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E-cigarette Case Study

Released by:

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Dear Customer,

Please find enclosed the test results for your samples described as:

1. E-cigarette Mouthpiece

The following test was performed:

1. Quadrupole Time of Flight Liquid Chromatography Mass Spectrometry (QTOF-LCMS)
2. Quadrupole Time-of-Flight Gas Chromatography Mass Spectrometry (QTOF GCMS)
3. Headspace Gas Chromatography Mass Spectrometry (HGCMS)

Objective

The objective of this work was to investigate the chemistry of extractable compounds present in the provided device.

Summary of Results

The provided samples were extracted with ethanol, water and hexane. The resulting extracts were analyzed by HGCMS, QTOF-GCMS and QTOF-LCMS. Method blank controls were created using the same extraction solvents and the glassware used in the sample extractions. The results are summarized in **Table 1** to **Table 7**.

Individual Test Results

A summary of the individual test results is provided below. All accompanying data, including spectra, has been included in the data section of this report.

Sample Preparation

Materials	CAS	Manufacturer	Lot (Expiration)
Distilled Ethanol	64-17-5	Pharmco	Lot C16A11002-00REZ-AC; Batch WO112961 (05/17/19)
Distilled Water (H_2O)	7732-18-5	Jordi Labs (ID 916)	
Distilled Hexane	110-54-3	Pharmco	Lot C15J16DRM-000HX95; Batch WO121935 (10/22/16)

The mouthpiece of the E-cigarette

Each mouthpiece of the E-cigarette (~1.5 g) wascut in half and placed in 20 mL scintillation vials. 5.03 mL of the extraction solvent (ethanol, water or hexane) was added into the vials. For ethanol and hexane extractions, the extraction vials were closed and placed in an oven at 50 ±2 ºC with shaking at 50 rpm. For water extraction, the extraction vial was closed and placed in an oven at 70 ±2 ºC with shaking at 50 rpm. Extraction solvent blank controls were prepared using the same solvent lots and volumes of solvents. These controls were incubated at the same temperatures with shaking at 50 rpm using the same equipment as the sample extractions. After a period of 72 ±2 hours, all samples and blanks were cooled to room temperature.

MS Data Interpretation

Mass spectral identifications are based on comparison with the NIST spectral library of over 796,613 compounds as well as Jordi proprietary databases. Manual data review is also conducted to confirm the database identifications. This includes a review of the predominate ions in the mass spectrum of each unknown followed by confirmation that these ions are also observed in the database spectra. Ion intensity ratios and chromatographic correlations of ion intensities are also considered when evaluating the match quality as appropriate.

If high mass accuracy data is available (QTOF-GCMS or QTOF-LCMS), molecular formula generation (MFG) can be conducted to determine the best matching elemental composition for the individual ions. MSMS spectra are also examined to aid in identification.

A rating of high, medium, low has been assigned in our identifications. These levels are assigned to provide an indication of the confidence level associated with a given identification. As stated in USP 1663, "Given the number and chemical diversity of organic extractables, it is unreasonable to expect that authentic reference compounds will be available (or can be made available) to confirm every identification. It is therefore necessary that levels of identification confidence be established and appropriately utilized." The data typically utilized from GC/MS and LC/MS analyses includes:

a) Mass spectrometric fragmentation behavior (MSMS)
b) Confirmation of molecular weight
c) Confirmation of elemental composition
d) Mass spectrum matches automated library or literature spectrum
e) Mass spectrum and chromatographic retention index match authentic reference compound

A low identification confidence means that the class of molecule cannot be identified based on the data obtained. A Medium identification confidence means that data has been obtained that is consistent with a class of molecules only. A high identification confidence indicates a strong match for a particular compound. Objective measures have been given the greatest weighting when determining the rating. This includes identifications for which all known fragment ions are observed, correct exact mass, correct isotope spacing and ratios or confirmation by a second method.

QTOF LCMS

Background: QTOF-LCMS combines high mass accuracy time of flight mass spectroscopy with the power of a liquid chromatography separation to provide detailed information about the elemental composition of unknowns.

The presence of an additional quadrupole mass spectrometer (Q) provides the added capability to perform fragmentation experiments. This increases the confidence of unknown identification. It is preferable that a standard of the suspected unknown be analyzed under identical conditions as the sample. If the fragmentation patterns, high accuracy mass data, isotope patterns and LC retention times match for the unknown and standard then there is a very high probability that the identification is correct. It is possible to gain significant information about the structure of an unknown, even in cases in which standards are not available by using the molecular formula generation (MFG) algorithms contained in the Mass Hunter qualitative software.

LCMS requires that the molecule of interest be ionized. Thus, data is typically plotted in positive and negative modes indicating the charge on the ions. Ion formation is accomplished through the formation of a molecular adduct using a charge carrying species. Typical charge carriers in positive ion mode include H^+, Na^+, K^+, NH_4^+ etc. Thus the observed mass is typically the mass of the compound plus the mass of the charge carrier.

The nature of the mobile phase and the ionization conditions determine the ions formed. In negative ion, the loss of hydrogen is generally observed which results in the loss of one mass unit (1.0078 amu). Other transformations are also possible including dehydration, dimer formation, etc.

A number of plots are used to aid in interpreting QTOF-LCMS data. This includes Base Peak Chromatograms (BPC), Extracted Ion Chromatograms (EIC), Extracted Compound Chromatogram (ECC), Mass spectra (MS) and Product Ion Spectra (MSMS). A BPC is formed by plotting the most intense ion at a given retention time. This spectrum is particularly useful for identifying the retention time of unknowns. EICs are formed by plotting a single mass at all retention times. This could be considered a plot of peak intensity (~compound concentration) for a single compound (and its isomers) versus retention time. ECC's are the sum of all the ions determined to be related to a single compound.

MS spectra plot the observed masses and their intensities at a single retention time. MS/MS spectra show the fragmentation pattern for a single compound. Mass Spectra plot the mass to charge ratio (m/z) and not the mass of the compound.

All structures indicated represent best estimates based on the data observed. In most cases the MS/MS fragmentation spectra have been consulted briefly to aid in identification of possible structures.

Sample Preparation

Ethanol and water extracts were subjected to QTOF-LCMS analysis without further preparation. Solvent exchange was performed by mixing 200 µL of the hexane extract with 1 mL of isopropanol and then concentrating the mixture to 200 µL at 50 ºC under a nitrogen stream prior to QTOF-LCMS analysis. Control extracts were analyzed using the same methodology.

Results

191 compounds were detected by LCMS from the hexane extraction sample. 86 compounds were detected by LCMS from the ethanol extraction sample. 5 compounds were detected by LCMS from the water extraction. The major componentin the ethanol and hexane extracts were consistent with cyclic siloxanes, linear siloxane related compounds and polyethylene glycol related compounds. In addition, compounds consistent with fatty acids and amides were also detected in the extracts.

Table 1-3 provides a summary of the LCMS results for the extraction samples. The top 25 compounds with the highest MS peak height have been identified along with all siloxane related compounds and polyethylene glycol related compounds for the purposes of this case study. **Figure 1-6** provide overlays of the base peak chromatograms (BPCs) obtained in positive and negative ionization modes, respectively.

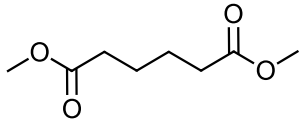
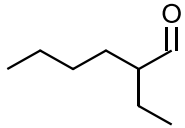
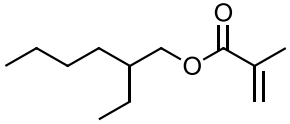
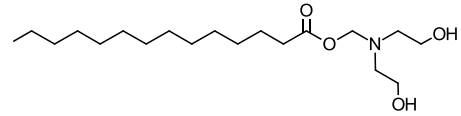
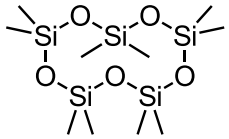
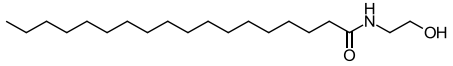
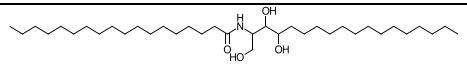
Table 1. Summary of LCMS Results (Hexane Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
2.497	175.0965		174.0891	$C_8\ H_{14}\ O_4$	98.01	0.78	 Dimethyl adipate	627-93-0	Medium
4.071	129.1275		128.1203	$C_8\ H_{16}\ O$	43.75	-1.09	 2-Ethyl-1-hexanal	123-05-7	Low
4.079	239.079		216.1726	$C_{12}H_{24}O_3$	99.73	-0.13	Unknown 1		Low
4.092	199.1693		198.162	$C_{12}\ H_{22}\ O_2$	99.72	-0.1	 2-Ethylhexyl methacrylate	688-84-6	Medium
4.582-4.776	*Var.*		*Var.*	$-C_2H_4O-$	-	-	PEG and related compounds (MSMS Fragment at 89.0589)		Medium
5.171		344.2808	345.2881	$C_{19}H_{39}NO_4$	99.23	-0.41	 [Bis(2-hydroxyethyl)amino]methyl myristate	88519-61-3	Low
5.371	371.1006		370.0932	$C_{10}\ H_{30}\ O_5\ Si_5$	91.58	1.92	 Decamethylcyclopentasiloxane (D5)	208-764-9	Medium
5.383		372.3124	327.3141	$C_{20}H_{41}NO_2$	98.85	-1.18	 Stearic ethanolamide	203-883-2	Low
5.391		582.5467	583.5538	$C_{36}H_{73}NO_4$	98.98	0.2	 N-(1,3,4-Trihydroxy-2-octadecanyl)octadecanamide		Low

