optimised concentration of benzylamine was determined to be 1.68 M. Although

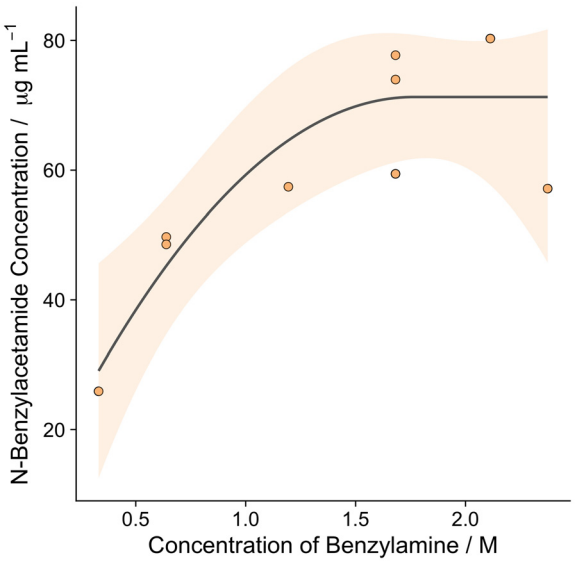


Fig. 2 Effect of increasing the concentration of benzylamine trapping reagent on detected *N*-benzylacetamide concentration used as a proxy for ketene. Standard error of the fit is shown in orange and the data is fitted to reach a maximum value.

a local maximum was found in the array of sampling conditions, there is no ketene standard to validate an absolute trapping efficiency. Consequently, our work and those of others should be treated as a lower limit for ketene production. Because the optimisation of trapping efficiency is specific to the impinger, vape device, and puff parameters used, we recommend impinger collection methods in future works to be tested against a range of conditions prior to sample collection. It is expected that the optimisation tests performed here are applicable to sample matrices broader than vape aerosols, including bioaerosols and atmospheric aerosols.

Control experiments were performed to understand the selectivity of the ketene analysis by benzylamine. Glacial acetic acid was added (up to 100 μL) to the impinger with benzylamine reagent to rule out the potential for the acetate group in acetic acid and other acetate analogues to form *N*-benzylacetamide which could interfere with the ketene trapping. Ketene was not observed (*i.e.* *N*-benzylacetamide peak) for acetic acid controls (Fig. S4). Another experiment was performed to evaluate the ability of benzylamine to react with the cannabinoid *O*-acetates, potentially producing *N*-benzylacetamide in the absence of vaping as a sampling artifact. An extract of Δ^8-THCO distillate was spiked with the same concentration benzylamine used in the vaping experiments, shaken for 1 hour and then analysed by GC-MS and LC-MS. This control showed no observation of *N*-benzylacetamide (Fig. S4) indicating benzylamine selectively reacts with the ketene formed during vaping, not the acetate of the cannabinoid, to form *N*-benzylacetamide. However, it was observed that benzylamine in the unvaped sample facilitated the conversion of Δ^8-THCO to Δ^8-THC at ~35% by mass upon storage,

compared to no conversion of the unvaped Δ^8-THCO distillate in the absence of benzylamine (Fig. S5) without an accompanying observation of *N*-benzylacetamide. These data suggest that while benzylamine can facilitate a conversion of Δ^8-THCO to Δ^8-THC, the reaction proceeds through a different (potentially base-catalysed) non-ketene pathway compared to the heat-assisted Δ^8-THCO degradation during vaping which yields ketene and the parent cannabinoid (Scheme 1, top). Finally, the data indicate that the conversion of Δ^8-THCO to Δ^8-THC due to instrument operating conditions, such as GC inlet temperature, can be ruled out. In the unvaped samples without benzylamine, there is no observable thermal degradation of Δ^8-THCO to Δ^8-THC in the GC chromatograms. Acetylated cannabinoid standards also did not decompose using the analytical methods in this work. The controls indicate ketene is solely a product of vaping and *N*-benzylamine is suitable trapping reagent for this purpose. Munger *et al.*,[9] also reported no interference of benzylamine to ketene formation from cannabinol-*O*-acetate (CBNO) without vaping. Despite the conversion of Δ^8-THCO to Δ^8-THC, the total quantified mass of cannabinoid, defined as the sum of Δ^8-THCO and Δ^8-THC, remains unchanged. Therefore, ketene formation could be normalised to total cannabinoid concentration to determine toxicant yields.

