Analysis of E-Cigarette Liquids for Toxicants Associated with EVALI

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ABSTRACT: E-cigarettes have been found to cause EVALI, but the cause of why has yet to be fully explained. For this reason, the purpose of this research was to screen two samples of e-cigarette liquid for toxicants using various instrumentation methods. The e-cigarette samples came from individuals who contracted EVALI. The results found that the nicotine concentrations were significantly different between the two samples. There were also no adulterations found in either sample. It was found that the aerosolization of the e-cigarette liquid increased the zinc concentration of the post-vape sample. Some flavoring compounds in the samples were found to be harmful if inhaled. These findings are significant since they help to determine what next steps need to be taken to improve the e-cigarette liquids and combat the EVALI epidemic.

Introduction

E-cigarettes, also known as vaping, are a recent development in the nicotine and tobacco industry, replacing conventional cigarettes at a rapidly increasing rate. The main feature of e-cigarettes is their ability to aerosolize various substances, allowing the user to inhale these products. (1) The e-cigarette is used to inhale nicotine, tetrahydrocannabinol (THC), cannabidiol (CBD), and other substances that are also found in conventional cigarettes. Due to the rise in e-cigarette usage, there has been an epidemic with connections to vaping, leading to illnesses and in some cases, death. (1) The exact substances found in e-cigarette liquid are still unclear, as the production of the liquid can vary and often black market liquids are used as well.

Studies have been conducted to determine possible toxicants in e-cigarette liquid, with many determining the presence of vitamin E acetate, terpenes, and THC. (2) Specifically, a study done by Taylor et al. found that the most common substances in THC-containing products were vitamin E acetate, medium chain triglyceride (MCT), CBD, and alpha tocopherol (2019). Although these substances were found in various e-cigarette liquids, their connection to possible health effects is still uncertain.

Due to the presence of potential toxicants in e-cigarette fluid, there have been found to be adverse health effects that specifically target the lungs. Patients who became hospitalized due to using e-cigarettes have presented symptoms that include coughing, difficult and labored breathing, and hypoxia (3). Some patients have to undergo steroid and oxygen therapy as a part of their recovery treatment. This type of injury is known as EVALI, which is E-cigarette or Vaping Associated Lung Injury (3). Over 2800 of these cases were reported to the Centers for Disease Control and Prevention (CDC) from August 2019 to February 2020 (4).

It is possible that there is a connection between THC products also containing vitamin E acetate, which can be found in e-cigarette fluid. This connection needs to be fully investigated before making a definite prognosis. It is not known which specific agents cause EVALI, making it of utmost importance to investigate the toxicants found in e-cigarette fluid.

The full effects of vaping have not been fully determined, due to it being a novel form of smoking. For this reason, the consequences that come from vaping cannot be fully explained. Through further investigation into the consequences of vaping, possible diagnoses and treatments, as well as the chemicals that are found to be toxicants, we can reach towards their removal from the supply chain (1,5).

Analysis of e-cigarette liquids through various instrumentation methods would help to determine what toxicants they contain. Two samples were obtained for evaluation from patients who were hospitalized due to EVALI: Sample 1 (101PTYR - USA Vape Lab - Naked Very Cool) and Sample 2 (101NBXY - Smoky Mountain Vapor - Banana Bread). Through gas chromatography - mass spectrometry (GC-MS), the flavoring compounds of the samples will be determined. The nicotine concentration of the samples will be quantified using high performance liquid chromatography (HPLC). The zinc concentration of the samples will be obtained through Flame Atomic Absorption (Flame AA). The purity of the samples will be analyzed using Fourier-transform infrared spectroscopy (FT-IR). Through these methods, more information about the potential cause of EVALI can be obtained and ultimately help reduce injuries caused by these substances.

Materials and Methods

FT-IR

FT-IR and the Hit Quality Index method was used to determine any general adulterations found in the samples. The Hit Quality Index will be used to match the sample spectrum against a reference spectrum in order to determine whether the sample is contaminant free or not - a HQI above a 0.99 signifies no contamination. This was accomplished by collecting a background and collecting sample spectra for each sample and the base by placing a drop of sample on the ATR window**.** Table 1 below displays the parameters of the instrument used to collect the sample spectrumes. Three spectra of each undiluted sample and the base reference sample were collected. Spectra was collected as *Absorbance* vs. *Wavenumber*.