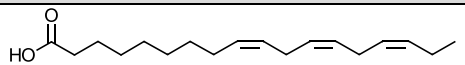
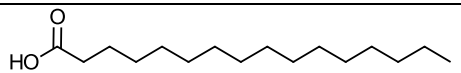
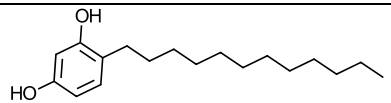
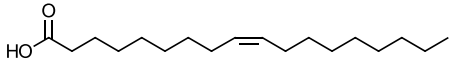
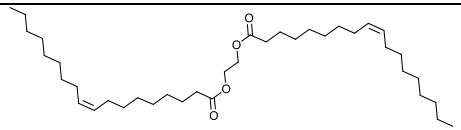
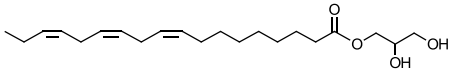
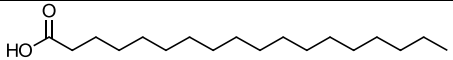
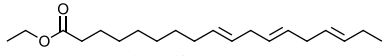
Table 1. Summary of LCMS Results (Hexane Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
5.403		323.2208	278.2226	$C_{18}H_{30}O_2$	84.34	7.3	 Linolenic acid	463-40-1	Medium
5.403		533.4559	534.463	$C_{34}H_{62}O_4$	89.28	3.39	Unkown 2		Low
5.408		255.2339 291.2102 301.2386	256.2411	$C_{16}H_{32}O_2$	95.12	-3.5	 Palmitic acid	57-10-3	Medium
5.417	279.2295 301.2113 579.4323		278.222	$C_{18}\ H_{30}\ O_2$	78.05	9.17	 4-Dodecyl-1,3-benzenediol	246-145-5	Low
5.459		271.2487	282.2559	$C_{18}H_{34}O_2$	86.26	-0.22	 Oleic acid	112-80-1	Medium
5.568		589.5181	590.5252	$C_{38}H_{70}O_4$	87.9	3.75	 Ethylene Glycol Dioleate	9005-07-6	Medium
5.571		351.2518	352.2590	$C_{21}H_{36}O_4$	83.75	6.67	 1-Monolinolenoyl-rac-glycerol	242-347-2	Low
5.581		283.2651	284.2723	$C_{18}H_{36}O_2$	95.66	-2.78	 Stearic acid	57-11-4	Medium
5.586	307.2600 329.2426 635.4947		306.2533	$C_{20}\ H_{34}\ O_2$	80.41	8.33	 Ethyl linolenate	1191-41-9	Low

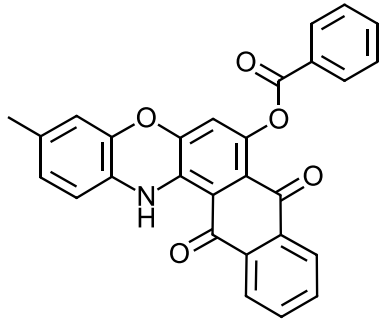
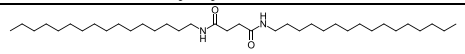
Table 1. Summary of LCMS Results (Hexane Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
5.798	448.1185 465.1450		447.1098	$C_{28}\ H_{17}\ N\ O_5$	66.48	2.03	 3-Methyl-8,13-dioxo-13,14-dihydro-8H-naphtho[2,3-a]phenoxazin-7-yl benzoate	136412-00-5	Low
5.8	445.1200 462.1466		444.1128	$C_{12}\ H_{36}\ O_6\ Si_6$	88.36	0.25	Dodecamethylcyclohexasiloxane (D6)	540-97-6	Medium
5.898	536.1655		518.1318	$C_{14}\ H_{42}\ O_7\ Si_7$	83.44	-0.42	Tetradecamethylcycloheptasiloxane (D7)	107-50-6	Medium
5.983	610.1841		592.1503	$C_{16}\ H_{48}\ O_8\ Si_8$	78.55	-0.01	Hexadecamethylcyclooctasiloxane (D8)	556-68-3	Medium
5.999		609.5574	564.5583	$C_{36}H_{72}N_2O_2$	89.72	1.98	 N,N'-Dihexadecylsuccinamide		Medium
6.052	684.2026		666.1689	$C_{18}\ H_{54}\ O_9\ Si_9$	75.05	0.38	Octadecamethylcyclononasiloxane (D9)	556-71-8	Medium
6.122	758.2215		740.1877	$C_{20}\ H_{60}\ O_{10}\ Si_{10}$	72.96	0.27	Eicosamethylcyclodecasiloxane (D10)	18772-36-6	Medium
6.19	834.2397		833.2315	$C_{43}\ H_{27}\ N_{15}\ O_5$	63.19	0.58	Unknown 2		Low
6.257	906.2590		888.2253	$C_{24}\ H_{72}\ O_{12}\ Si_{12}$	35.71	0.28	Tetracosamethylcyclododecasiloxane (D12)	18919-94-3	Medium
6.331	980.2773		962.2435	$C_{26}\ H_{78}\ O_{13}\ Si_{13}$	70.93	0.79	Hexacosamethylcyclotridecasiloxane (D13)	23732-94-7	Medium
6.412	1055.2970		1036.262	$C_{28}\ H_{84}\ O_{14}\ Si_{14}$	7.03	0.69	Octacosamethylcyclotetradecasiloxane (D14)	149050-40-8	Medium
6.502	1128.3150		1110.281	$C_{30}\ H_{90}\ O_{15}\ Si_{15}$	98.88	0.8	Triacontamethylcyclopentadecasiloxane (D15)	23523-14-0	Medium
6.594	1202.3337		1184.3	$C_{32}\ H_{96}\ O_{16}\ Si_{16}$	99.03	0.8	Dotriacontamethylcyclohexadecasiloxane (D16)	150026-95-2	Medium
6.689	1276.3523		1258.318	$C_{34}\ H_{102}\ O_{17}\ Si_{17}$	98.83	0.98	Tetratriacontamethylcycloheptadecasiloxane (D17)	150026-96-3	Medium
6.808	1350.3710		1332.337	$C_{36}\ H_{108}\ O_{18}\ Si_{18}$	98.72	0.96	Hexatriacontamethylcyclooctadecasiloxane (D18)	23523-12-8	Medium

Table 1. **Summary of LCMS Results (Hexane Extract)**									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
6.917	1424.3898		1406.356	C_{38} H_{114} O_{19} Si_{19}	48.87	-0.16	Octatriacontamethylcyclononadecasiloxane (D19)	150026-97-4	Medium
7.036	1498.4086		1480.374	C_{40} H_{120} O_{20} Si_{20}	49.27	1.11	Tetracontamethylcycloeicosasiloxane (D20)	150026-99-6	Medium
7.162	1572.4268		1554.393	C_{42} H_{126} O_{21} Si_{21}	49.03	1.28	Dotetracontamethylcyclohenicosasiloxane (D21)		Medium
7.294	1646.4445		1628.411	C_{44} H_{132} O_{22} Si_{22}	48.21	1.61	Tetratetracontamethylcyclodocosasiloxane (D22)		Medium

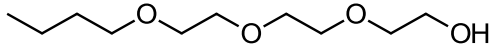
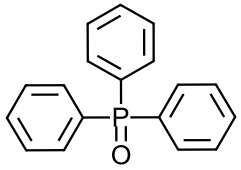
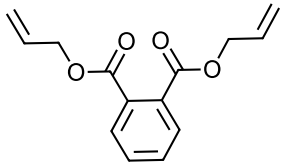
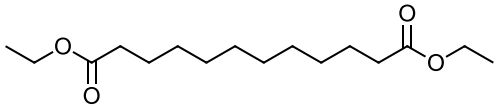
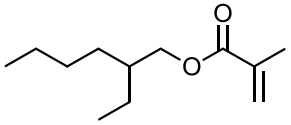
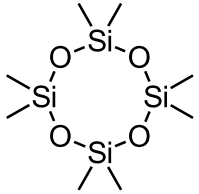
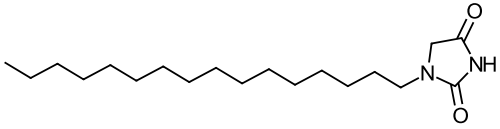
Table 2. Summary of LCMS Results (Ethanol Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
2.63	207.1589		206.1518	$C_{10}H_{22}O_4$	87.11	-0.19	 Triethylene glycol monobutyl ether	143-22-6	Medium
3.628	279.0933		278.086	$C_{18}H_{15}OP$	99.77	0.08	 Triphenylphosphine oxide	791-28-6	Medium
3.743	247.0965		246.0893	$C_{14}H_{14}O_4$	99.65	-0.16	 DAP-M / Diallyl phthalate (DASP)	131-17-9	Medium
4.792	287.2219 304.2484 309.2039		286.2147	$C_{16}H_{30}O_4$	99.48	-0.85	 Diethyl dodecanedioate	505-54-4	Medium
4.793	199.1694		198.1621	$C_{12}H_{22}O_2$	98.43	-0.7	 2-Ethylhexyl methacrylate	688-84-6	Medium
4.844	297.0822		296.0749	$C_8H_{24}O_4Si_4$	96.17	1.01	 Octamethylcyclotetrasiloxane (D4)	556-67-2	Medium
5.309	325.2848 347.2668		324.2775	$C_{19}H_{36}N_2O_2$	98.53	0.53	 1-Hexadecyl-2,4-imidazolidinedione	85117-82-4	Low

