Supplementary Information (SI) for Analyst.

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

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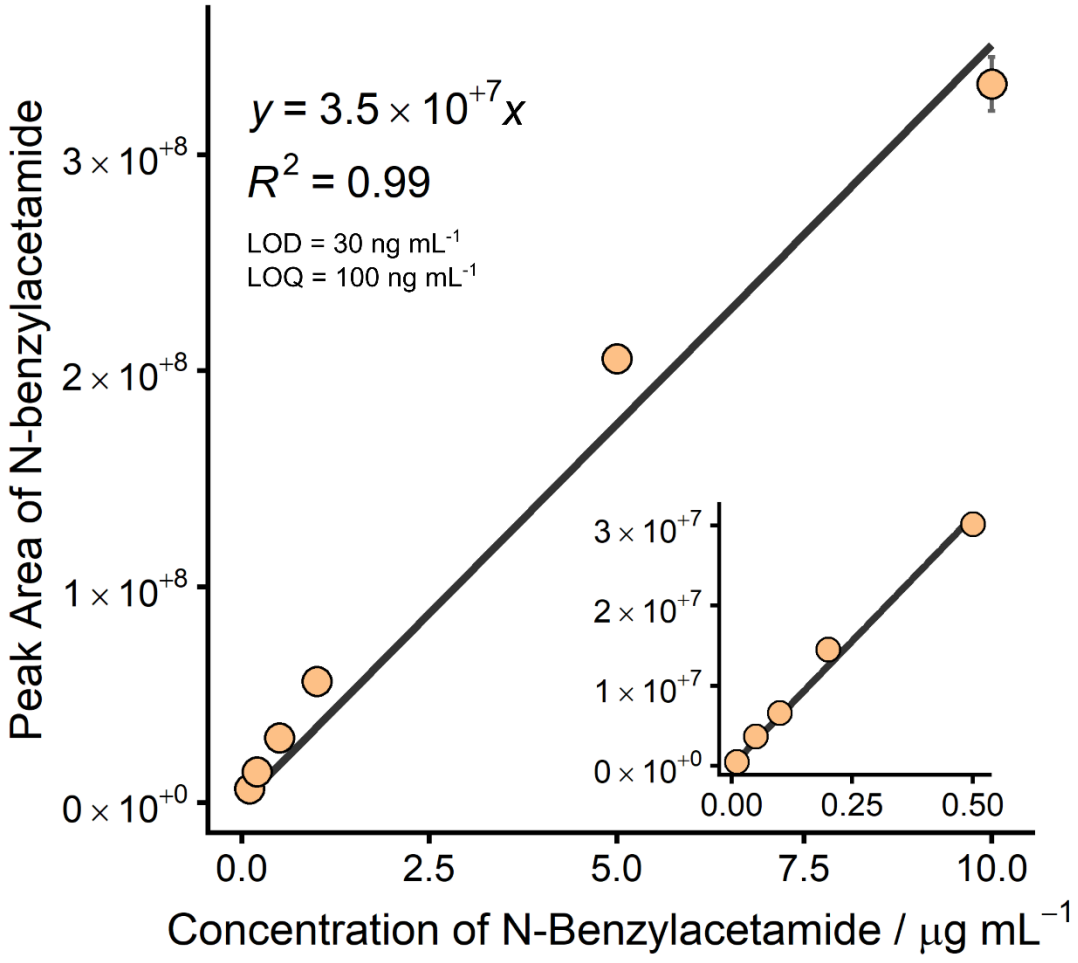


Figure S1: HPLC-HRMS calibration curve of N-benzylacetamide used to determine ketene concentration in the vaping samples. An inset plot of the low concentration calibration is shown in the lower right panel. The linearity, limit of detection and limit of quantification are shown in the top left.