Table 1: FT-IR Instrument Parameters

Parameter	Value
Make and Model	Perkin Elmer Spectrum One
Detector Type	Deuterated triglycine sulfate infrared detector
Scan Range (cm-1)	4000 - 650
Number of Acquisitions	64
Sample Cell Type	Diamond ATR

GC-MS

In order to determine the flavoring compounds in our samples, they were analyzed using the GC-MS instrument (Tables 2, 3). A methanol blank was run on the instrument to verify that no significant contamination was present. Liquid-phase microextraction was used to prepare the samples. This consisted of placing a couple of drops of our e-cigarette sample in an autosampler vial. A syringe was loaded with 2 μL of methanol and pushed into the autosampler vial, stopping before touching the sample. The methanol was exposed to the sample for 30 seconds in order to absorb any flavoring compounds. The samples were manually injected into the GC compartment.

Table 2: GC Instrument Parameters

Parameter	Value
Make and Model	Agilent 7890A
Column	Agilent 19091S-433
Carrier	UHP He at 9 psi
Injection Port Temperature (°C)	260
Initial Oven Temperature (°C)	35
Oven Ramp Rate (°C/min)	20
Final Oven Temperature (°C)	250
Detector Type	Mass Spectrometer

Table 3: MS Instrument Parameters

Parameter	Value
Make and Model	Agilent 5975C with an Agilent G1888
Source	Electron Ionization
Mass Filter	Quadrupole
Detector	Electron Multiplier
Acquisition Mode	Scan
Scan Range	40-350 m/z
Scan Speed	Normal
Source Temperature (°C)	230

Flame AA

Flame AA spectroscopy (Table 4) was used to determine the zinc concentration found for one of our vape juice samples post and pre vaping. The samples were examined pre- and post- vaping to determine if the vaporization process had an effect on the zinc concentration levels of the samples.

We vaped a small portion of the sample in 30 second intervals using a laboratory vaping apparatus consisting of a peristaltic pump in order to obtain a sufficient amount of aerosolized sample. The sample obtained was the post vape sample. The pre vape sample consisted of the e-cigarette juice. The pre and post samples were diluted with ultrapure water (18.2 MΩ). We prepared a standard curve out of five zinc standards. The standards ranged from 1.94 ppm to 10.64 ppm. The standards had to be diluted from 1:10 in order to be analyzed.

Table 4: Flame AA Instrument Parameters

Parameter	Value
Make and Model	Perkin Elmer PinAAcle 900F
Wavelength (nm)	213.86
Number of Replicates/Sample	3
Slit Width (nm)	10
Oxidant Flow (L/min)	10
C2H2 Flow (L/min)	2.18
Working range (mg/L)	1
Cathode Lamp	Zinc

HPLC

The HPLC instrument (Table 5) was used to determine the nicotine concentration found in our samples. Standards and quality control samples were used in order to ensure there was no contamination, passing standard curve, and passing QC. The nicotine standards were prepared ranging from 9.93 ppm to 99.9 ppm using ultrapure water (18.2 MΩ). The standards and samples were placed into 2 mL autosampler vials for injection by the autosampler. The samples were prepared by placing 2 drops of each sample into a tared vial, and filled with 2 mL of ultrapure water (18.2 MΩ). The masses of the sample combined with the ultrapure water was collected for data analysis.

Table 5: HPLC Instrument Parameters

Parameter	Value
Make and Model	Agilent 1220 HPLC
Detector Type	Variable Wavelength
Mobile phase	75% water/ 25% Acetonitrile/ 0.1% formic acid
Detector Wavelength (nm)	260
Injection Volume (μL)	10
Output range (nm)	190 - 400
Column	Alltech Prevail Select C18

Results and Discussion

FT-IR

The results for the FT-IR and the Hit Quality Index (HQI) analysis is shown below in Figure 1. The samples and the reference spectra were compared to determine if any adulteration was present. Sample 1 had a HQI of 0.9996 and sample 2 had a HQI of 0.9962. No adulterations present in the samples would lead to a HQI of >0.99 or above.. As can be seen in Figure 1, the spectra for each sample matched the reference closely, with little discrepancies.

Due to incorrect setting on the instrument, the data collected for sample 1, did not match the format from sample 2 and the reference. To correct this issue, a function was created that models the spectrum; sample 2 data was used to correct the wavelength spacing for the y-values from sample 1.

