Supporting Information for:

Characterizing the Chemical Landscape in Commercial E-cigarette Liquids and Aerosols by Liquid Chromatography – High-Resolution Mass spectrometry

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Number of pages: 17; Tables: 5; Figures: 6

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Table S1: Orbitrap LC-MS instrumental parameters for full scan and dd-MS^2 in positive and negative polarity modes.

MS parameters				
Non-target analysis			**Compound confirmation**	
	Full Scan	**dd-MS^2 Scan**	**Full Scan**	**Parallel Reaction Monitoring Scan**
Polarity	**-/+**	**-/+**	**-/+**	**-/+**
Resolution (m/Δm at m/z 200)	120,000	60,000	60,000	15,000
AGC Target	3e6	1e5	3e6	1e5
Maximum IT (ms)	100	50	100	50
Loop count	N/A	5	N/A	N/A
Isolation window (m/z)	N/A	4.0 (positive ESI), 1.0 (negative ESI)	N/A	1.0 (positive and negative)
Stepped NCE	N/A	20, 30, 40	N/A	30, 65, 100
Intensity threshold	N/A	1.6e5	N/A	N/A
Dynamic exclusion (s)	N/A	10.0	N/A	N/A
Scan range (m/z)	80-1200	80-1200	100-500	100-500
Inclusion list	N/A	N/A	N/A	11 compounds of interest

Electrospray ionization (ESI) source parameters	
Spray voltage (kV)	3 (positive), 2 (negative)
Capillary temperature (°C)	300
Sheath gas (au)	20
Aux gas (au)	5
S-lens RF level	60

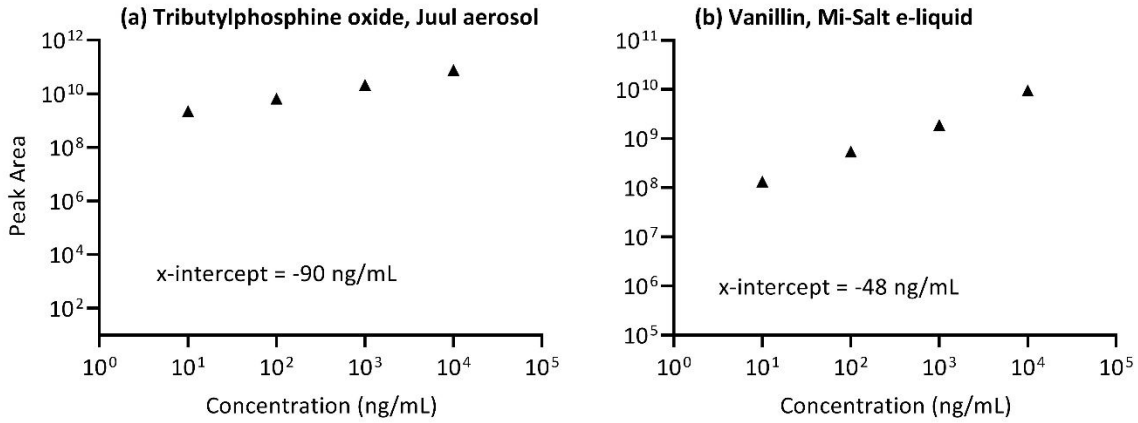

Figure S1: Example standard addition calibration curves for (a) tributylphosphine oxide in Juul aerosol and (b) vanillin in Mi-Salt e-liquid. Un-spiked calibrators (0 ng/mL) not shown due to log scale.

Table S2: Overview of quality control mix compounds and analytical results. Mass error is calculated based on the detected adduct mass (n=8 replicates).

Analyte	Molecular formula	Log Kow**	Ion polarity response (+/-)	Theoretical mass $[M+H]^+$	Detected $[M+H]^+$	Mass error (ppm)	RT (min) Mean ± SD	Abundance Mean ± SD
Epinephrine	$C_9H_{13}NO_3$	-1.4	+	184.0968	184.0967	0.5	1.81 ± 0.01	$3.9x10^8 \pm 1.4x10^8$
Acephate	$C_4H_{10}NO_3PS$	-0.8	+	184.0192	184.0192	0.5	9.62 ± 0.49	$1.0x10^7 \pm 5.8x10^6$
Iohexol	$C_{19}H_{26}I_3N_3O_9$	-3.0	+	821.8876	821.8882	1.0	10.35 ± 0.41	$1.1x10^8 \pm 3.6x10^7$
Atenolol	$C_{14}H_{22}N_2O_3$	0.2	+	267.1703	267.1703	0.5	11.34 ± 0.13	$4.7x10^9 \pm 4.8x10^8$
Caffeic acid*	$C_9H_8O_4$	1.2	-	179.0350	179.0343	2.8	14.01 ± 0.14	$2.7x10^9 \pm 3.0x10^8$
Caffeine	$C_8H_{10}N_4O_2$	-0.1	+	195.0877	195.0877	0.4	14.19 ± 0.08	$7.7x10^9 \pm 8.0x10^8$
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	-1.1	+	332.1405	332.1402	0.5	14.36 ± 0.12	$1.3x10^8 \pm 1.7x10^8$
Oxytetracycline*	$C_{22}H_{24}N_2O_9$	-1.6	-	459.1409	459.1408	0.3	15.72 ± 0.31	$1.7x10^8 \pm 9.3x10^7$
Dicamba*	$C_8H_6Cl_2O_3$	2.2	-	218.9621	218.9617	1.2	16.34 ± 0.16	$8.8x10^7 \pm 8.8x10^7$
(-)-Erythromycin	$C_{37}H_{67}NO_{13}$	2.7	+	734.4685	734.4678	0.6	17.19 ± 0.10	$7.2x10^7 \pm 5.3x10^7$
Carbamazepine	$C_{15}H_{12}N_2O$	2.5	+	237.1022	237.1020	0.6	17.20 ± 0.03	$1.2x10^{10} \pm 1.3x10^9$
4-Hydroxybenzaldehyde*	$C_7H_6O_2$	1.4	+	123.0441	123.0441	0.5	17.72 ± 0.19	$4.5x10^6 \pm 4.3x10^6$
Boscalid	$C_{18}H_{12}Cl_2N_2O$	4.9	+	343.0399	343.0396	0.5	18.54 ± 0.04	$5.8x10^9 \pm 5.5x10^8$
Avobenzone	$C_{20}H_{22}O_3$	4.8	+	311.1642	311.1637	1.5	19.84 ± 0.02	$4.3x10^8 \pm 2.0x10^8$
Linoleic acid*	$C_{18}H_{32}O_2$	6.8	-	279.2330	279.2328	0.4	22.81 ± 0.03	$1.7x10^9 \pm 2.4x10^9$