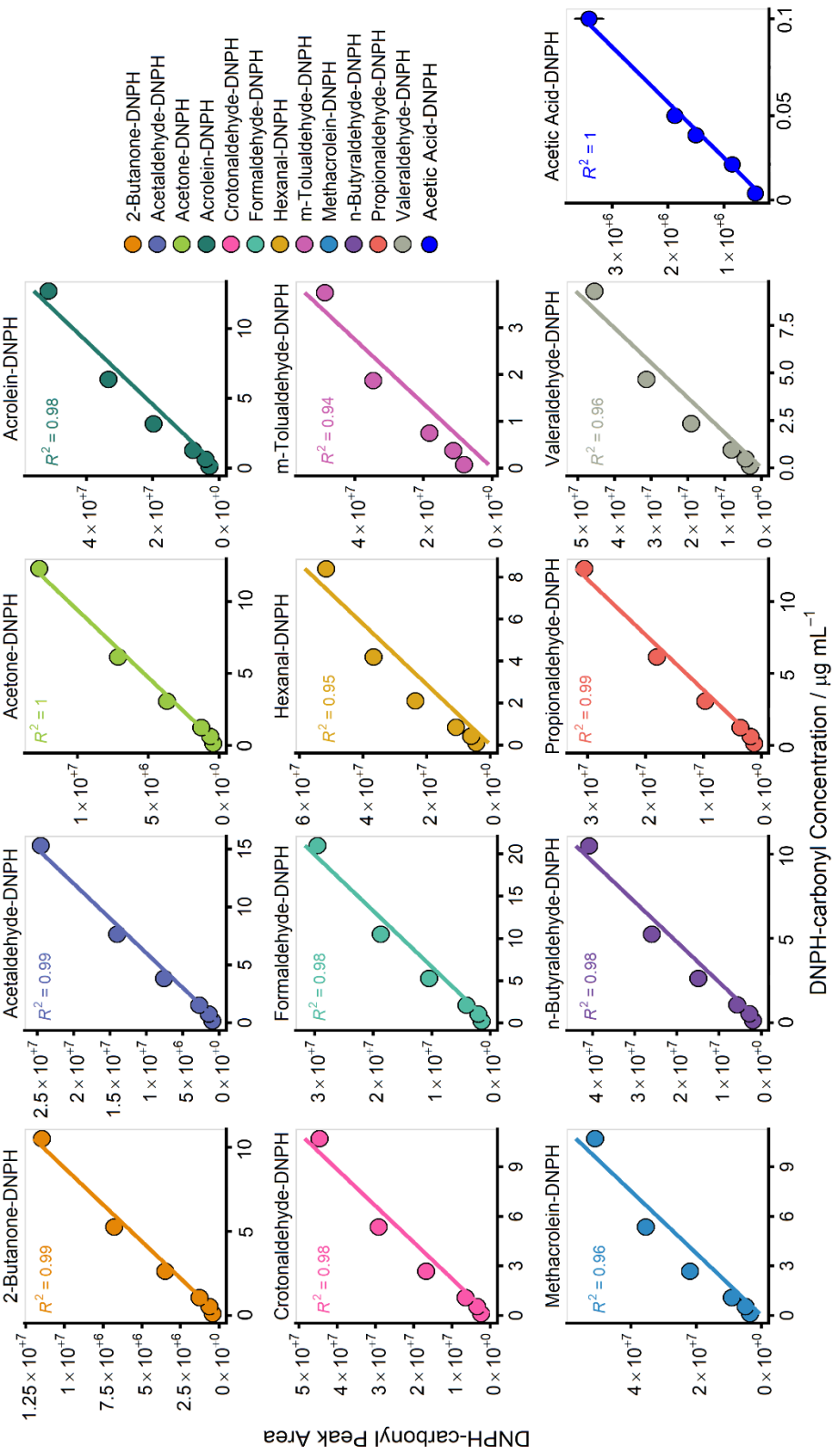


Figure S2: HPLC-HRMS calibration curve for each DNPH hydrazone derivative of the vaping carbonyls quantified in this study. The linearity of each curve is shown in the top left.