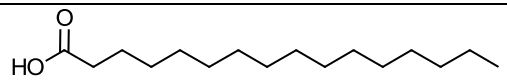
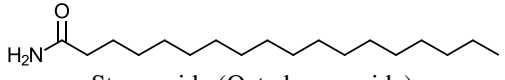
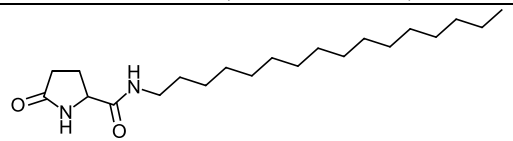
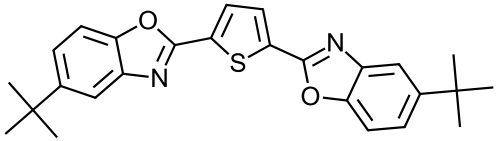
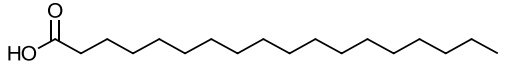
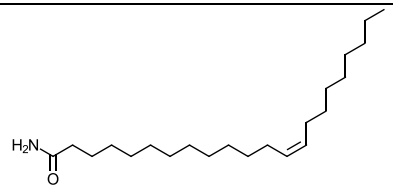
Table 2. Summary of LCMS Results (Ethanol Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
5.363	371.1008		370.0934	C_{10} H_{30} O_5 Si_5	92.25	1.41	Decamethylcyclopentasiloxane (D5)	541-02-6	Medium
5.41		255.2333	256.2405	C16 H32 O2	98.11	-1.06	 Palmitic acid	57-10-3	Medium
5.411	284.2946 306.2764		283.2872	C_{18} H_{37} N O	96.83	1.21	 Stearamide (Octadecanamide)	124-26-5	Medium
5.498	353.3159 375.2977		352.3085	C_{21} H_{40} N_2 O_2	98.96	1.23	 N-Hexadecyl-5-oxoprolinamide	55478-49-4	Low
5.505	431.1780 453.1597		430.1707	C_{26} H_{26} N_2 O_2 S	97.42	1.87	 BBOT / 2,5-bis(5-tert-Butyl-2-benzoxazolyl)thiophene	7128-64-5	Medium
5.585		283.2646	284.2718	C18 H36 O2	97.47	-1.04	 Stearic acid	253-480-0	Medium
5.606	338.3417 360.3233 675.6751		337.3343	C_{22} H_{43} N O	99.63	0.43	 Erucamide	112-84-5	Medium
5.8	462.1462		444.1123	C_{12} H_{36} O_6 Si_6	88.26	1.1	Dodecamethylcyclohexasiloxane (D6)	540-97-6	Medium

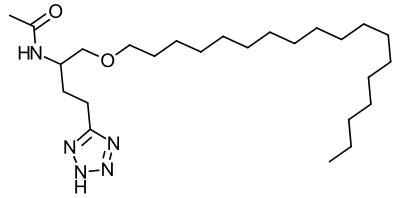
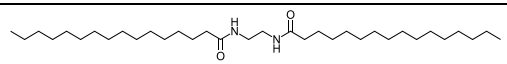
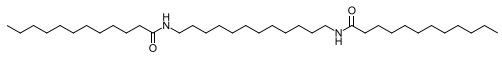
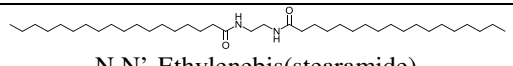
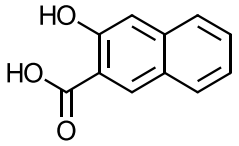
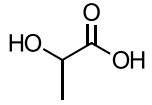
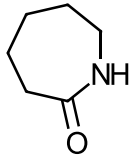
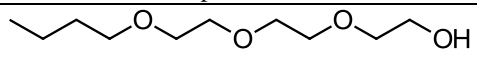
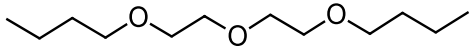
Table 2. Summary of LCMS Results (Ethanol Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
5.821	452.3943		451.387	$C_{25}H_{49}N_5O_2$	93.45	3.63	 N-[1-(Octadecyloxy)-4-(1H-tetrazol-5-yl)-2-butanyl]acetamide	192563-92-1	Low
5.895	536.1656		518.1318	$C_{14}H_{42}O_7Si_7$			Tetradecamethylcycloheptasiloxane (D7)	107-50-6	Medium
5.898	539.1642		538.1555	$C_{32}H_{27}O_6P$	65.45	-1.73	Unknown 1		Low
5.934	537.5347 559.5163 1096.0405		536.5273	$C_{34}H_{68}N_2O_2$	98.23	1.36	 N,N'-1,2-Ethanediyldihexadecanamide	5518-18-3	Medium
5.976	610.1840		592.1503	$C_{16}H_{48}O_8Si_8$	79.47	0.11	Hexadecamethylcyclooctasiloxane (D8)	556-68-3	Medium
6.004	565.5658 587.5474 1152.1012		564.5584	$C_{36}H_{72}N_2O_2$	97.36	1.8	 N,N'-1,12-Dodecanediyldidodecanamide	151493-20-8	Medium
6.045	684.2028		666.169	$C_{18}H_{54}O_9Si_9$	76.03	0.79	Octadecamethylcyclononasiloxane (D9)	556-71-8	Medium
6.067	593.5966 615.5783		592.5893	$C_{38}H_{76}N_2O_2$	96.19	2.36	 N,N'-Ethylenebis(stearamide)	110-30-5	Medium
6.186	832.2405		814.2067	$C_{22}H_{66}O_{11}Si_{11}$	71.74	0.05	Docosamethylcycloundecasiloxane (D11)	18766-38-6	Medium
6.248	906.2591		888.2253	$C_{24}H_{72}O_{12}Si_{12}$	71.47	0.2	Tetracosamethylcyclododecasiloxane (D12)	18919-94-3	Medium
6.325	980.2775		962.2438	$C_{26}H_{78}O_{13}Si_{13}$	71.19	0.55	Hexacosamethylcyclotridecasiloxane (D13)	23732-94-7	Medium
6.407	1054.2966		1036.263	$C_{28}H_{84}O_{14}Si_{14}$	71.33	0.33	Octacosamethylcyclotetradecasiloxane (D14)	149050-40-8	Medium
6.487	1128.3153		1110.281	$C_{30}H_{90}O_{15}Si_{15}$	99.44	0.53	Triacontamethylcyclopentadecasiloxane (D15)	23523-14-0	Medium
6.582	1202.3344		1184.3	$C_{32}H_{96}O_{16}Si_{16}$	99.58	0.3	Dotriacontamethylcyclohexadecasiloxane (D16)	150026-95-2	Medium

Table 2. Summary of LCMS Results (Ethanol Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
6.693	1276.3508		1258.317	C_{34} H_{102} O_{17} Si_{17}	94.93	2.33	Tetratriacontamethylcycloheptadecasiloxane (D17)	150026-96-3	Medium
6.794	1350.3720		1332.338	C_{36} H_{108} O_{18} Si_{18}	99.41	0.45	Hexatriacontamethylcyclooctadecasiloxane (D18)	23523-12-8	Medium
6.912	1424.3875		1406.353	C_{38} H_{114} O_{19} Si_{19}	92.96	2.67	Octatriacontamethylcyclononadecasiloxane (D19)	150026-97-4	Medium
7.03	1498.4053		1480.371	C_{40} H_{120} O_{20} Si_{20}	90.7	3.07	Tetracontamethylcycloeicosasiloxane (D20)	150026-98-5	Medium
7.153	1572.4257		1554.392	C_{42} H_{126} O_{21} Si_{21}	95.78	1.84	Dotetracontamethylcyclohenicosasiloxane (D21)	23523-13-9	Medium
7.279	1646.4403		1628.407	C_{44} H_{132} O_{22} Si_{22}	84.67	4.12	Tetratetracontamethylcyclodocosasiloxane (D22)	1177831-23-0	Medium

Table 3. Summary of LCMS Results (Water Extract)									
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	CAS Number	Confidence Level
0.349		187.0421	188.0494	C_{11} H_8 O_3	62.34	-10.81	 3-Hydroxy-2-naphthoic acid	92-70-6	Medium
0.351		89.0243	90.0316	C_3 H_6 O_3	87.76	1.28	 Lactic acid	50-21-5	Medium
1.566	114.0913 136.0723		113.0841	C_6 H_{11} N O	87.49	0.17	 Caprolactam	105-60-2	Medium
2.652	207.1591 224.1854		206.1517	C_{10} H_{22} O_4	86.53	0.25	 Triethylene glycol monobutyl ether	143-22-6	Medium
4.205	219.1955 236.2217 241.1773		218.1882	C_{12} H_{26} O_3	86.84	0	 Diethylene glycol dibutyl ether	112-73-2	Medium

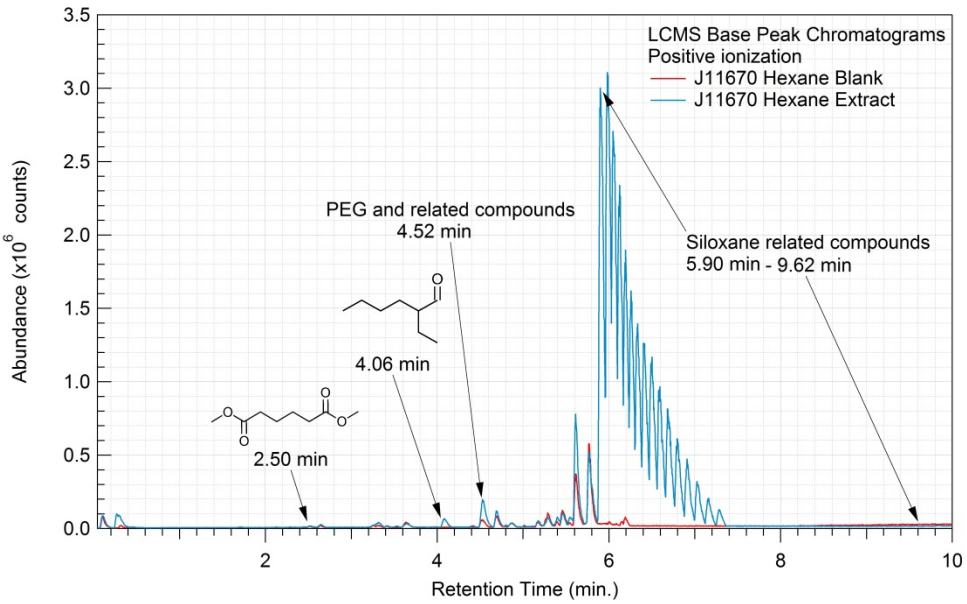


Figure 1- Overlay of LCMS base peak chromatograms of hexane extracts, positive ionization.