Table S3: Spikes of quality control mix into PG/VG base and Juul e-liquid.

	PG/VG spike (n=2)		Juul spike (n=1)		Aqueous (n=8)	
	Mean abundance	RT (min) range	Abundance	RT (min)	Abundance mean ± SD	RT (min) mean ± SD
Epinephrine	$6.5x10^8$	1.80-1.80	$2.3 x10^8$	1.77	$3.9x10^8 \pm 1.4x10^8$	1.81 ± 0.01
Acephate	$6.8 x10^6$	8.79-9.48	$4.3 x10^5$	9.71	$1.0x10^7 \pm 5.8x10^6$	9.62 ± 0.49
Iohexol	$7.5 x10^7$	9.77-10.29	$7.1 x10^7$	10.60	$1.1x10^8 \pm 3.6x10^7$	10.35 ± 0.41
Atenolol	$4.5 x10^9$	11.21-11.35	$3.3 x10^9$	11.44	$4.7x10^9 \pm 4.8x10^8$	11.34 ± 0.13
Caffeic acid	$2.1 x10^9$*	13.86	ND	ND	$2.7x10^9 \pm 3.0x10^8$	14.01 ± 0.14
Caffeine	$6.0 x10^9$	14.05-14.14	$4.1 x10^9$	14.18	$7.7x10^9 \pm 8.0x10^8$	14.19 ± 0.08
Ciprofloxacin	$4.5 x10^8$	14.13-14.31	$6.2 x10^8$	14.18	$1.3x10^8 \pm 1.7x10^8$	14.36 ± 0.12
Oxytetracycline	$7.3 x10^7$*	16.01	ND	ND	$1.7x10^8 \pm 9.3x10^7$	15.72 ± 0.31
Dicamba	$1.7 x10^8$*	16.14	ND	ND	$8.8x10^7 \pm 8.8x10^7$	16.34 ± 0.16
(-)-Erythromycin	$1.4 x10^8$*	17.12-17.13	$5.1 x10^6$	17.14	$7.2x10^7 \pm 5.3x10^7$	17.19 ± 0.10
Carbamazepine	$7.8 x10^9$	17.16-17.18	$7.5 x10^9$	17.19	$1.2x10^{10} \pm 1.3x10^9$	17.20 ± 0.03
4-Hydroxybenzaldehyde	$4.2 x10^9$	17.46-17.51	$6.0 x10^9$	17.48	$4.5x10^6 \pm 4.3x10^6$	17.72 ± 0.19
Boscalid	$2.7 x10^9$	18.46-18.47	$3.7 x10^9$	18.52	$5.8x10^9 \pm 5.5x10^8$	18.54 ± 0.04
Avobenzone	$7.1 x10^7$	19.81-19.84	$6.5 x10^8$	19.82	$4.3x10^8 \pm 2.0x10^8$	19.84 ± 0.02
Linoleic acid	$2.4 x10^8$*	22.79	ND	ND	$1.7x10^9 \pm 2.4x10^9$	22.81 ± 0.03