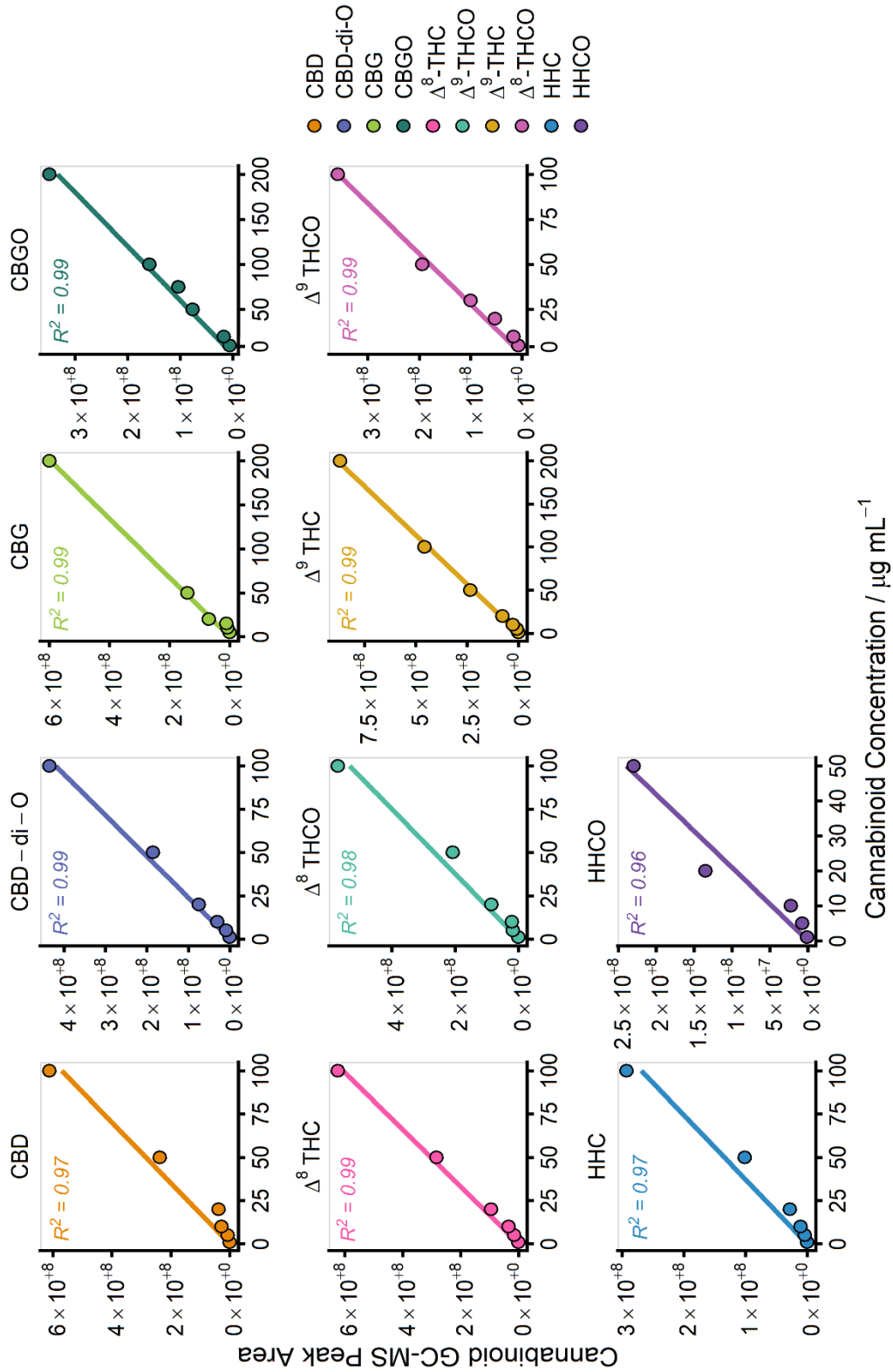


Figure S3: GC-MS calibration curves of each tested cannabinoid and the O-Acetate analogue. The linearity of each curve is shown in the top left.