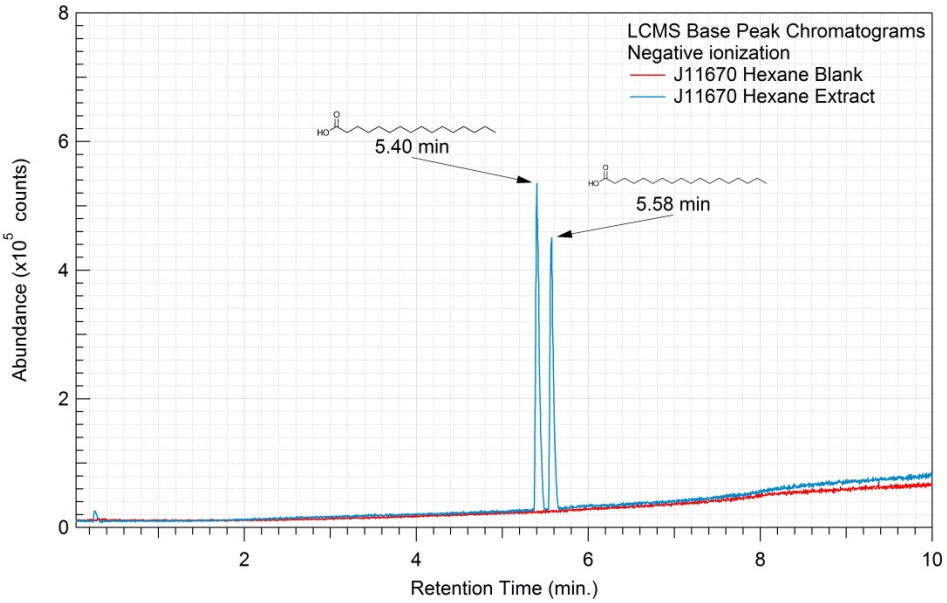


Figure 2 - Overlay of LCMS base peak chromatograms of hexane extracts, negative ionization.

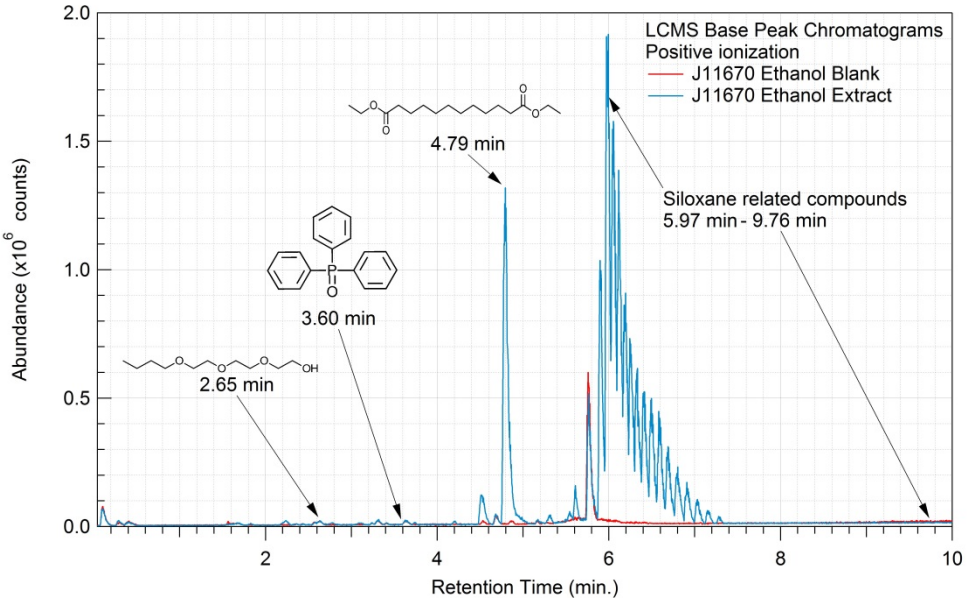


Figure 3- Overlay of LCMS base peak chromatograms of ethanol extracts, positive ionization.

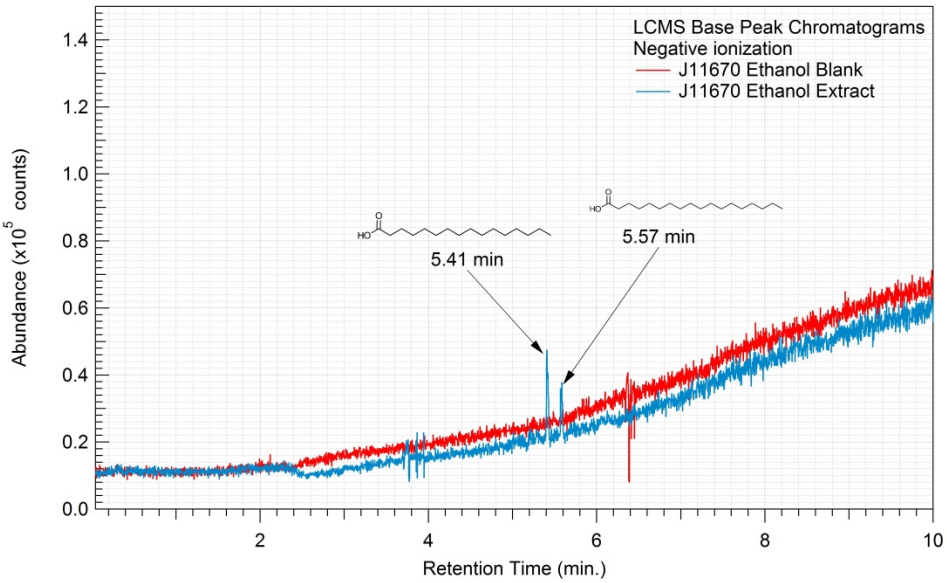


Figure 4 - Overlay of LCMS base peak chromatograms of ethanol extracts, negative ionization.

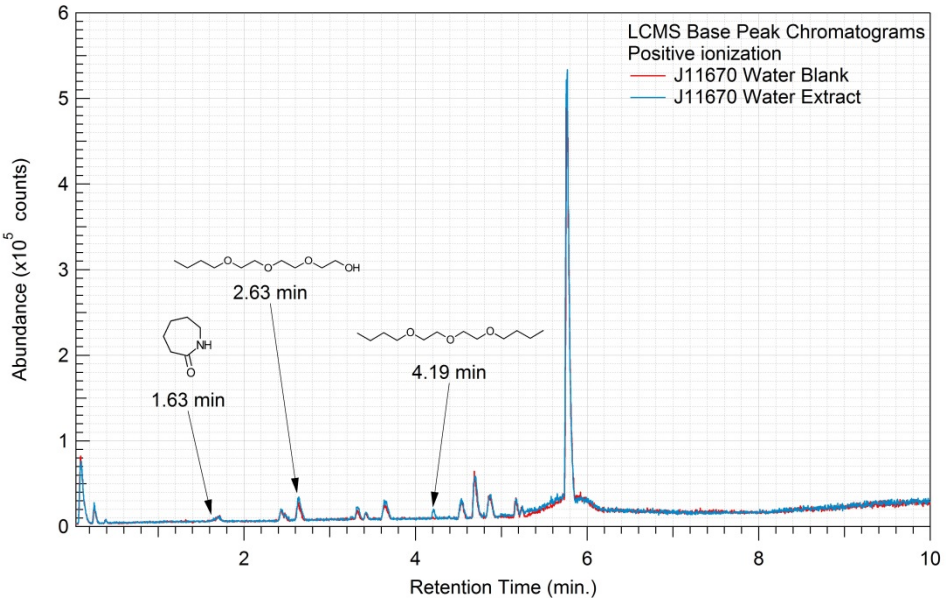


Figure 5- Overlay of LCMS base peak chromatograms of water extracts, positive ionization.