*n=1

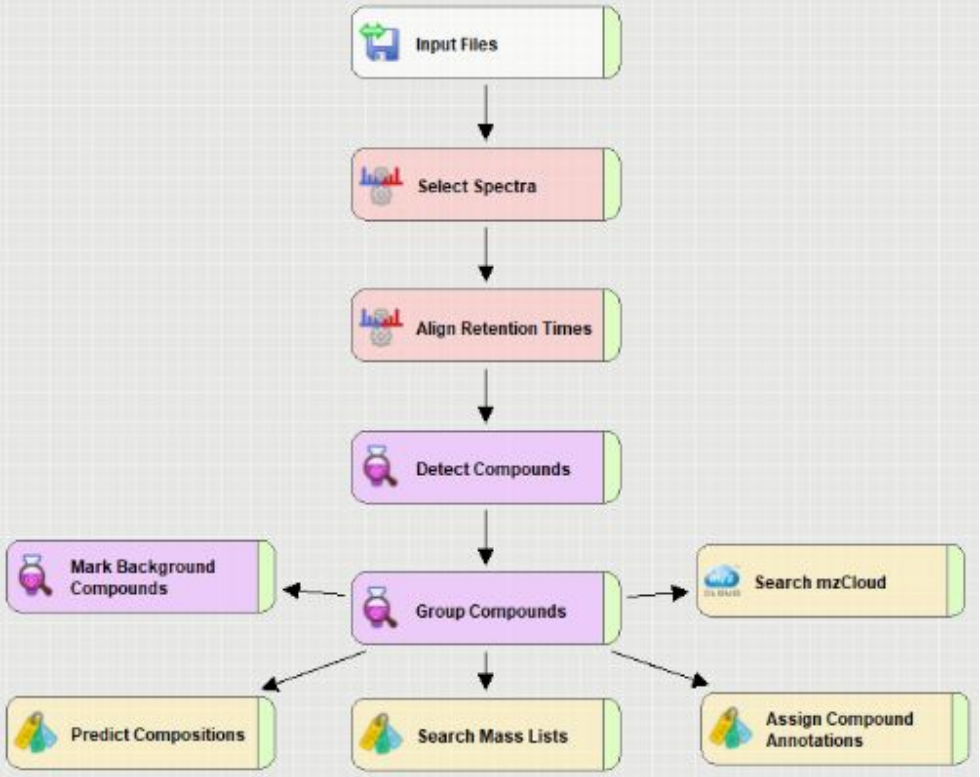

Figure S2: Compound Discoverer workflow tree.

Table S4A: Compound Discoverer workflow parameters used.

Select spectra	
Precursor selection	Use MS(n-1) Precursor
Use Isotope Pattern in Precursor Reevaluation	True
Provide Profile Spectra	Automatic
Store Chromatograms	False
Lower/Upper RT Limits	lowest and highest available RT used
First/Last Scan	first and last available scan used
Ignore Specified Scans	
Lowest/Highest Precursor Charge State	0 – no maximum
Min – Max Singly-Charged Precursor Mass	50 - 5000 Da
Total Intensity Threshold	0
Minimum Peak Count	1
Mass Analyzer	(Not specified)
MS Order	Any
Activation Type	(Not specified)
Min -Max Normalized Collision Energy	0 - 1000
Scan Type	Any
Polarity Mode	Any
Unrecognized Charge Replacements	1
Unrecognized Mass Analyzer Replacements	ITMS
Unrecognized MS Order Replacements	MS2
Unrecognized Activation Type Replacements	CID
Unrecognized Polarity Replacements	+
Unrecognized MS Resolution @200 Replacements	60000
Unrecognized MSn Resolution @200 Replacements	30000
Align Retention Times	
Alignment Model	Adaptive curve
Alignment Fallback	Use Linear Model
Maximum Shift (min)	2
Shift Reference File	True
Mass Tolerance	5 ppm
Remove Outlier	True

Table S4B: Compound Discoverer workflow parameters used.

Detect Compounds	
Mass Tolerance	5 ppm
Intensity Tolerance (%)	30
S/N Threshold	3
Min Peak Intensity	100,000
Ions	$[2M+H]^{+1}$; $[2M+K]^{+1}$; $[2M+Na]^{+1}$; $[2M-H]^{-1}$; $[M+2H]^{+2}$; $[M+H]^{+1}$; $[M+H+K]^{+2}$; $[M+H+Na]^{+2}$; $[M+H-H2O]^{+1}$; $[M+H-NH_3]^{+1}$; $[M+K]^{+1}$; $[M+Na]^{+1}$; $[M-2H]^{-2}$; $[M-2H+K]^{-1}$; $[M-H]^{-1}$
Min Element Counts to be Used for Isotope Pattern Matching	C H
Max Element Counts	C90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5
Filter peaks	True
Max. Peak Width	0.5
Remove Singlets	True
Min. # Scans per Peak	5
Min. # Isotopes	2
Group Compounds	
Mass Tolerance	5 ppm
RT Tolerance	0.2 min
Preferred Ions for Selecting Fragmentation Data	$[M+H]^{+1}$; $[M-H]^{-1}$
Mark Background Compounds	
Max Sample/ Blank	5
Max Blank/ Sample	Skip rule
Hide Background	True
Assign Compound Annotations	
Mass Tolerance	5 ppm
Data Source Order	mzCloud search, ChemSpider Search, Predicted Compositions, MassList Search
Scoring Rules: Use mzLogic	True
Scoring Rules: Use Spectral Distance	True
SFit Threshold	20
SFit Range	20

Table S4C: Compound Discoverer workflow parameters used.

Search mzCloud	
Compound Classes	All
Precursor Mass Tolerance	10 ppm
FT Fragment Mass Tolerance	10 ppm
IT Fragment Mass Tolerance	0.4 Da
Library	Autoprocessed; Reference
Post Processing	Recalibrated
Max. # Results	10
Annotate Matching Fragments	True
DDA: Identity Search	HighChem HighRes
DDA: Match Activation Type	True
DDA: Match Activation Energy	Match with tolerance of +/- 20
DDA: Apply Intensity Threshold	True
DDA: Similarity Search	None
DDA: Match Factor Threshold	60
Use DIA Scans for Search	False
Search ChemSpider	
Databases	Drugbank, EPA DSSTox, EPA Toxcast, KEGG, MassBank, MeSH, NIST Chemistry WebBook, PubMed
Search Mode	By formula or mass
Mass Tolerance	5 ppm
Max # Results per Compound	100
Max # Predicted Compositions	3

Table S4D: Compound Discoverer workflow parameters used.