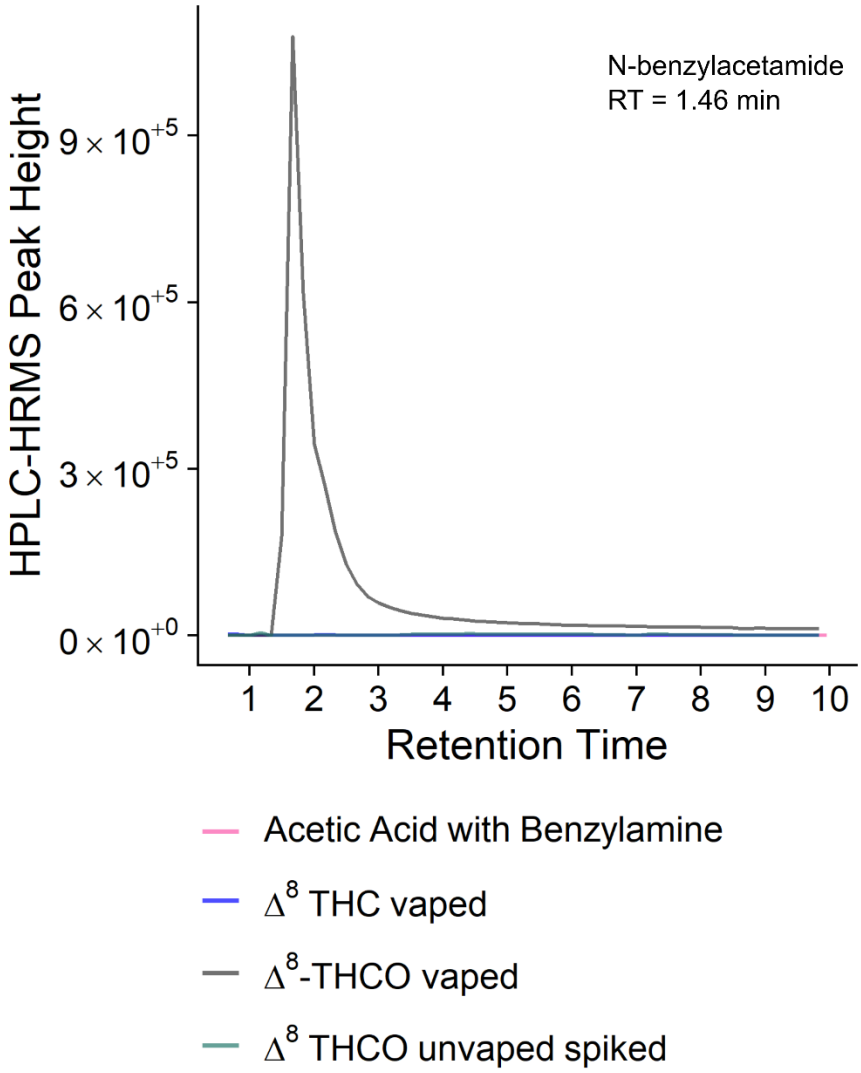


Figure S4: Extracted ion chromatograms for the N-Benzylacetamide peak detected by HPLC-HRMS for the vaped Δ^8-THCO experiment (grey), control data from vaping Δ^8-THC (blue), spiking the unvaped Δ^8-THCO distillate with trapping reagent benzylamine (green) and a control reaction of acetic acid with benzylamine (pink)