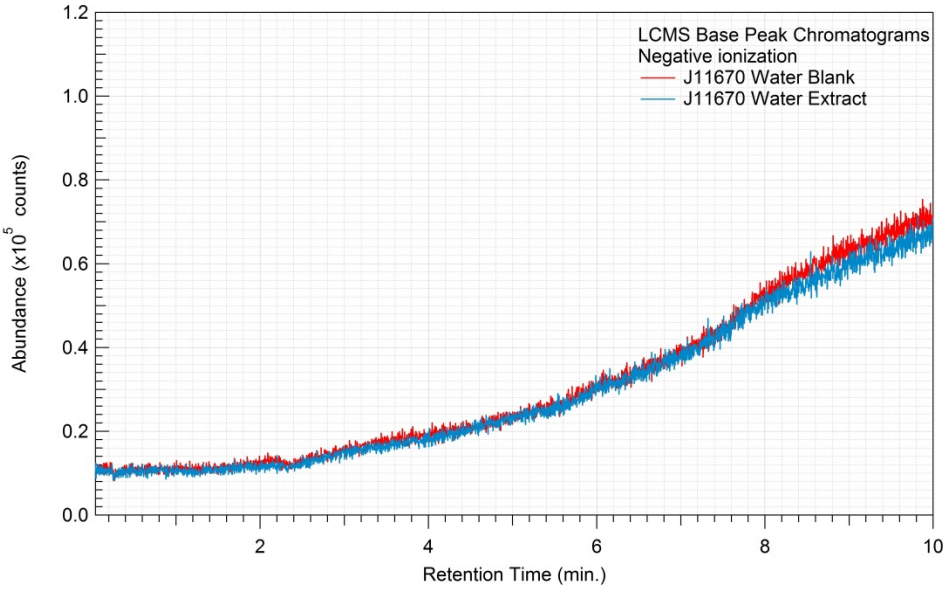


Figure 6 - Overlay of LCMS base peak chromatograms of water extracts, negative ionization.

QTOF GCMS

GCMS analysis was performed in electron impact mode. The spectra collected using electron impact (EI) ionization can be compared to the NIST mass spectral database for identification. In addition, fragments can be identified using the accurate mass data collected. This ionization mode is high energy and generally causes a large amount of analyte fragmentation. In many cases the EI mass spectra collected only contain fragment ions making definitive unknown identification impossible for compounds not present in the mass spectral database. Chemical ionization (CI) provides less energy and causes significantly less fragmentation. The CI data collected can, in most cases, be used to determine the molecular formula for a particular compound using the molecular formula generation (MFG) algorithm.

Relative quantitation compares the peak response of an unknown compound to that of a surrogate standard. It should be noted that peak response is not only affected by compound concentration but also by compound volatility and ionization efficiency. All concentration values should therefore be considered estimates.

Ethanol and hexane extracts were subjected to QTOF-GCMS analysis without further preparation. The water extract (1 mL) was extracted with DCM (1:1, v/v) to give the analytical solutions, which were subjected to QTOF-GCMS analysis.

Results

Tables 4-6 provide proposed identifications and estimated concentrations of components detected in the water, ethanol and hexane extracts, respectively. **Figure 7-9** provide overlays of the GCMS chromatograms obtained for those samples and the corresponding controls for each.

Table 4
QTOF-GCMS Results – Water Extract

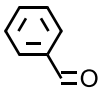
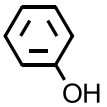
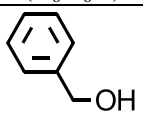
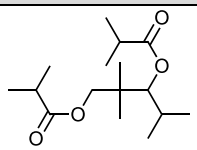
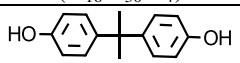
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
7.78	 Benzaldehyde (C_7H_6O)	100-52-7	NIST	High	167621.14	0.0406	0.20
8.14	 Phenol (C_6H_6O)	108-95-2	NIST	High	951742.37	0.2307	1.16
9.00	 Benzyl alcohol (C_7H_8O)	100-51-6	NIST	High	429477.56	0.1041	0.52

Table 4
QTOF-GCMS Results – Water Extract

RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
14.75	 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate ($C_{16}H_{30}O_4$)	6846-50-0	NIST	Medium	152325.50	0.0283	0.14
18.74	 Bisphenol A ($C_{15}H_{16}O_2$)	80-05-7	NIST	High	92781.98	0.0230	0.12

Quantitation Standard Compound

RT	Possible Identification	Conc. (µg/mL)	Peak Area	RT of Analytes
8.452	Decane	0.50	2062586.90	4-11.5
14.702	Hexadecane	0.50	2688928.50	11.5-16.0
17.542	Eicosane	0.50	2440456.68	16.0-18.5
19.905	Tetracosane	0.50	2016202.44	18.5-33

$$Estimated\ Conc.\ (\mu g/mL) = \frac{Peak\ Area\ of\ Analyte}{Peak\ Area\ of\ Standard} \times Conc.of\ Standard\ (0.500\ \mu g/mL)$$

$$Mass\ per\ device\ (\mu g) = \frac{Estimated\ concentration\ \left(\frac{\mu g}{mL}\right) \times Total\ volume\ extract\ (5.03\ mL)}{number\ of\ devices\ (1)} \div concentration\ factor\ (1)$$

* Average peak area for two injections
N.A. – Not Applicable

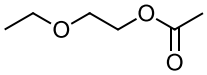
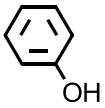
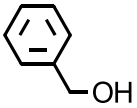
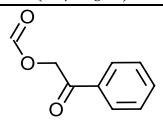
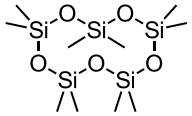
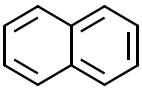
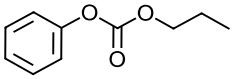
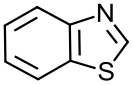
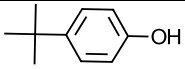
Table 5 QTOF-GCMS Results – Ethanol Extract							
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
7.09	 2-Ethoxyethyl acetate ($C_6H_{12}O_3$)	111-15-9	NIST	Medium	178508.51	0.0409	0.21
8.28	 Phenol (C_6H_6O)	108-95-2	NIST	High	3305118.00	0.7571	3.81
9.08	 Benzyl alcohol (C_7H_8O)	100-51-6	NIST	High	1365277.37	0.3128	1.57
9.55	 Ethanone, 2-(formyloxy)-1-phenyl- ($C_9H_8O_3$)	55153-12-3	NIST	Medium	157419.40	0.0361	0.18
10.56	 Cyclopentasiloxane, decamethyl- ($C_{10}H_{30}O_5Si_5$)	541-02-6	NIST	High	1519514.68	0.3481	1.75
10.75	Unknown	N.A.	NIST	Low	89465.90	0.0205	0.10
10.98	 Naphthalene ($C_{10}H_8$)	91-20-3	NIST	High	321367.60	0.0736	0.37
11.39	 Carbonic acid, phenyl propyl ester ($C_{10}H_{12}O_3$)	13183-16-9	NIST	Medium	151319.34	0.0347	0.17
11.43	 Benzothiazole (C_7H_5NS)	95-16-9	NIST	High	421916.53	0.0967	0.49
12.04	 Phenol, p-tert-butyl- ($C_{10}H_{14}O$)	98-54-4	NIST	Medium	157950.24	0.0293	0.15

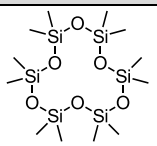
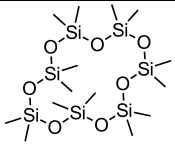
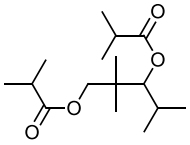
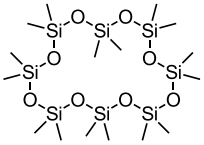
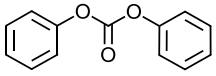
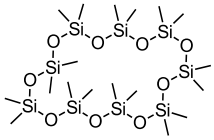
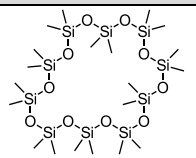
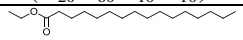
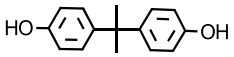
Table 5 QTOF-GCMS Results – Ethanol Extract							
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
12.33	 Cyclohexasiloxane, dodecamethyl- ($C_{12}H_{36}O_6Si_6$)	540-97-6	NIST	High	4088455.03	0.7571	3.81
13.86	 Cycloheptasiloxane, tetradecamethyl- ($C_{14}H_{42}O_7Si_7$)	107-50-6	NIST	High	7549636.97	1.3981	7.03
14.74	 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate ($C_{16}H_{30}O_4$)	6846-50-0	NIST	High	11646523.82	2.1568	10.85
15.21	 Cyclooctasiloxane, hexadecamethyl- ($C_{16}H_{48}O_8Si_8$)	556-68-3	NIST	High	14301350.48	2.6485	13.32
15.45	Hydrocarbon ($<C_{20}$)	N.A.	NIST	Medium	160333.15	0.0297	0.15
15.53	 Diphenyl carbonate ($C_{13}H_{10}O_3$)	102-09-0	NIST	High	1560620.75	0.2890	1.45
16.17	Hydrocarbon ($<C_{20}$)	N.A.	NIST	Low	242850.33	0.0485	0.24
16.38	 Cyclononasiloxane, octadecamethyl- ($C_{18}H_{54}O_9Si_9$)	556-71-8	NIST	High	15460816.67	3.0875	15.53