Predict compositions	
Mass Tolerance	5 ppm
Minimum Element Counts to be Used for Prediction	C H
Maximum Element Counts	C90 H190 Br3 Cl4 N10 O18 P3 S5
Min – Max RDBE	0 - 40
Min – Max H/C	0.1 – 3.5
Max # Candidates	10
Max # Internal Candidates	200
Pattern Matching: Intensity Tolerance	30%
Pattern Matching: Intensity Threshold	0.1%
Pattern Matching: S/N Threshold	3
Pattern Matching: Min. Spectral Fit	30%
Pattern Matching: Min. Pattern Coverage	90%
Pattern Matching: Use Dynamic Recalibration	True
Use Fragments Matching	True
Fragments Matching: Mass Tolerance	5 ppm
Fragments Matching: S/N Threshold	3
Search Mass Lists	
Mass Lists	12 lists including suspect lists for flame retardants, flavonoids, phenols, pesticides, parabens, and others derived from EPA Comptox and other sources
Use RT	True
RT Tolerance	2 min
Mass Tolerance	5 ppm
Post-Processing Nodes	Differential Analysis, Descriptive Statistics
Differential Analysis: Log10 Transform Values	True

Table S5: Non-target workflow results for known compounds in the QC mixture and reference standard mixture. Sfit% and mzCloud best match scores were obtained from a single CD analysis of data from multiple runs using the non-target instrumental method. For the standard mix, data from aerosols and e-liquids over two runs was included. For the QC mix, data from all replicates (n=8) over 4 runs were included.

		Compound Discoverer	
Analyte	**Molecular formula**	**Mean SFit (%)**	**Mean mzCloud score (%)**
QC Mixture (n=8)			
Epinephrine	$C_9H_{13}NO_3$	81	80
Acephate	$C_4H_{10}NO_3PS$	No results	No results
Iohexol	$C_{19}H_{26}I_3N_3O_9$	No results	No results
Atenolol	$C_{14}H_{22}N_2O_3$	55	96
Caffeic acid	$C_9H_8O_4$	86	85
Caffeine	$C_8H_{10}N_4O_2$	67	97
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	No results	No results
Oxytetracycline	$C_{22}H_{24}N_2O_9$	No results	No results
Dicamba	$C_8H_6Cl_2O_3$	No results	No results
(-)-Erythromycin	$C_{37}H_{67}NO_{13}$	91	87
Carbamazepine	$C_{15}H_{12}N_2O$	84	92
4-Hydroxybenzaldehyde	$C_7H_6O_2$	81	81
Boscalid	$C_{18}H_{12}Cl_2N_2O$	48	No results
Avobenzone	$C_{20}H_{22}O_3$	86	72
Linoleic acid	$C_{18}H_{32}O_2$	70	95
Standards Mixture			
Caffeine	$C_8H_{10}N_4O_2$	61	96
Isophorone	$C_9H_{14}O$	49	93
Tributyl O-acetylcitrate (Citroflex A 4)	$C_{20}H_{34}O_8$	81	92
Tributylphosphine oxide	$C_{12}H_{27}OP$	52	86
Triethyl citrate (Citroflex 2)	$C_{12}H_{20}O_7$	65	94
Vanillin	$C_8H_8O_3$	47	91
Harmaline	$C_{13}H_{14}N_2O$	53	85

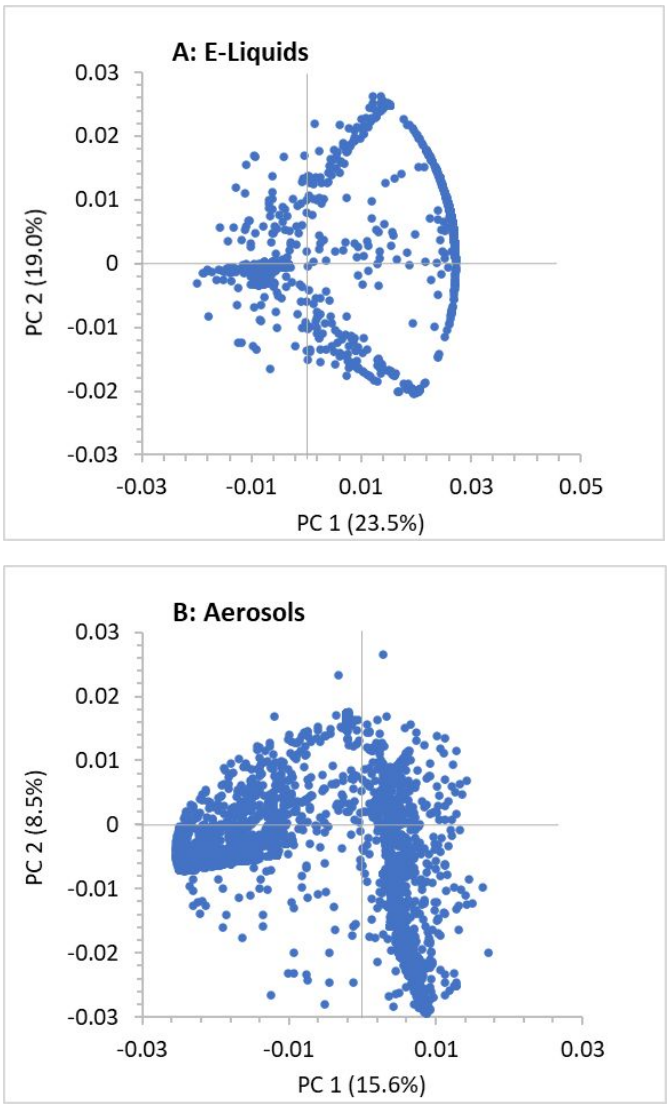

Figure S3: PCA loading plots for (A) e-liquids and (B) aerosols (vaping session 1) of Juul, Mi-Salt (Smok), and Vuse.