Quantification of ketene from cannabinoid vaping

Using the optimised impinger collection method, we found the average ketene production per distillate ranged between 7.5–42.7 μg mg^{-1} or 0.8–4.3% by mass of the *O*-acetylated cannabinoid (Fig. 3A). It was observed, as expected,[12] that all five non-acetylated cannabinoids did not yield ketene upon vaping (Fig. 3B). Unvaped distillates were also confirmed to not produce ketene (Fig. S4). The greatest ketene production was observed from CBD-di-O distillate. A one-way ANOVA test followed by pairwise Tukey Honest Significant Difference tests between distillate mean ketene yield found that the average yield from CBD-di-O was statistically significant compared to other distillates ($p < 0.01$) as shown by the red asterisk in Fig. 3A. However, the average ketene production between Δ^8-THCO, Δ^9-THCO, CBG-di-O and HHCO was not statistically different from each other. Previously, Munger *et al.*,[9] observed CBD-di-O produced 13 times more ketene by mass compared to Δ^8-THCO during hot flash vaporisation. Whereas in this study, using a ceramic-coil cannabis vaping device, the yield of ketene from CBD-di-O was on average 3.1 times greater than that for Δ^8-THCO. The higher ketene yields from CBD-di-O could be due to the presence of two acetate groups in the chemical structure of CBD-di-O compared to the single acetate groups in other tested distillates. However, CBG-di-O also has two acetate groups but produced significantly less ketene (1%) compared to CBD-di-O (4.3%). Previous research found CBD in solution to be thermolabile and susceptible to oxidation which may increase the ketene yield relative to other tested cannabinoids.[47] However, further work is needed to evaluate the difference in toxicant production between CBD-di-O and CBG-di-O.

The cannabinoid mass that transformed from the *O*-acetylated form to ketene and the non-acetylated cannabinoid form was studied for mass closure using Δ^8-THCO distillate in a pure methanol impinger without any chemical reagents. It was found that 1.2% of Δ^8-THCO converted to Δ^8-THC upon vaping (Fig. S5). Compared to the 1.4 ± 0.5% of ketene produced per total cannabinoid mass, we conclude that the conversion of *O*-acetyl group to ketene (Scheme 1, top) occurs with quantitative (unity) conversion yield.

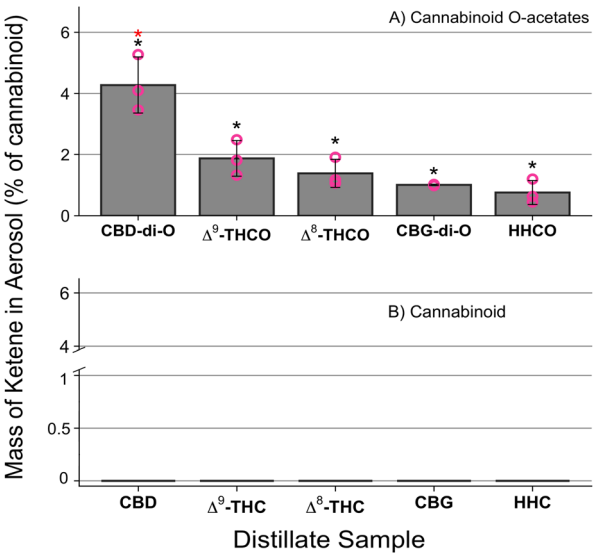


Fig. 3 Average ketene production represented as the percentage mass of the total vaped cannabinoid concentration for *O*-acetylated cannabinoids (Panel A) and their parent cannabinoid (Panel B) in vaped samples. Individual repeat experiments are shown as coloured markers. Asterisks indicate a significant difference determined by a one-way ANOVA test between the parent and *O*-acetate cannabinoids (black) or between the different cannabinoid *O*-acetate distillates (red).