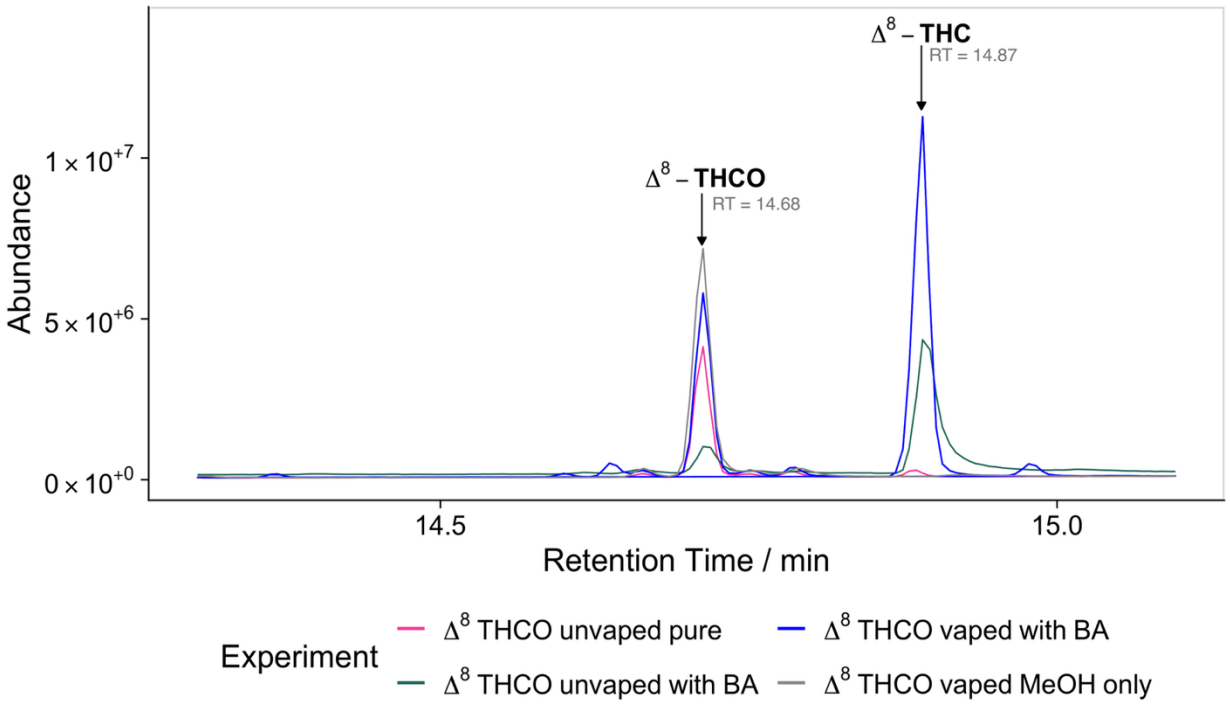


Figure S5: GC-MS chromatograms of Δ⁸-THCO distillate unvaped diluted in methanol (pink), in the vaping experiments with benzylamine (BA) trapping reagent (blue), in a control experiment of unvaped distillate spiked with benzylamine (BA) reagent (green) and in a vaping experiment without benzylamine (grey).

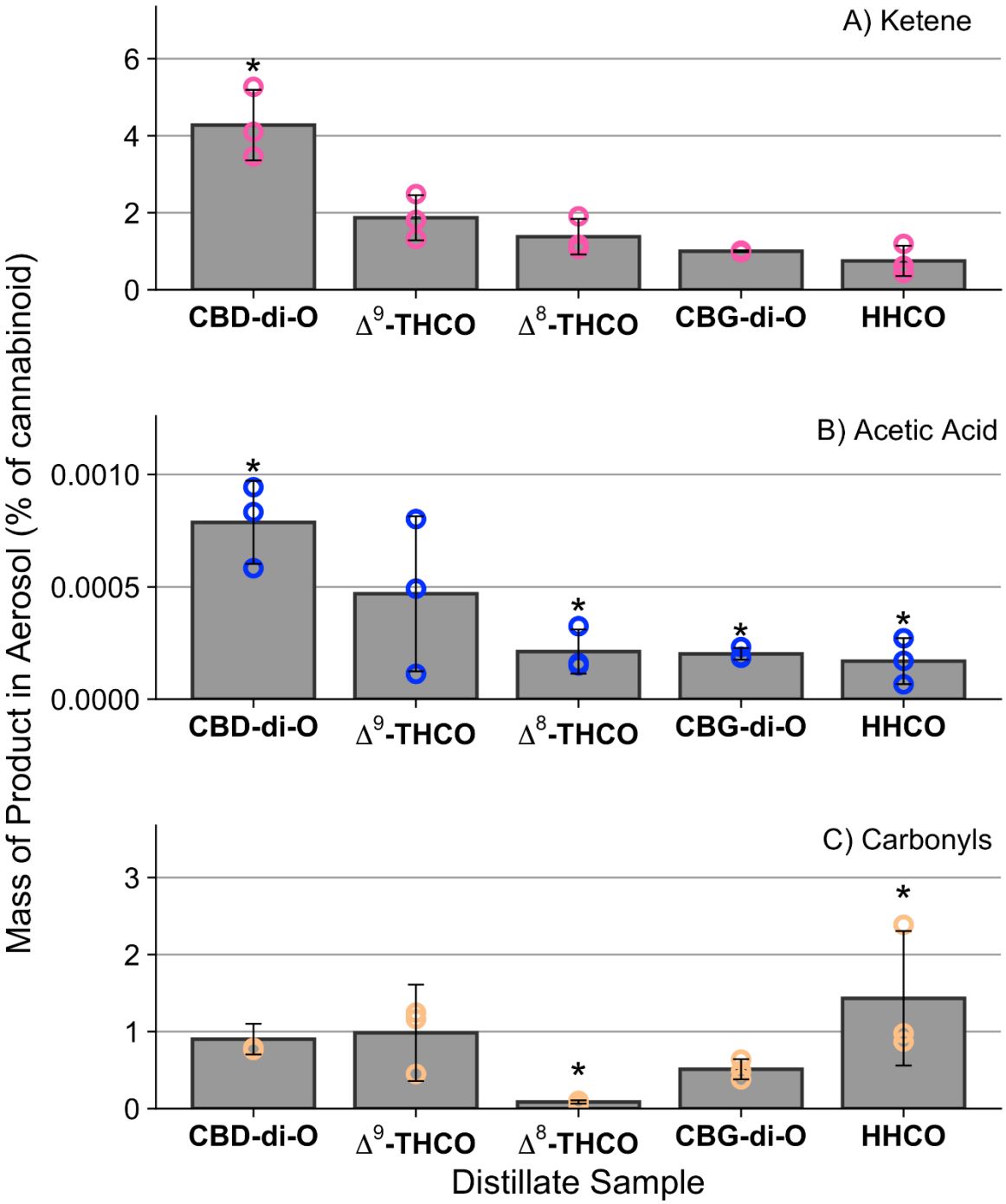
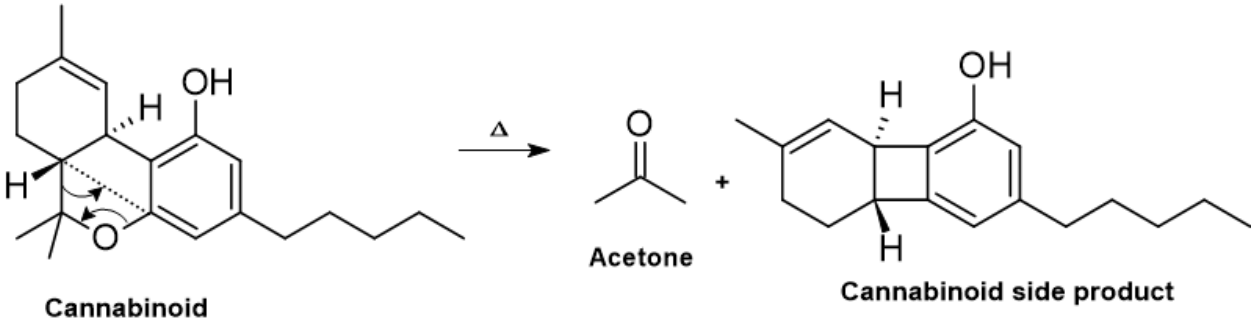