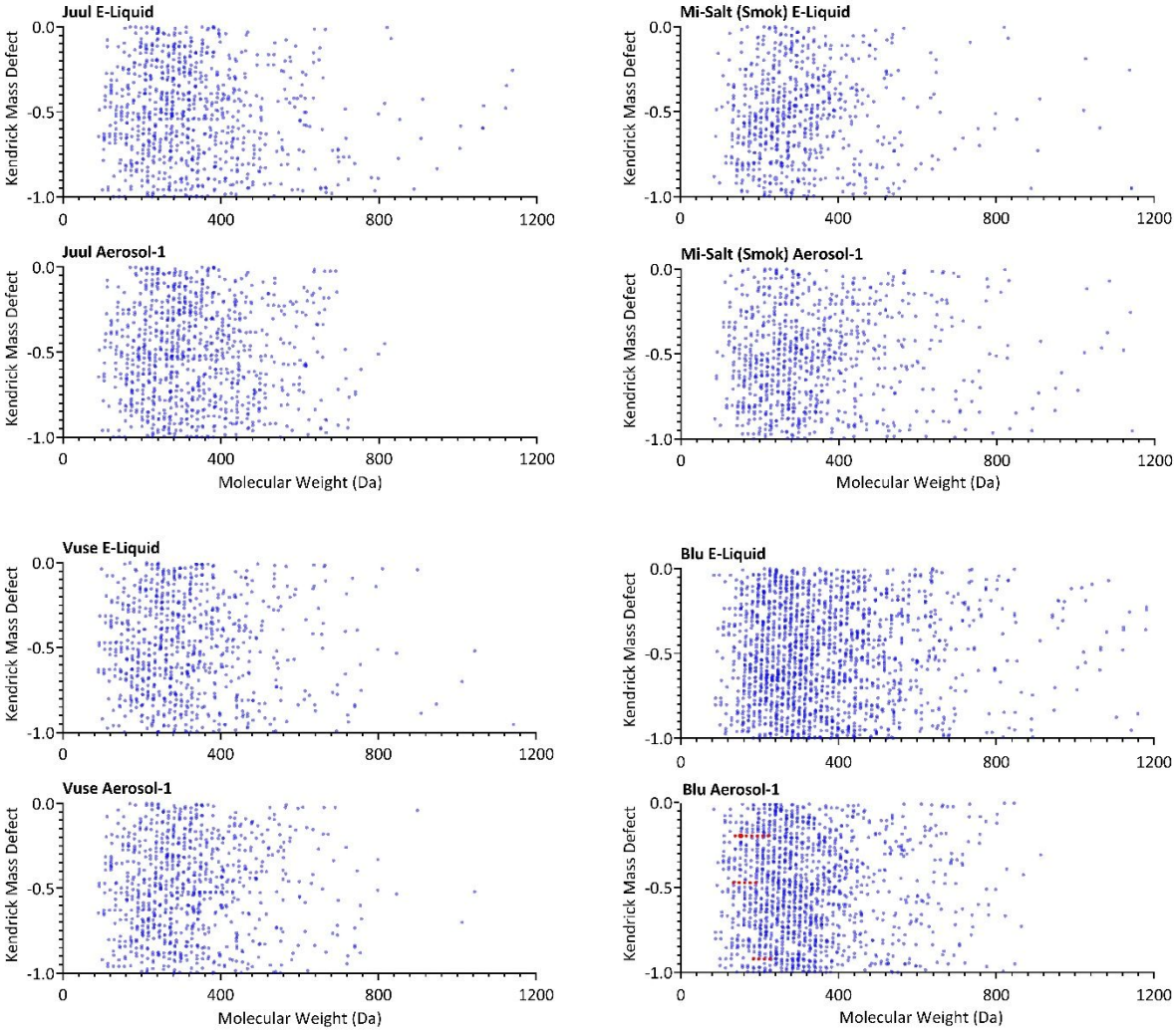

Figure S4: Kendrick Mass Defect plots for methylene homologous series in e-liquids and aerosols (vaping session 1) of all commercial products analyzed. Red symbols in Blu Aerosol-1 represent three series of homologous compounds detected only after vaping in Blu, all classified as lipid-like by MSCC and sharing similar retention times.