Table 5
QTOF-GCMS Results – Ethanol Extract

RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
17.42	 Cyclodecasiloxane, eicosamethyl- ($C_{20}H_{60}O_{10}Si_{10}$)	18772-36-6	NIST	High	14447544.06	2.8852	14.51
17.52	 Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$)	628-97-7	NIST	Medium	406871.73	0.0813	0.41
18.37	Cyclicsiloxane	N.A.	NIST	Medium	13196903.46	2.6354	13.26
18.75	 Bisphenol A ($C_{15}H_{16}O_2$)	80-05-7	NIST	High	477732.27	0.1133	0.57
19.24	Cyclicsiloxane	N.A.	NIST	Medium	13515355.57	3.2064	16.13
20.03	Cyclicsiloxane	N.A.	NIST	Medium	14673558.86	3.4812	17.51
20.78	Cyclicsiloxane	N.A.	NIST	Medium	15377802.68	3.6482	18.35
21.48	Cyclicsiloxane	N.A.	NIST	Medium	14506221.33	3.4415	17.31
22.13	Cyclicsiloxane	N.A.	NIST	Medium	14658254.18	3.4775	17.49
22.75	Cyclicsiloxane	N.A.	NIST	Medium	10420417.25	2.4721	12.43
23.33	Cyclicsiloxane	N.A.	NIST	Medium	7729500.63	1.8338	9.22
23.97	Cyclicsiloxane	N.A.	NIST	Medium	5468223.24	1.2973	6.53
24.68	Cyclicsiloxane	N.A.	NIST	Medium	4034769.86	0.9572	4.81
25.53	Cyclicsiloxane	N.A.	NIST	Medium	4331894.84	1.0277	5.17
26.59	Cyclicsiloxane	N.A.	NIST	Medium	4103031.52	0.9734	4.90
27.85	Cyclicsiloxane	N.A.	NIST	Medium	3341203.20	0.7927	3.99
29.39	Cyclicsiloxane	N.A.	NIST	Medium	3721621.53	0.8829	4.44
31.32	Cyclicsiloxane	N.A.	NIST	Medium	3569889.65	0.8469	4.26

Quantitation Standard Compound

RT	Possible Identification	Conc. (µg/mL)	Peak Area	RT of Analytes
8.452	Decane	0.50	2182649.42	4-11.5
14.702	Hexadecane	0.50	2699924.56	11.5-16.0
17.542	Eicosane	0.50	2503741.89	16.0-18.5
19.905	Tetracosane	0.50	2107565.90	18.5-33

$$Estimated\ Conc.(\mu g/mL) = \frac{Peak\ Area\ of\ Analyte}{Peak\ Area\ of\ Standard} \times Conc.of\ Standard\ (0.500\ \mu g/mL)$$

$$Mass\ per\ device\ (\mu g) = \frac{Estimated\ concentration\ \left(\frac{\mu g}{mL}\right) \times Total\ volume\ extract\ (5.03\ mL)}{number\ of\ devices\ (1)} \div concentration\ factor\ (1)$$

* Average peak area for two injections
N.A. – Not Applicable

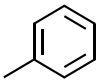
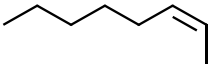
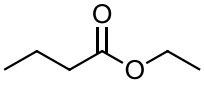
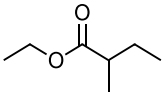
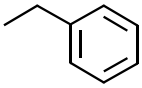
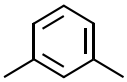
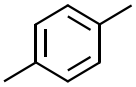
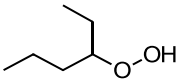
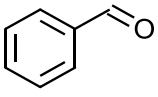
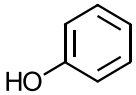
Table 6 QTOF-GCMS Results – Hexane Extract							
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
3.25	 Toluene (C_7H_8)	108-88-3	NIST	High	377014.03	0.084	0.42
3.83	 2-Octene (C_8H_{16})	111-67-1	NIST	High	162125.35	0.036	0.18
3.88	 Ethyl ester Butanoic acid ($C_6H_{12}O_2$)	105-54-4	NIST	High	915681.86	0.205	1.03
5.26	 2-Methyl-ethyl ester Butanoic acid ($C_7H_{14}O_2$)	7452-79-1	NIST	High	107458.36	0.024	0.12
5.53	 Ethyl Benzene (C_8H_{10})	100-41-4	NIST	High	794922.21	0.178	0.89
5.75	 m-Xylene (C_8H_{10})	108-38-3	NIST	High	925669.51	0.207	1.04
6.36	 p-Xylene (C_8H_{10})	106-42-3	NIST	Medium	691670.64	0.155	0.78
7.54	 1-ethylbutyl Hydroperoxide ($C_6H_{14}O_2$)	24254-56-6	NIST	Medium	221672.81	0.050	0.25
7.77	 Benzaldehyde (C_7H_6O)	100-52-7	NIST	High	3204769.60	0.716	3.60
8.15	 Phenol (C_6H_6O)	108-95-2	NIST	High	450306.66	0.101	0.51

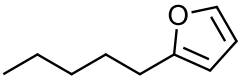
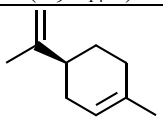
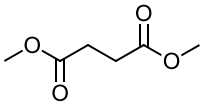
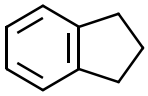
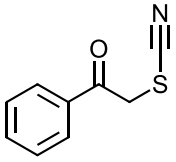
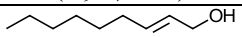
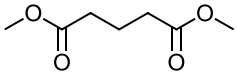
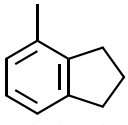
Table 6 QTOF-GCMS Results – Hexane Extract							
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
8.33	 2-pentyl-Furan ($C_9H_{14}O$)	3777-69-3	NIST	Medium	108799.45	0.024	0.12
8.92	 1-methyl-4-(1-methylethenyl)-Cyclohexene ($C_{10}H_{16}$)	5989-54-8	NIST	High	490785.63	0.110	0.55
8.99	 dimethyl ester Butanedioic acid ($C_6H_{10}O_4$)	106-65-0	NIST	High	119615.21	0.027	0.13
9.02	 Indane (C_9H_{10})	496-11-7	NIST	High	270228.81	0.060	0.30
9.47	 Phenacyl thiocyanate (C_9H_7NOS)	5399-30-4	NIST	High	740913.19	0.166	0.83
9.96	 2-Nonen-1-ol ($C_9H_{18}O$)	22104-79-6	NIST	Medium	134743.82	0.030	0.15
10.36	 dimethyl ester Pentanedioic acid ($C_7H_{12}O_4$)	1119-40-0	NIST	High	478475.11	0.107	0.54
10.42	 Methyl Indene ($C_{10}H_{12}$)	824-22-6	NIST	High	96086.03	0.021	0.11

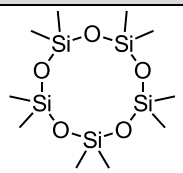
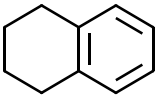
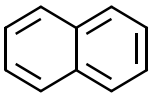
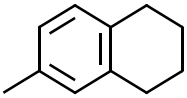
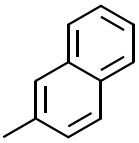
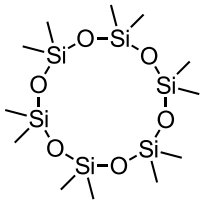
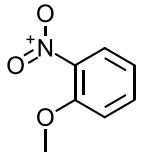
Table 6 QTOF-GCMS Results – Hexane Extract							
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
10.55	 decamethyl-Cyclopentasiloxane ($C_{10}H_{30}O_5Si_5$)	541-02-6	NIST	High	1301383.33	0.291	1.46
10.71	 1,2,3,4-tetrahydro-Naphthalene ($C_{10}H_{12}$)	119-64-2	NIST	High	394890.38	0.088	0.44
10.97	 Naphthalene ($C_{10}H_8$)	91-20-3	NIST	High	255677.97	0.057	0.29
11.84	 1,2,3,4-tetrahydro-6-methyl-Naphthalene ($C_{11}H_{14}$)	1680-51-9	NIST	Medium	145831.84	0.027	0.13
12.15	 2-methyl Naphthalene ($C_{11}H_{10}$)	91-57-6	NIST	High	190292.63	0.035	0.17
12.33	 dodecamethyl-Cyclohexasiloxane ($C_{12}H_{36}O_6Si_6$)	540-97-6	NIST	High	9562212.63	1.739	8.75
12.69	 1-methoxy-2-nitro-Benzene	91-23-6	NIST	High	2014412.42	0.366	1.84

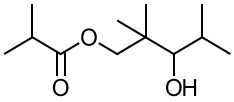
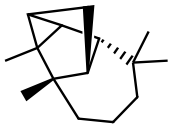
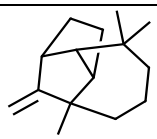
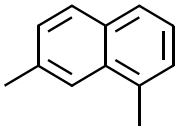
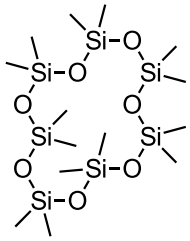
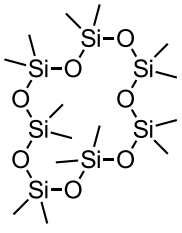
Table 6 QTOF-GCMS Results – Hexane Extract							
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
	($C_7H_7NO_3$)						
12.87	 3-hydroxy-2,2,4-2-methyl-trimethylpentyl ester Propanoic acid, ($C_{12}H_{24}O_3$)	77-68-9	NIST	Medium	276537.75	0.050	0.25
12.94	 Longicyclene ($C_{15}H_{24}$)	1137-12-8	NIST	Medium	307282.61	0.056	0.28
13.03	Hydrocarbon ($<C_{16}$)	NA	NIST	Medium	141226.42	0.026	0.13
13.27	 Longifolene ($C_{15}H_{24}$)	475-20-7	NIST	High	991455.93	0.180	0.91
13.35	 1,7-dimethyl-Naphthalene ($C_{12}H_{12}$)	575-37-1	NIST	High	134859.46	0.025	0.12
13.56	 tetradecamethyl-Cycloheptasiloxane ($C_{14}H_{42}O_7Si_7$)	107-50-6	NIST	Medium	136043.30	0.025	0.12
13.87	 tetradecamethyl-Cycloheptasiloxane ($C_{14}H_{42}O_7Si_7$)	107-50-6	NIST	High	17178142.44	3.123	15.71