Despite differences in quantification metrics, the ketene production yield was compared between this study and previous studies of cannabinoid *O*-acetates. In this study, the average total mass of ketene produced upon vaping ranged between 84.6–141.7 μg across all tested distillates which is greater than the estimated 78 μg ketene yield from the flash vaporisation of 40 mg of CBNO.[9] This could be due to the optimisation of the impinger collection methodology in this study and therefore it is possible previous exposures may have underestimated the ketene production from cannabinoid usage. Many prior studies have normalised toxicant formation from vaping to the number of puffs. Following this approach this study yields a ketene production of 5.64–9.45 μg per puff which is within the higher range previously reported for Vitamin E Acetate vaping (0.77–12.9 μg per puff).[12,22] Incorporating the molar mass of ketene and the puff volume, we can convert the ketene production (μg perpuff) to 25–42 ppm (by mole) in each puff at room temperature. Even the lowest ketene levels measured in this work significantly exceeds the safety limit set by NIOSH (5 ppm) per each puff.[21]

It should be noted, however, that puff content might be highly variable for vaping viscous material such as cannabis distillates. Despite using the same number of puffs for each

distillate in this study the cannabinoid mass detected in the vape aerosol varied between 2.0–14.6 mg on average. This observation suggests that a generic metric such as "number of puffs" or "estimated puff volume", which assumes the distillates vape at the same rate, is insufficient to assess yields of toxicants. Therefore, we suggest normalising toxicant formation in cannabis vapes by the cannabinoid mass because it reflects variations in vaping efficiency and gives insight into chemical conversion rates. Normalising by cannabinoid mass also has the benefit that conclusions drawn over the toxicity of different distillates will not be reflective of vape device behaviour or inconsistencies.

Quantification of carbonyls from cannabinoid vaping

Other potential products from the *O*-acetate decomposition upon vaping are acetic acid and diacetyl, which were quantified alongside ketene (Scheme 1). However, diacetyl was below the detection limit for all determinations. Acetic acid production (Fig. S6) followed the same pattern as ketene yields in Fig. 2 which suggests production primarily from the *O*-acetate group. However, the production yield (<0.1%) is negligible compared to ketene. This observation confirms that the *O*-acetate group of the cannabinoid *O*-acetate primarily forms ketene upon vaping. Vaping can also lead to the production of other carbonyls besides diacetyl, which show a different trend to ketene in Fig. S6. These trends suggest the carbonyl formation proceeds from a different chemical moiety than the *O*-acetyl group.

On average, the total carbonyl production from the cannabinoid *O*-acetates was observed to range from 0.9–14.8 μg mg^{-1} or 0.1–1.5% by cannabinoid mass. A maximum yield was observed in HHCO and a minimum in Δ^8-THCO (Fig. 4). The carbonyls predominantly produced from vaping cannabinoid *O*-acetates were acetone, *n*-butyraldehyde and acetaldehyde. Acetone has been previously reported as a major product of cannabis vaping and prior studies have similarly observed the formation of *n*-butyraldehyde and acetaldehyde.[17,27–29] In this study 12 carbonyls were successfully quantified, however, there are a greater number of carbonyl compounds formed that have not been quantified, including cannabinoid carbonyls with up to 23 carbons, and future work would benefit from synthesis of carbonyl DNPH hydrazone standards for increased targeted quantification or by conducting a non-target analysis for further product elucidation.[48]

Non-acetylated cannabinoids were found to have similar or greater carbonyl yields (0.1–7.1%) compared to their acetylated analogues. The largest carbonyl yields were observed from Δ^8-THC and Δ^9-THC. The dominant carbonyl product for non-acetylated cannabinoids in Fig. 4 was acetone, similarly to the findings for *O*-acetates and in agreement with previous studies.[27,28] It is notable that HHC, which lacks aliphatic C=C bonds (Scheme 2), formed almost exclusively acetone, while the other distillates show a higher diversity of carbonyl products. These observations suggest the mechanism of acetone formation from both acetylated and non-acetylated cannabinoids originate from a structurally similar moiety. We hypothesise that direct thermal production *via* the alkyl side chain or the dimethyl carbon (Scheme S1) is possible. It is also possible that carbonyls may originate from oxidant attack on the aliphatic C=C bonds as shown in Robertson *et al.*[27] Additional studies are needed to confirm mechanistic pathways.