Figure S6: Product yields of A) ketene, B) acetic acid, and C) carbonyls by cannabinoid mass for the cannabinoid O-acetate distillates upon vaping. The axis labels are ordered by descending yield. The black asterisks indicate statistically significant differences determined by a one way ANOVA test. For A) CBD-di-O is statistically significant from all other distillates. For B) CBD-di-O is statistically significant from all other distillates except d9-THCO. For C) CBG-di-O and d8-THCO are statistically significant from CBD-di-O.



Scheme S1: A proposed direct thermal degradation pathway toward formation of the dominant carbonyl, acetone, from vaping cannabinoids and their O-acetate derivatives via the dimethyl carbon that is present in numerous of the tested cannabinoids. In the O-acetate analogues the phenyl moiety will have a O-C(O)CH_3 group in place of OH in the above scheme. Robertson et al.[1] proposed formation of acetone and other carbonyls via radical oxidation initiated pathways. Additional investigations are needed to confirm which pathway(s) are operative during vaping.

Table S1: Tabulated data for ketene concentration, standard deviation (S.D.), total cannabinoid found in 15 puffs, and the percentage mass of ketene normalized by cannabinoids. These determinations are performed in triplicate. The ketene data are not reported for non-acetylated cannabinoid vaped aerosol because they were all below detection limit.

Distillate vaped	Normalised ketene (µg/mg)	S.D. ketene (µg/mg)	Total mass cannabinoid (mg)	Percent mass of ketene (%)
CBD-di-O	42.75	9.16	2.03	4.28
CBG-di-O	10.02	0.23	13.60	1.00
HHCO	7.51	3.93	14.61	0.75
Δ^8-THCO	13.80	4.60	7.36	1.38
Δ^9-THCO	18.72	5.85	9.20	1.87

Table S2: Tabulated data for carbonyl concentration from O-acetylated cannabinoids, including carbonyl production yields, standard deviation (S.D.), and the percentage mass of carbonyl normalized by cannabinoids. These determinations were performed in triplicate. Total cannabinoid mass for the O-acetylated cannabinoids are reported in Table S1. Only carbonyls for which there are detectable signals from any of the vape samples are reported. ND = not detected.