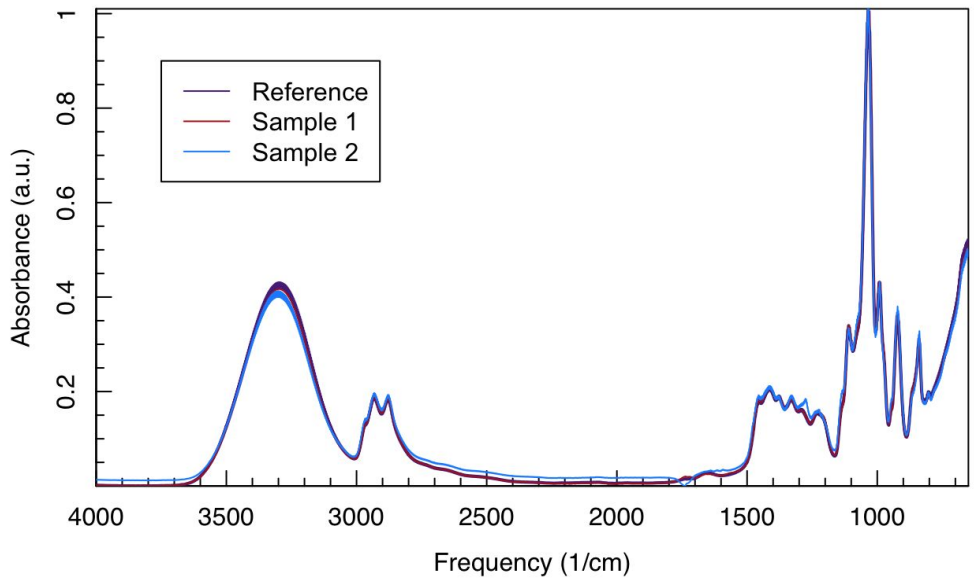

Figure 1: The figure above displays the average of each sample and the reference sample. Sample 1 in red refers to 101PTYR, and sample 2 in blue refers to 101NXBY. The reference sample is shown in purple.

GC-MS

Sample 1: 101PTYR

The results from the gas chromatography analysis performed on sample 1 are shown below in Figures 2 and 3. Figure 2 displays the gas chromatogram for sample 1. The two compounds chosen for analysis were ethyl-2-methyl butyrate, the retention time at 5.6 minutes, and butyl pentanoate, the retention time at 7.7 minutes, shown by the dotted green line and dashed blue line, respectively.

The mass spectrum shown below in Figure 3A was determined by using MassBank of North America (MoNA) to be ethyl 2-methylbutyrate. After further analysis of the mass spectrum, we were able to determine a reasonable molecular ion of 130.1 as can be seen in Figure 3B. The calculation for the numbers for carbons for ethyl 2-methyl butyrate was calculated to be 7 and the number of rings or double bonds was calculated to be 1.

The mass spectrum shown below in Figure 3C was determined by using MassBank of North America (MoNA) to be butyl pentanoate. The most reasonable molecular ion is located at 103.1 as can be seen in Figure 3D. The number of carbons was determined to be 10 and the number of rings or double bonds was calculated to be 1.

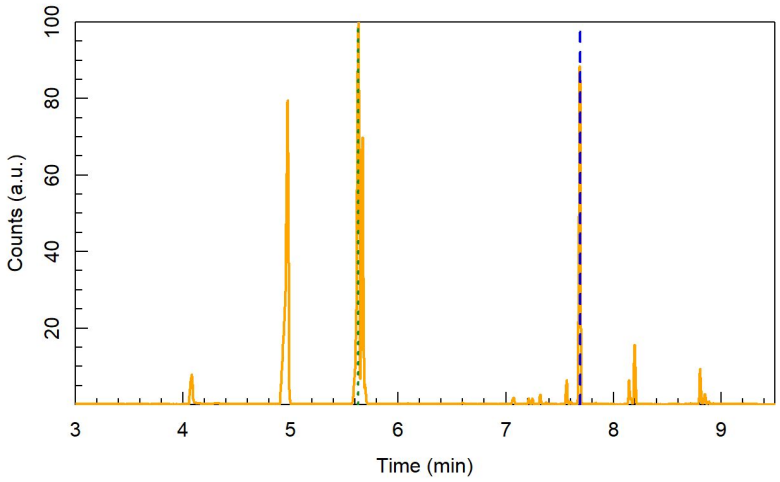

Figure 2: The chromatogram of Sample 1. The dotted green line represents the location of the mass spectrum of ethyl 2-methylbutyrate. The dashed blue line represents the location of the mass spectrum of butyl pentanoate.