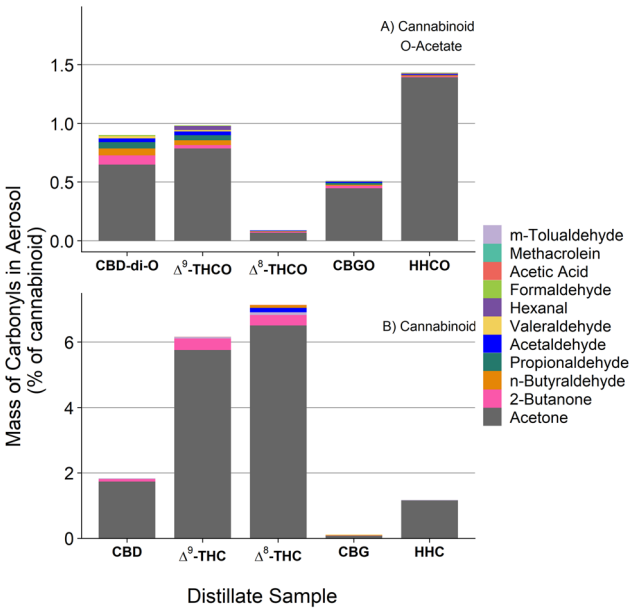


Fig. 4 Average total carbonyl production as a percentage mass of the total vaped cannabinoid concentration for each distillate, shown as a stacked colour bar with the carbonyl speciation in descending percentage mass.

Impact of operating conditions on toxicant formation

Finally, it remains unclear if user device behaviour could limit the formation of ketene and to the same effect, carbonyl production. For instance, it has been shown that device temperature and power can affect cannabinoid decomposition and increase products from VEA vaping.[24,49] Munger *et al.*[10] also suggested that oxygen plays a role in decreasing the activation barrier of ketene formation from thermal decomposition. Metal catalysis on the heated coil surface[50] also likely plays a role in generating ketenes with less heat than theoretically expected.

The CCell Palm Pro has adjustable air vents; a fully closed vent has been described as providing a smaller but "hotter", and "more robust" puff while fully open vents lead to formation of less dense clouds.[51] This translates to less air flow and hotter temperatures when the vent is closed. Due to the viscosity of the distillates, it is not possible to record the temperature of the vaping coil in this study. It was observed that the ketene yield in the vent closed scenario was greater than in the vent opened case (Fig. 5), indicating temperature may be a more important factor for formation compared to aerobic conditions. Whereas for carbonyls, the total yield increased when the vent was opened, despite the lower coil temperature (Fig. 5). While ketenes appear to have a thermally-controlled formation pathway, carbonyls have also been proposed to form *via* reaction with reactive oxygen species such as hydroxy rad-

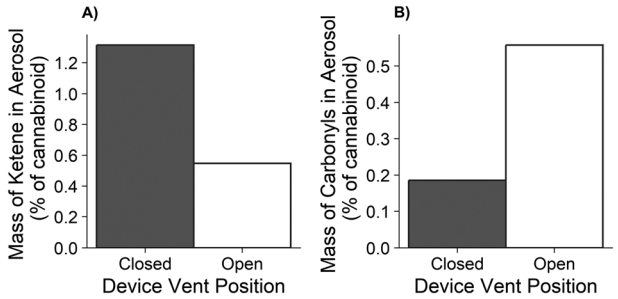


Fig. 5 Product yields of (A) ketene and (B) total carbonyls per cannabinoid mass for vent open and vent closed operation of the CCELL Palm Pro vaping device for Δ^8-THCO.

icals in vapes.[33,43] These preliminary results indicate that oxygen may be more important for carbonyl production compared to temperature; however, further testing may be necessary to systematically test toxicant production under different conditions and elucidate the mechanisms which exacerbate their production.