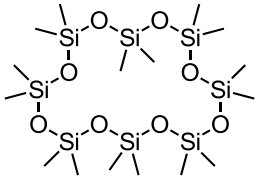
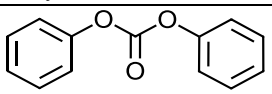
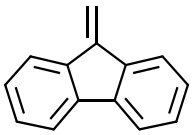
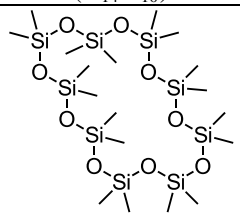
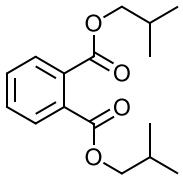
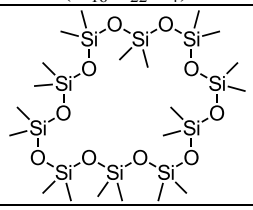
Table 6 QTOF-GCMS Results – Hexane Extract							
RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
14.71	Hydrocarbon ($\leq C_{16}$)	NA	NIST	Medium	267965.79	0.049	0.25
14.75	Unknown (m/z: 149, 166)	NA	NIST	Low	132168.32	0.024	0.12
15.08	Unknown (m/z: 105, 182)	NA	NIST	Low	240923.59	0.044	0.22
15.22	 hexadecamethyl-Cyclooctasiloxane ($C_{16}H_{48}O_8Si_8$)	556-68-3	NIST	High	18147754.65	3.300	16.60
15.47	Hydrocarbon ($<C_{20}$)	NA	NIST	Medium	414697.09	0.075	0.38
15.53	 Diphenyl carbonate ($C_{13}H_{10}O_3$)	102-09-0	NIST	High	1150450.99	0.209	1.05
16.19	Hydrocarbon ($<C_{20}$)	NA	NIST	Medium	131104.25	0.025	0.13
16.27	 9-methylene-9H-Fluorene ($C_{14}H_{10}$)	4425-82-5	NIST	Medium	169480.15	0.033	0.17
16.39	 octadecamethyl-Cyclononasiloxane ($C_{18}H_{54}O_9Si_9$)	556-71-8	NIST	High	17981011.50	3.485	17.53
16.74	 Diisobutyl phthalate ($C_{16}H_{22}O_4$)	84-69-5	NIST	High	271590.17	0.053	0.26
17.43		18772-36-6	NIST	High	16645094.82	3.226	16.23

Table 6
QTOF-GCMS Results – Hexane Extract

RT	Possible Identification	CAS	ID Source	Confidence Level	Peak Area*	Estimated Conc. (µg/mL)	Mass per Device (µg)
	eicosamethyl-Cyclodecasiloxane ($C_{20}H_{60}O_{10}Si_{10}$)						
18.38	Cyclicsiloxane	N.A.	NIST	Medium	15605962.46	3.025	15.21
19.24	Cyclicsiloxane	N.A.	NIST	Medium	16195695.35	3.721	18.72
20.04	Cyclicsiloxane	N.A.	NIST	Medium	17861482.26	4.104	20.64
20.79	Cyclicsiloxane	N.A.	NIST	Medium	18767629.62	4.312	21.69
21.49	Cyclicsiloxane	N.A.	NIST	Medium	17370974.40	3.991	20.08
22.14	Cyclicsiloxane	N.A.	NIST	Medium	15453599.59	3.551	17.86
22.76	Cyclicsiloxane	N.A.	NIST	Medium	10677117.45	2.453	12.34
23.34	Cyclicsiloxane	N.A.	NIST	Medium	6862299.04	1.577	7.93
23.97	Cyclicsiloxane	N.A.	NIST	Medium	4481888.90	1.030	5.18
24.69	Cyclicsiloxane	N.A.	NIST	Medium	3278556.46	0.753	3.79
25.54	Cyclicsiloxane	N.A.	NIST	Medium	2135818.96	0.491	2.47
26.58	Cyclicsiloxane	N.A.	NIST	Medium	1710828.28	0.393	1.98
27.84	Cyclicsiloxane	N.A.	NIST	Medium	1503823.26	0.346	1.74

Quantitation Standard Compound

RT	Possible Identification	Conc. (µg/mL)	Peak Area	RT of Analytes
8.45	Decane	0.5	2237675.80	4-11.5 min
14.70	Hexadecane	0.5	2750002.81	11.5-16 min
17.54	Eicosane	0.5	2579709.24	16-18.5 min
19.90	Tetracosane	0.5	2176086.66	18.5-33 min

$$Estimated\ Conc.\,(\mu g/mL) = \frac{Peak\ Area\ of\ Analyte}{Peak\ Area\ of\ Standard} \times Conc.of\ Standard\ (0.500\ \mu g/mL)$$

$$Mass\ per\ device\ (\mu g) = \frac{Estimated\ concentration\ \left(\frac{\mu g}{mL}\right) \times Total\ volume\ extract\ (5.03\ mL)}{number\ of\ devices\ (1)}$$

* Average peak area for two injections
N.A. – Not Applicable

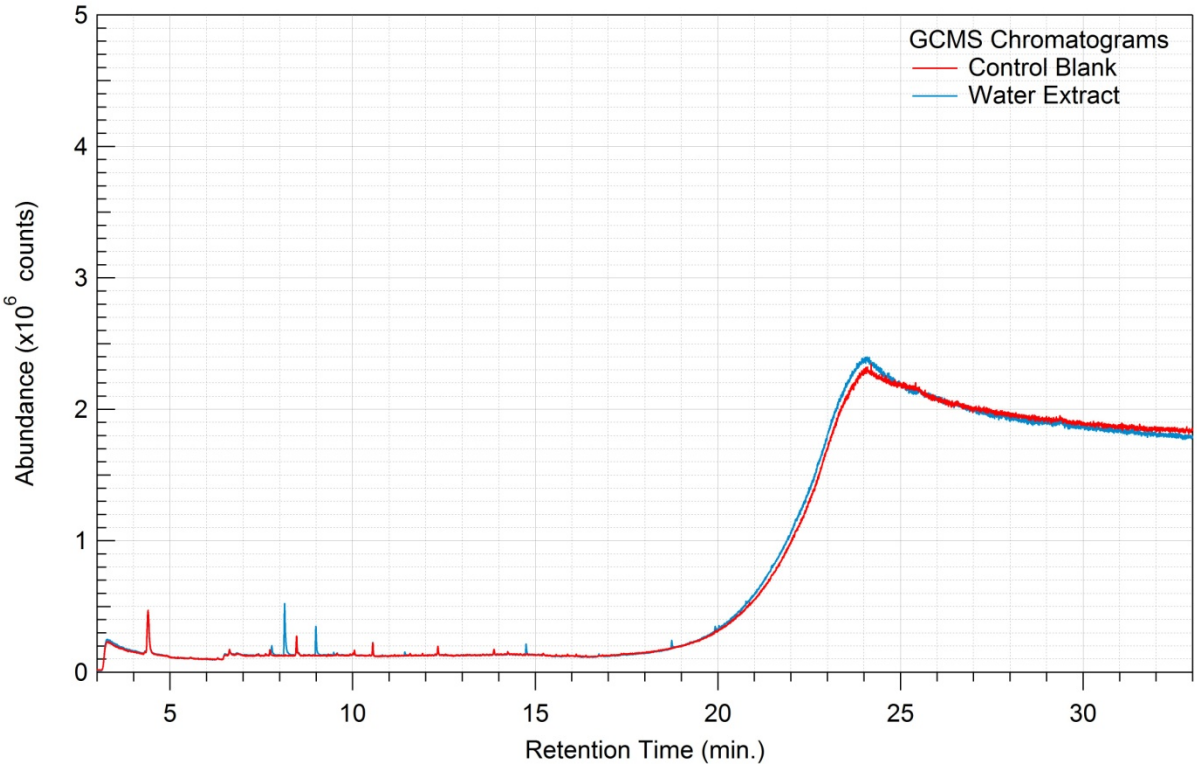


Figure 7. Overlay of GCMS chromatograms of the water extract and a control blank.