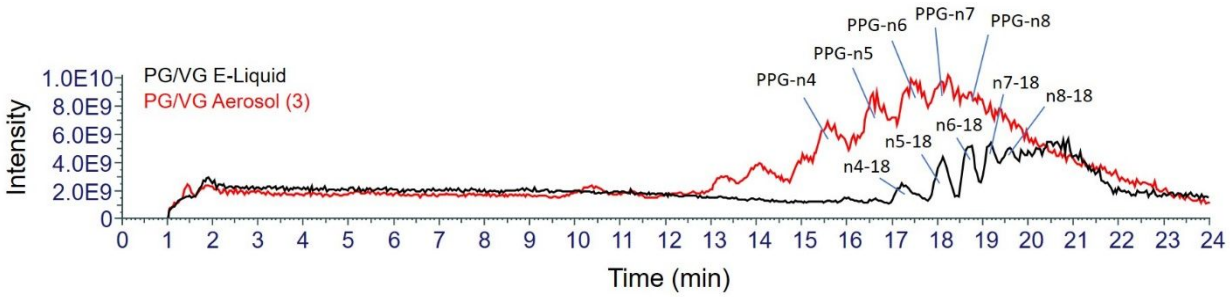

Figure S5: Total ion chromatograms for PG/VG, both the e-liquid and aerosol (interval 3), with polypropylene glycol (PPG) molecules at various chain lengths (e.g., PPG-n4 contains 4 PPG units) labeled.

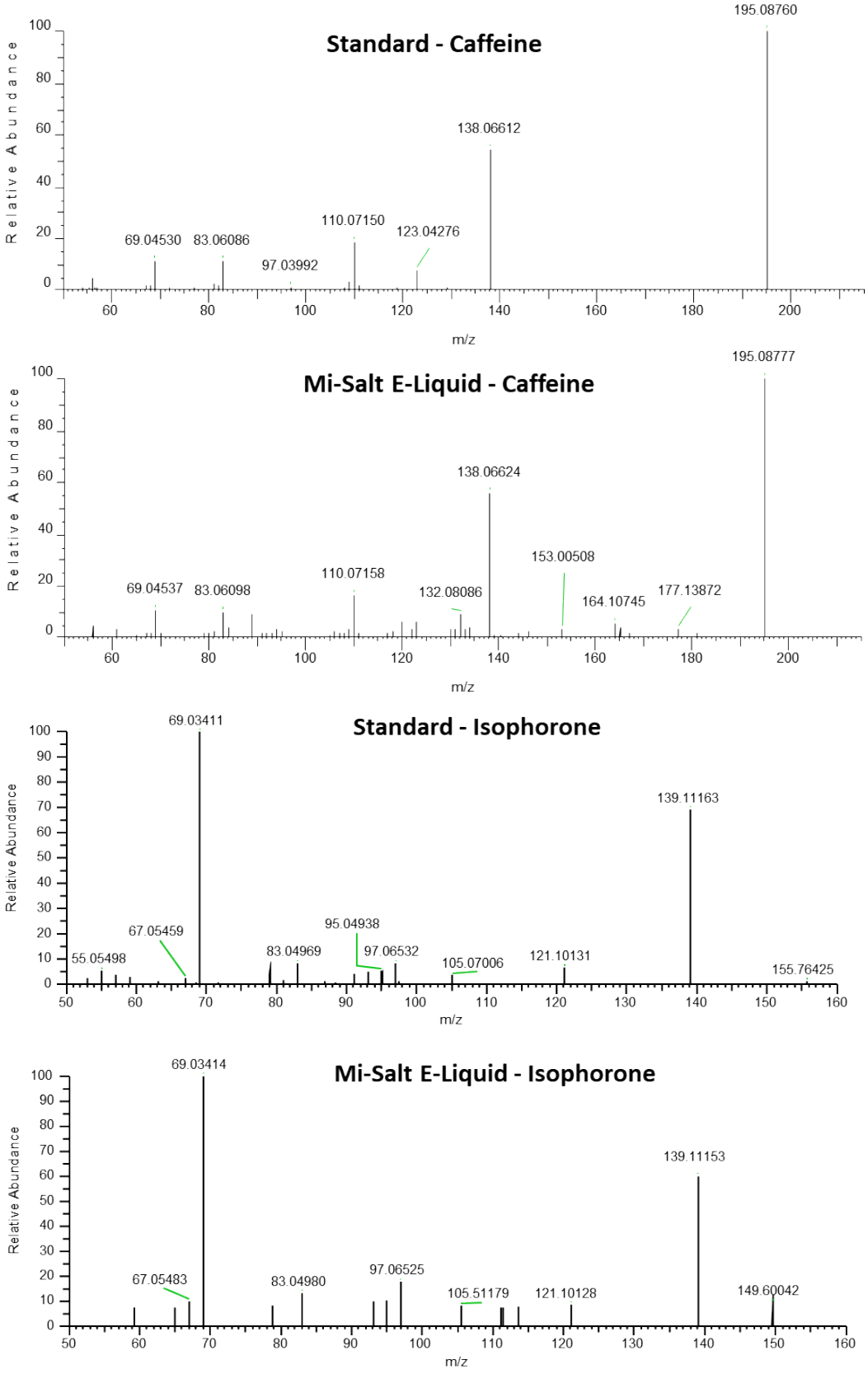

Figure S6A: MS^2 spectra of reference standard and e-cig sample for (top) caffeine, (bottom) isophorone.