Conclusions

In this study, the yields of toxicants such as ketene and carbonyls from vaping a range of cannabinoids and their *O*-acetate analogues were evaluated with an optimised sample analysis methodology. It was observed that multiple factors can impact the collection procedure for gaseous ketene, but that ketene production is the predominant reactive fate of the acetyl group during vaping. It was found that using the vape device with the vent closed produced significantly higher ketene compared to vent open due to higher temperatures, despite lower oxygen levels. Most of the ketene yields in this study from vaping cannabinoid *O*-acetates with an authentic cannabis e-cigarette (Fig. 2) are newly reported, and all reported ketene yields exceeded NIOSH safety limits. In agreement with previous work, ketene was not observed in absence of vaping nor in vaping non-acetylated analogues of cannabinoids.[9]

For a few cases for which intercomparison can be performed with previous studies, the yields found here were generally higher by up to a factor of 2. The source of discrepancy is not clear; however, other studies focused on the quantification of ketene from vaping have used lower impinger volumes and trapping reagent concentrations compared to this work. It is likely that all studies, including this one, underestimate ketene formation, as there is currently no standard with which to quantify absolute trapping efficiency. However, these results highlight the importance of optimising the collection methods in each study to maximise potential yield. While high yields of ketene reported here are a significant finding for understanding the toxicological impacts of vaping, future studies will need to consider whether vaping injury effects can be traced back to ketene itself, or whether its rapid hydration reaction modifies its inhalation toxicity.[18]

The greatest summed production yield of ketene and carbonyl toxicants by mass of cannabinoid was observed for CBD-di-O, which has two acetyl groups and an exocyclic C=C bond in the terpene moiety that appear to be more labile to decomposition upon vaping. Overall, the yields of toxicants ranged from 0.8–4.3% for ketene and 0.1–1.5% for total carbonyls, respectively, from vaping cannabinoid *O*-acetates. Whereas for the non-acetylated analogues, there is a lack of ketene production, but carbonyl yields ranged between 0.1–7.1% by cannabinoid mass. Ultimately, this work advocates for increased scrutiny of acetylated and other synthetic cannabinoid products that are widely available to consumers to avoid further cases of lung damage associated with e-cigarettes and vaping.

Author contributions

R. L. E. and T. B. N. designed the experiments. R. L. E. and C. N. C. developed the analytical protocol, collected data and performed the data analysis. H. C. H. performed preliminary experiments. R. L. E. and T. B. N. wrote the manuscript with inputs from all co-authors. S. C. T. N., A. K. E. and T. B. N. obtained the funding for this work.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: Fig. S1–S3: calibration curve of ketene, DNPH hydrazones and cannabinoids tested in this study, Fig. S4–S5: HPLC–HRMS and GC-MS chromatograms of *N*-benzylacetamide and cannabinoids respectively in control experiments. Fig. S6: yields of ketene, acetic acid and total carbonyls for *O*-acetate cannabinoids. Tables S1–S3: ketene and carbonyl quantification. See DOI: https://doi.org/10.1039/d6an00190d.