Distillate vaped	Compound detected	Carbonyl production (µg/ mg)	S.D. Carbonyl production (µg/ mg)	Percent mass of carbonyl (%)
CBD-di-O	2-Butanone-DNPH	0.786	0.000	0.079
CBG-di-O	2-Butanone-DNPH	0.203	0.032	0.020
Δ^8-THCO	2-Butanone-DNPH	0.082	0.018	0.008
Δ^9-THCO	2-Butanone-DNPH	0.289	0.288	0.029
HHCO	2-Butanone-DNPH	0.070	0.000	0.007
CBD-di-O	Acetaldehyde-DNPH	0.319	0.000	0.032
CBG-di-O	Acetaldehyde-DNPH	0.119	0.023	0.012
Δ^8-THCO	Acetaldehyde-DNPH	0.027	0.000	0.003
Δ^9-THCO	Acetaldehyde-DNPH	0.319	0.135	0.032
HHCO	Acetaldehyde-DNPH	0.076	0.000	0.008
CBD-di-O	Acetic Acid-DNPH	0.008	0.002	0.001
CBG-di-O	Acetic Acid-DNPH	0.002	0.000	0.000
Δ^8-THCO	Acetic Acid-DNPH	0.002	0.001	0.000
Δ^9-THCO	Acetic Acid-DNPH	0.005	0.003	0.000
HHCO	Acetic Acid-DNPH	0.002	0.001	0.000
CBD-di-O	Acetone-DNPH	6.493	1.981	0.649
CBG-di-O	Acetone-DNPH	4.469	1.130	0.447
Δ^8-THCO	Acetone-DNPH	0.673	0.144	0.067
Δ^9-THCO	Acetone-DNPH	7.874	4.811	0.787
HHCO	Acetone-DNPH	13.937	8.602	1.394
CBD-di-O	Formaldehyde-DNPH	0.082	0.000	0.008
CBG-di-O	Formaldehyde-DNPH	ND	ND	ND
Δ^8-THCO	Formaldehyde-DNPH	ND	ND	ND
Δ^9-THCO	Formaldehyde-DNPH	0.040	0.025	0.004
HHCO	Formaldehyde-DNPH	0.031	0.005	0.003
CBD-di-O	Hexanal-DNPH	ND	ND	ND
CBG-di-O	Hexanal-DNPH	ND	ND	ND
Δ^8-THCO	Hexanal-DNPH	ND	ND	ND
Δ^9-THCO	Hexanal-DNPH	0.340	0.315	0.034
HHCO	Hexanal-DNPH	0.052	0.000	0.005
CBD-di-O	Methacrolein-DNPH	ND	ND	ND
CBG-di-O	Methacrolein-DNPH	0.014	0.006	0.001
Δ^8-THCO	Methacrolein-DNPH	ND	ND	ND
Δ^9-THCO	Methacrolein-DNPH	ND	ND	ND
HHCO	Methacrolein-DNPH	ND	ND	ND

CBD-di-O	Propionaldehyde-DNPH	0.551	0.000	0.055
CBG-di-O	Propionaldehyde-DNPH	0.161	0.020	0.016
Δ^8-THCO	Propionaldehyde-DNPH	0.032	0.000	0.003
Δ^9-THCO	Propionaldehyde-DNPH	0.441	0.257	0.044
HHCO	Propionaldehyde-DNPH	ND	ND	ND
CBD-di-O	Valeraldehyde-DNPH	0.198	0.000	0.020
CBG-di-O	Valeraldehyde-DNPH	0.054	0.037	0.005
Δ^8-THCO	Valeraldehyde-DNPH	ND	ND	ND
Δ^9-THCO	Valeraldehyde-DNPH	0.132	0.039	0.013
HHCO	Valeraldehyde-DNPH	0.049	ND	0.005
CBD-di-O	m-Tolualdehyde-DNPH	ND	ND	ND
CBG-di-O	m-Tolualdehyde-DNPH	ND	ND	ND
Δ^8-THCO	m-Tolualdehyde-DNPH	0.008	0.000	0.001
Δ^9-THCO	m-Tolualdehyde-DNPH	ND	ND	ND
HHCO	m-Tolualdehyde-DNPH	ND	ND	ND
CBD-di-O	n-Butyraldehyde-DNPH	0.584	0.000	0.058
CBG-di-O	n-Butyraldehyde-DNPH	0.093	0.060	0.009
Δ^8-THCO	n-Butyraldehyde-DNPH	0.043	0.042	0.004
Δ^9-THCO	n-Butyraldehyde-DNPH	0.398	0.380	0.040
HHCO	n-Butyraldehyde-DNPH	0.105	0.119	0.011