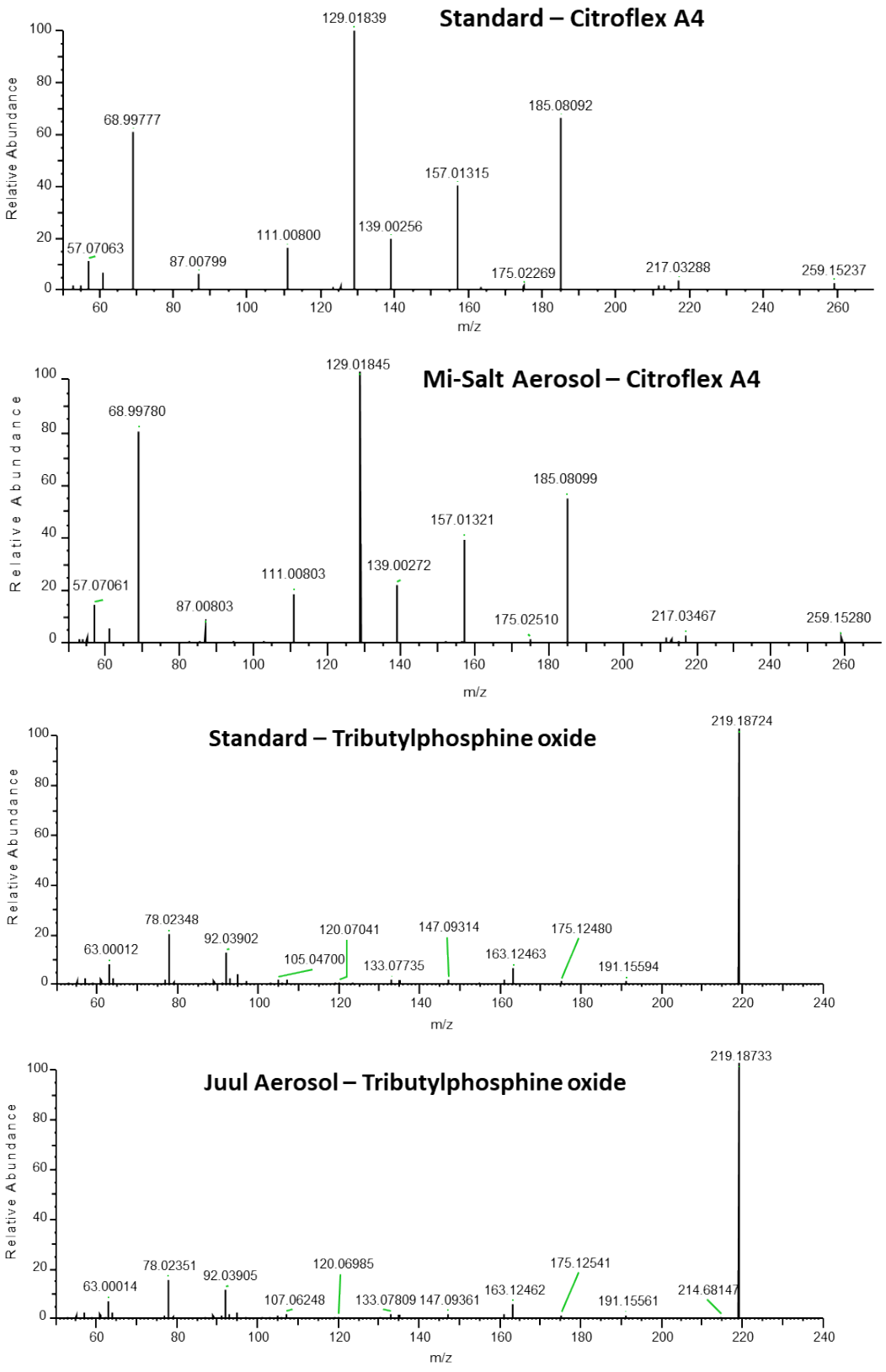

Figure S6B: MS^2 spectra of reference standard and e-cig sample for (top) citroflex A4, (bottom) tributylphosphine oxide.