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References

1 L. C. Bidwell, R. Martin-Willett and H. C. Karoly, *Drug Alcohol Rev.*, 2021, **40**, 900–913.
2 E. C. Leas, *Am. J. Public Health*, 2021, **111**, 1927–1931.
3 J. Meehan-Atrash and I. Rahman, *Chem. Res. Toxicol.*, 2021, **35**, 73–76.
4 E. Boakye, O. H. Obisesan, S. M. I. Uddin, O. El-Shahawy, O. Dzaye, A. D. Osei, E. J. Benjamin, A. C. Stokes, R. M. Robertson, A. Bhatnagar and M. J. Blaha, *Prev. Med.*, 2021, **153**, 106800.
5 J. Morgan, G. Gschwend, M. Houston, A. Jones and C. Kelso, *Drug Alcohol Depend.*, 2022, **240**, 109632.
6 P. Sharma, D. B. Mathews, Q. A. Nguyen, G. L. Rossmann, C. A. Patten and C. J. Hammond, *Clin. Med. Insights Pediatr.*, 2023, **17**, 11795565231162296.
7 M. E. Morean, D. R. Davis, G. Kong, K. W. Bold, D. R. Camenga, S. Suttiratana, J. Lee, L. Rajeshkumar and S. Krishnan-Sarin, *Drug Alcohol Depend.*, 2021, **228**, 109104.
8 M. Diaby, O. Agbonlahor, B. S. Fennell, J. L. Hart and D. T. Mattingly, *J. Cannabis Res.*, 2025, **7**, 26.
9 K. R. Munger, R. P. Jensen and R. M. Strongin, *Chem. Res. Toxicol.*, 2022, **35**, 1202–1205.
10 K. R. Munger, K. M. Anreise, R. P. Jensen, D. H. Peyton and R. M. Strongin, *JACS Au*, 2024, **4**, 2403–2410.
11 N. L. Benowitz, C. Havel, P. Jacob, D. F. O'Shea, D. Wu and J. Fowles, *J. Med. Toxicol.*, 2023, **19**, 37–39.
12 P. Wang, P. Jacob, Z. M. Wang, J. Fowles, D. F. O'Shea, J. Wagner and K. Kumagai, *Chem. Res. Toxicol.*, 2024, **37**, 1415–1427.
13 J. Meehan-Atrash and I. Rahman, *Chem. Res. Toxicol.*, 2021, **34**, 2169–2179.
14 R. F. LeBouf, A. Ranpara, J. Ham, M. Aldridge, E. Fernandez, K. Williams, D. A. Burns and A. B. Stefaniak, *Front. Public Health*, 2022, **9**, 765168.
15 D. Wu and D. F. O'Shea, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 6349–6355.
16 B. Duffy, L. Li, S. Lu, L. Durocher, M. Dittmar, E. Delaney-Baldwin, D. Panawennage, D. LeMaster, K. Navarette and D. Spink, *Toxics*, 2020, **8**, 8.
17 Y. Li, J. Dai, L. N. Tran, K. E. Pinkerton, E. R. Spindel and T. B. Nguyen, *Chem. Res. Toxicol.*, 2022, **35**, 1095–1109.
18 N. R. Council, *Acute Exposure Guideline Levels for Selected Airborne Chemicals*, 2014, DOI: 10.17226/18707.
19 G. D. Nielsen, *Regul. Toxicol. Pharmacol.*, 2018, **99**, 89–97.
20 P. Rasin and P. Prabhakaran, *Chem. Res. Toxicol.*, 2025, **38**, 1147–1149.
21 CDC – NIOSH Pocket Guide to Chemical Hazards – Ketene, https://www.cdc.gov/niosh/npg/npgd0367.html, (accessed 16 October 2025).
22 J. Lynch, L. Lorenz, J. L. Brueggemeyer, A. Lanzarotta, T. M. Falconer and R. A. Wilson, *Front. Chem.*, 2021, **9**, 734793.
23 X. Tang, L. Cancelada, V. H. Rapp, M. L. Russell, R. L. Maddalena, M. I. Litter, L. A. Gundel and H. Destaillats, *Environ. Sci. Technol.*, 2021, **55**, 6160–6170.
24 E. J. Kim, E. Kwon, S. J. Oh, M. R. Choi, S. R. Lee, B. H. Jung, W. Lee and J. Hong, *J. Chromatogr. A*, 2025, **1749**, 465909.
25 J. Meehan-Atrash, W. Luo, K. J. McWhirter and R. M. Strongin, *ACS Omega*, 2019, **4**, 16111–16120.
26 J. Meehan-Atrash, W. Luo and R. M. Strongin, *ACS Omega*, 2017, **2**, 6112–6117.