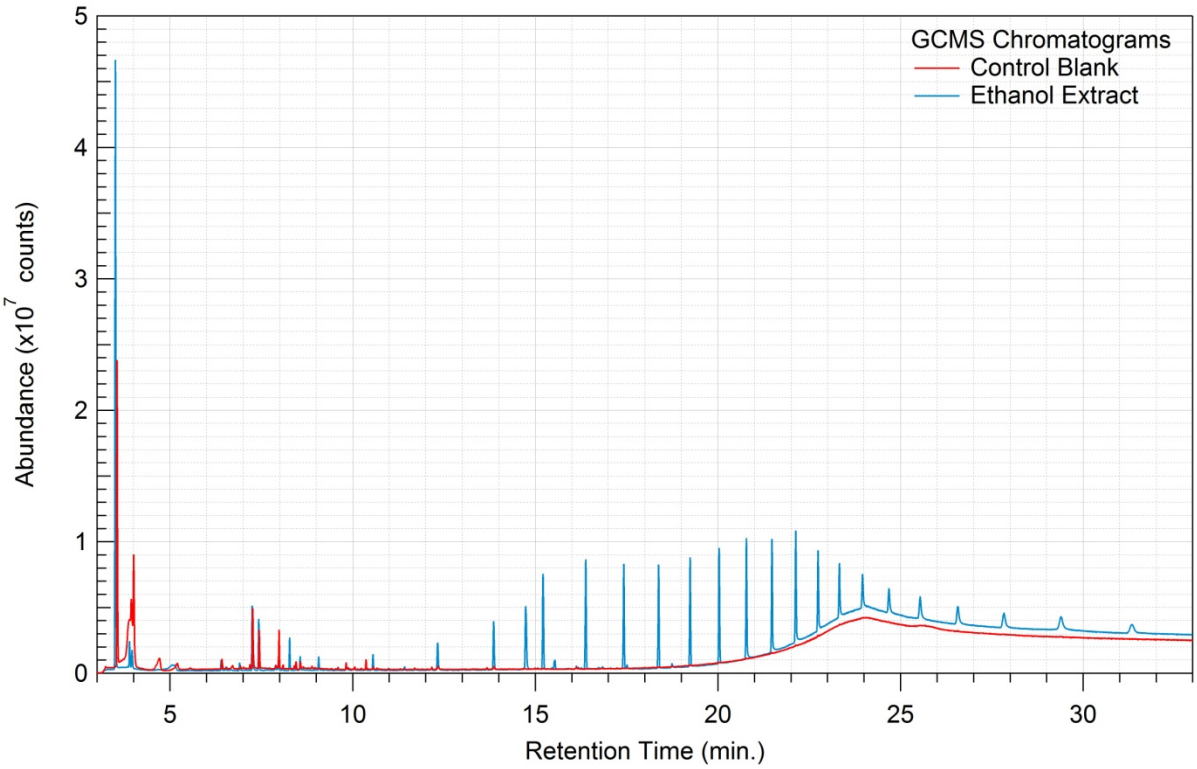


Figure 8. Overlay of GCMS chromatograms of the ethanol extract and a control blank.

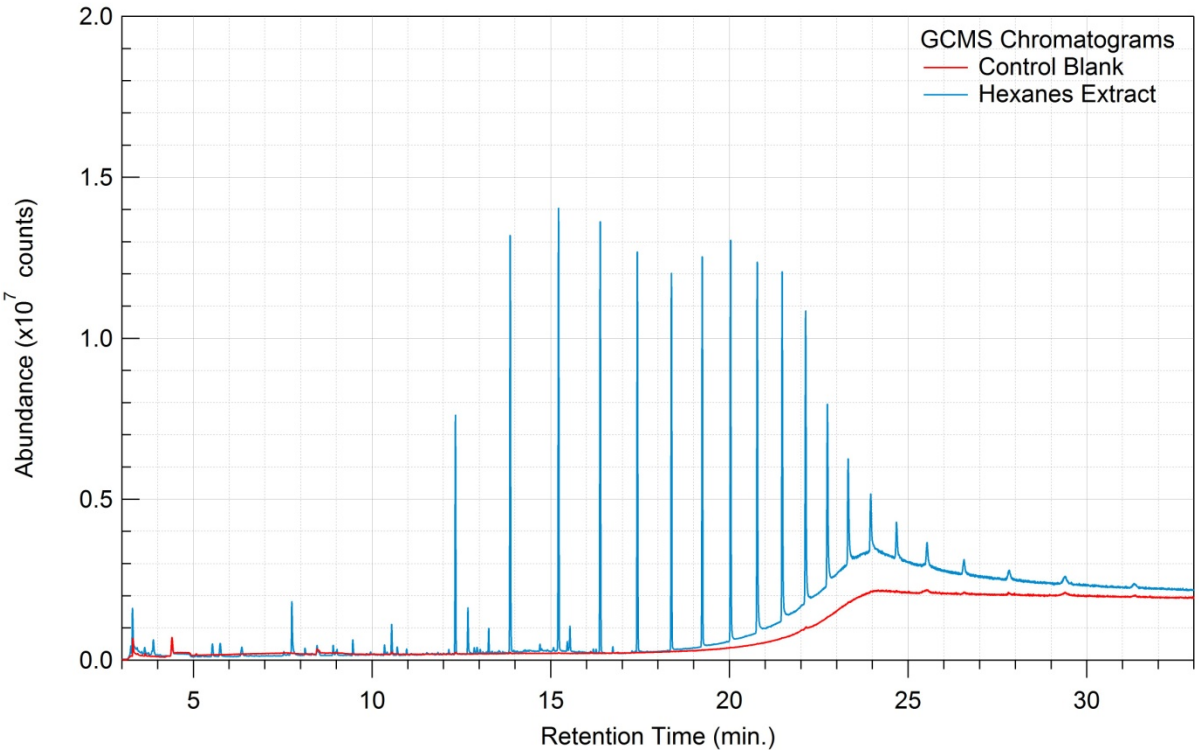


Figure 9. Overlay of GCMS chromatograms of the hexane extract and a control blank.

HGCMS

Sample Preparation

The mouthpiece of the E-cigarette was sealed in a 20ml headspace sampling vial. The sample was heated at 100°C for 10 minutes prior to injection of the headspace gasses. For headspace analysis, approximately 1 ml of the headspace gasses present were transferred to the GCMS for analysis.

Results

The major component detected in the sample are consistent with octyl methoxycinnamate, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB), and a variety of siloxane compounds.

The identifications of the major compounds detected in the sample are summarized in **Table 7**. An overlay of the HGCMS chromatograms collected from the sample and an air blank is shown in **Figure 10**.

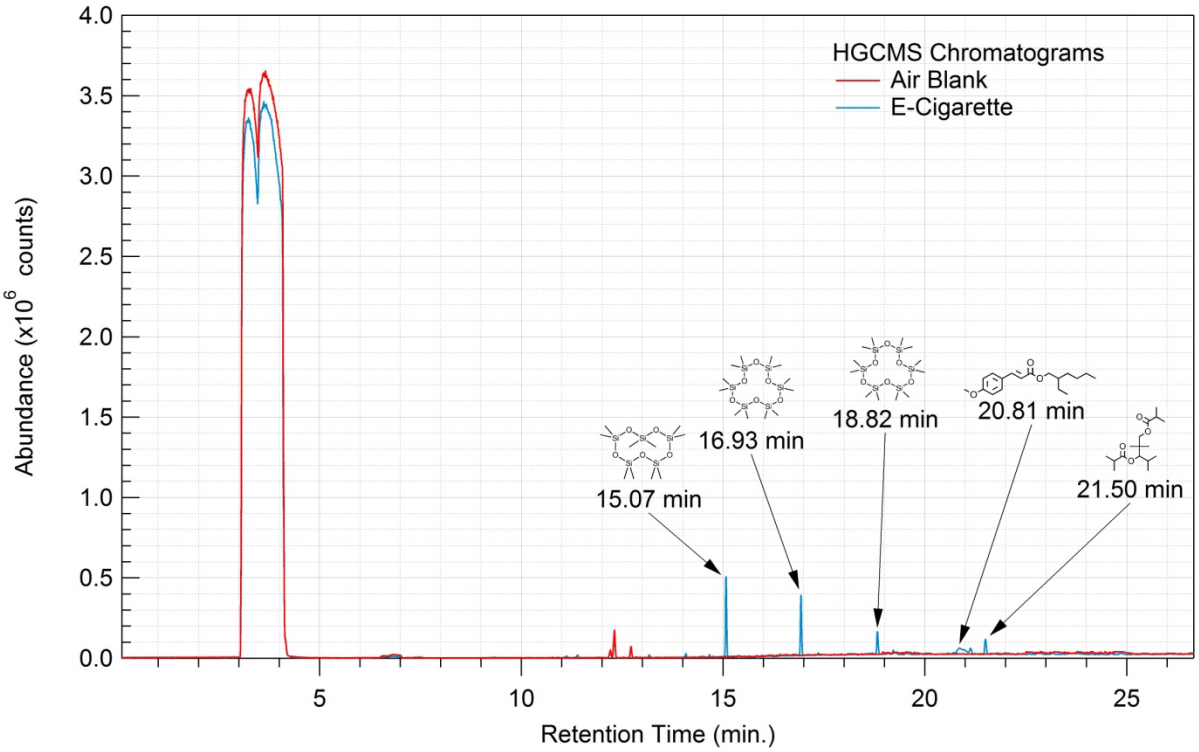
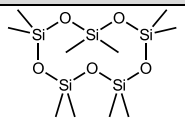
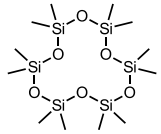
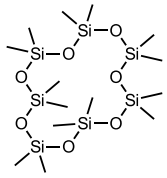
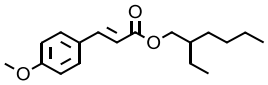
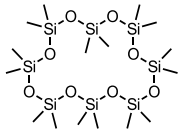
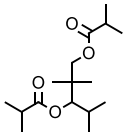


Figure 10. An overlay of HGCMS chromatograms collected for sample and an air blank

Table 7 HGCMS Results			
RT (mins)	Probable ID	CAS #	Source
15.076	 Decamethyl cyclopentasiloxane	541-02-6	NIST
16.929	 Dodecamethyl cyclopentasiloxane	540-97-6	NIST
18.823	 Tetradecamethyl cyclopentasiloxane	107-50-6	NIST
20.855	 Octyl methoxycinnamate	5466-77-3	NIST
21.128	 Hexadecamethyl cyclopentasiloxane	556-68-3	NIST
21.502	 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB)	6846-50-0	NIST

Analysis Conditions

This section of a Jordi report provides information on the methods used including instrument type, temperatures, solvents, sample preparation, etc. The specific conditions have been removed for this case study.

Closing Comments

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Sincerely,

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