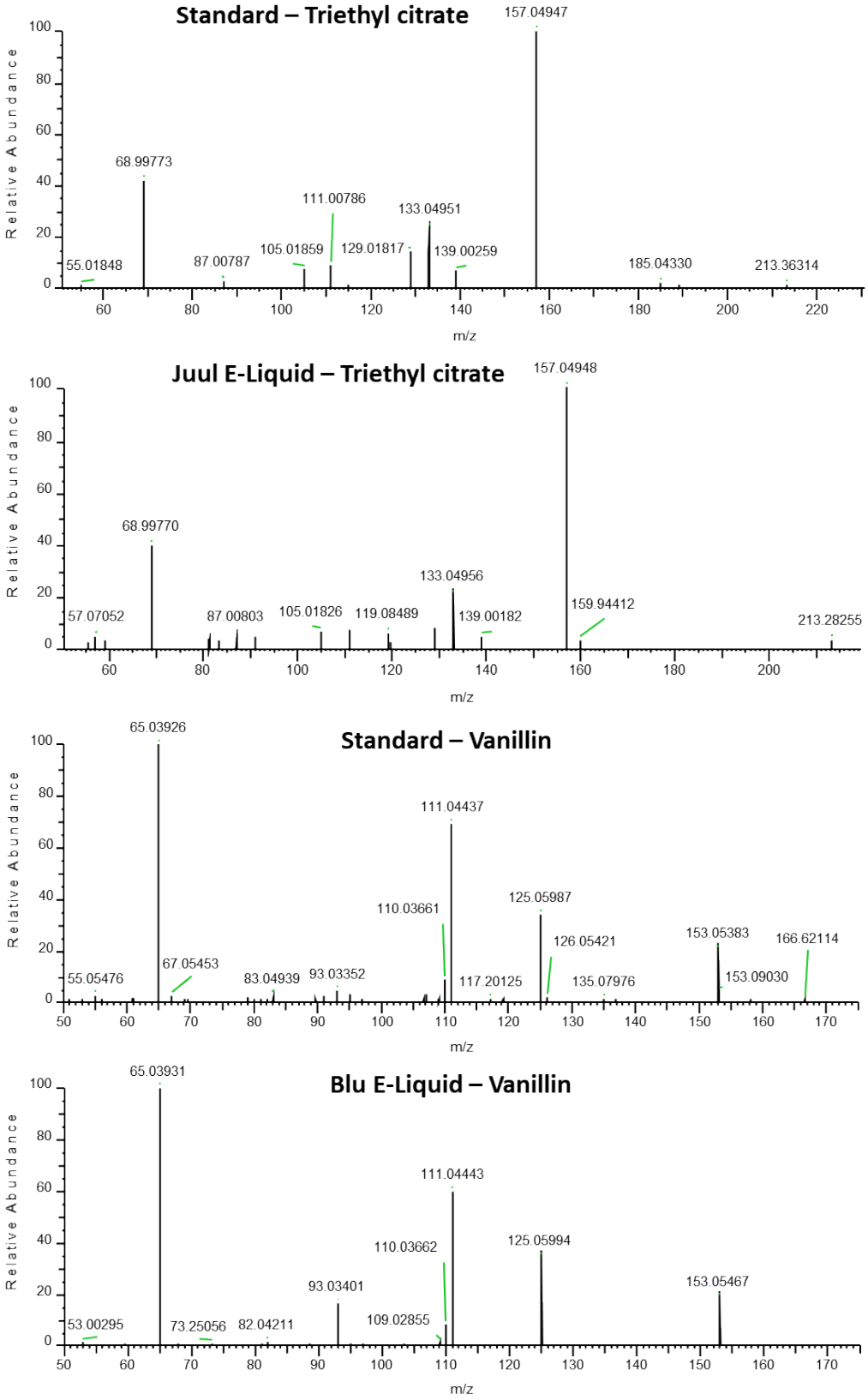

Figure S6C: MS² spectra of reference standard and e-cig sample for (top) triethyl citrate, (bottom) vanillin.

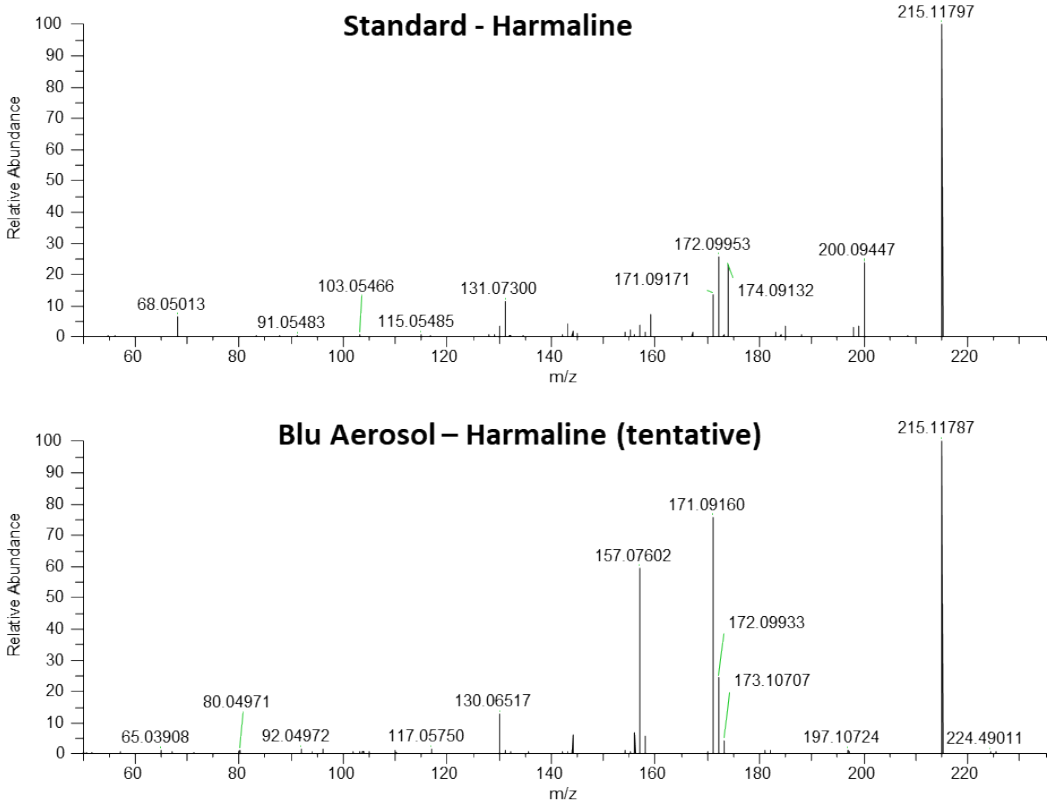

Figure S6D: MS^2 spectra of reference standard and e-cig sample for harmaline.