27 N. E. Robertson, J. Connolly, N. Shevchenko, M. Mascal, K. E. Pinkerton, S. C. T. Nicklisch and T. B. Nguyen, *Chem. Res. Toxicol.*, 2024, **37**, 1965–1975.
28 E. Y. Chiu, L. N. Tran, H. C. Hunsaker, S. C. T. Nicklisch and T. B. Nguyen, *ACS ES&T Air*, 2025, **2**, 1805–1815.
29 H. C. Hunsaker, N. E. Robertson, B. A. Poulin and T. B. Nguyen, *ACS ES&T Air*, 2025, **2**, 1714–1724.
30 J. Meehan-Atrash, W. Luo, K. J. McWhirter, D. G. Dennis, D. Sarlah, R. P. Jensen, I. Afreh, J. Jiang, K. C. Barsanti, A. Ortiz and R. M. Strongin, *RSC Adv.*, 2021, **11**, 11714–11723.
31 K. R. Munger, K. M. Anreise and R. M. Strongin, *Front. Toxicol.*, 2025, **7**, 1568207.
32 W. D. Troutt and M. D. Didonato, *J. Altern. Complement Med.*, 2017, **23**, 879–884.
33 Y. Li, A. E. Burns, L. N. Tran, K. A. Abellar, M. Poindexter, X. Li, A. K. Madl, K. E. Pinkerton and T. B. Nguyen, *Chem. Res. Toxicol.*, 2021, **34**, 1640–1654.
34 S. J. Campbell, S. Stevanovic, B. Miljevic, S. E. Bottle, Z. Ristovski and M. Kalberer, *Environ. Sci. Technol.*, 2019, **53**, 6729–6737.
35 K. P. Yu, Y. P. Chen, J. Y. Gong, Y. C. Chen and C. C. Cheng, *J. Aerosol Sci.*, 2016, **101**, 133–143.
36 C. C. Tseng and C. S. Li, *J. Aerosol Sci.*, 2005, **36**, 593.
37 S. R. Mallampati, C. McDaniel and A. R. Wise, *ACS Omega*, 2021, **6**, 17126–17135.
38 K. R. R. Mahanama and J. M. Daisey, *Environ. Sci. Technol.*, 1996, **30**, 1477–1484.
39 N. Borduas-Dedekind, K. J. Gemmell, M. M. Jayakody, R. J. M. Lee, C. Sardena and S. Zala, *Environ. Sci.: Atmos.*, 2024, **4**, 611–619.
40 M. Dill, S. Barhdadi, C. Vanhee and E. Deconinck, *Anal. Methods*, 2025, **17**, 1997–2014.
41 V. B. Mikheev, T. P. Klupinski, A. Ivanov, E. A. Lucas, E. D. Strozier and C. Fix, *Aerosol Sci. Technol.*, 2020, 993–998.
42 A. Lanzarotta, T. M. Falconer, R. Flurer and R. A. Wilson, *Anal. Chem.*, 2020, **92**, 2374–2378.
43 L. N. Tran, G. Rao, N. E. Robertson, H. C. Hunsaker, E. Y. Chiu, B. A. Poulin, A. K. Madl, K. E. Pinkerton, R. D. Britt and T. B. Nguyen, *Chem. Res. Toxicol.*, 2024, **37**, 991–999.
44 J. Zhao, Y. Feng, G. Tian, C. Taylor and N. S. Arden, *Comput. Biol. Med.*, 2021, **132**, 104333.
45 Y. C. Chen, I. J. Wang, C. C. Cheng, Y. C. Wu, C. H. Bai and K. P. Yu, *Aerobiologia*, 2021, **37**, 243–252.

46 K. P. Yu, Y. P. Chen, J. Y. Gong, Y. C. Chen and C. C. Cheng, *J. Aerosol Sci.*, 2016, **101**, 133–143.
47 A. I. Fraguas-Sánchez, A. Fernández-Carballido, C. Martin-Sabroso and A. I. Torres-Suárez, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2020, **1150**, 122188.
48 M. W. Tehrani, M. N. Newmeyer, A. M. Rule and C. Prasse, *Chem. Res. Toxicol.*, 2021, **34**, 2216–2226.
49 A. Canchola, R. Meletz, R. A. Khandakar, M. Woods and Y. H. Lin, *PLoS One*, 2022, **17**, e0265365.
50 N. A. Saliba, A. El Hellani, E. Honein, R. Salman, S. Talih, J. Zeaiter and A. Shihadeh, *J. Anal. Appl. Pyrolysis*, 2018, **134**, 520–525.
51 CCELL Palm Pro Vape Pen Review, https://mipod.com/blogs/mipodblog/ccell-palm-pro-vaporizer-review, (accessed 4 January 2026).