Table S3: Tabulated data for carbonyl concentration from non-acetylated cannabinoids, including total cannabinoid mass in 15 puffs, carbonyl production yields, and the percentage mass of carbonyl normalized by cannabinoids. These determinations were performed in a single replicate. Only carbonyls for which there are detectable signals from any of the vape samples are reported. ND = not detected.

Distillate vaped	Compound detected	Total mass cannabinoid (mg)	Carbonyl production (µg/ mg)	Percent mass of carbonyl (%)
CBD	Acetic Acid-DNPH	0.68	0.02	0.002
CBG	Acetic Acid-DNPH	11.96	0.00	0.000
Δ^8-THC	Acetic Acid-DNPH	0.16	0.08	0.008
Δ^9-THC	Acetic Acid-DNPH	0.18	0.05	0.005
HHC	Acetic Acid-DNPH	0.55	0.02	0.002
CBD	Acetaldehyde-DNPH	0.68	ND	ND
CBG	Acetaldehyde-DNPH	11.96	0.02	0.002
Δ^8-THC	Acetaldehyde-DNPH	0.16	1.35	0.135
Δ^9-THC	Acetaldehyde-DNPH	0.18	ND	ND
HHC	Acetaldehyde-DNPH	0.55	ND	ND
CBD	Acetone-DNPH	0.68	17.34	1.734
CBG	Acetone-DNPH	11.96	0.83	0.083
Δ^8-THC	Acetone-DNPH	0.16	65.07	6.507
Δ^9-THC	Acetone-DNPH	0.18	57.60	5.760
HHC	Acetone-DNPH	0.55	11.54	1.154
CBD	2-Butanone-DNPH	0.68	0.81	0.081
CBG	2-Butanone-DNPH	11.96	0.06	0.006
Δ^8-THC	2-Butanone-DNPH	0.16	3.26	0.326
Δ^9-THC	2-Butanone-DNPH	0.18	3.51	0.351
HHC	2-Butanone-DNPH	0.55	ND	ND
CBD	n-Butyraldehyde-DNPH	0.68	ND	ND
CBG	n-Butyraldehyde-DNPH	11.96	0.01	0.001
Δ^8-THC	n-Butyraldehyde-DNPH	0.16	0.84	0.084
Δ^9-THC	n-Butyraldehyde-DNPH	0.18	ND	ND
HHC	n-Butyraldehyde-DNPH	0.55	ND	ND
CBD	Valeraldehyde-DNPH	0.68	ND	ND
CBG	Valeraldehyde-DNPH	11.96	0.16	0.016
Δ^8-THC	Valeraldehyde-DNPH	0.16	ND	ND
Δ^9-THC	Valeraldehyde-DNPH	0.18	ND	ND
HHC	Valeraldehyde-DNPH	0.55	ND	ND
CBD	m-Tolualdehyde-DNPH	0.68	0.13	0.013
CBG	m-Tolualdehyde-DNPH	11.96	0.01	0.001
Δ^8-THC	m-Tolualdehyde-DNPH	0.16	0.79	0.079
Δ^9-THC	m-Tolualdehyde-DNPH	0.18	0.45	0.045
HHC	m-Tolualdehyde-DNPH	0.55	0.14	0.014

References

1. Robertson NE, Connolly J, Shevchenko N, Mascal M, Pinkerton KE, Nicklisch SCT, Nguyen TB. Chemical Composition of Aerosols from the E-Cigarette Vaping of Natural and Synthetic Cannabinoids. Chemical Research in Toxicology. 2024;37(12):1965-75. doi: 10.1021/acs.chemrestox.4c00326.