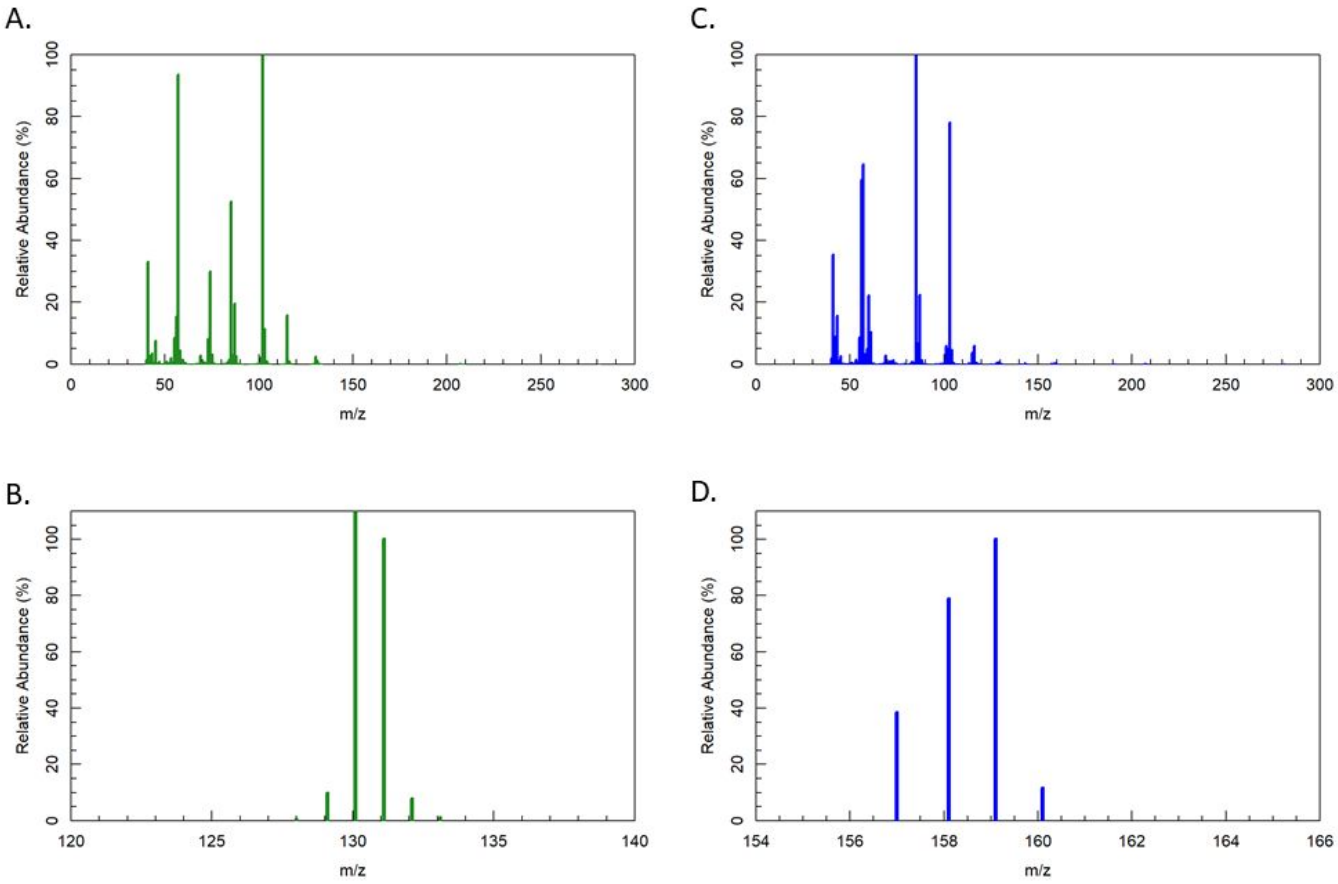

Figure 3: A. Mass spectrum of ethyl 2-methylbutyrate. The peak for this compound is around 5.6 minutes in Figure 2, highlighted by the dotted green line. B. This is the molecular ion region for ethyl 2-methylbutyrate. The y-axis has been rescaled to make M+. = 100%. C. Mass spectrum of butyl pentanoate. The peak for this compound is around 7.7 minutes in Figure 2, highlighted by the dashed blue line. D. This is the molecular ion region for butyl pentanoate. The y-axis has been rescaled to make M+. = 100%.

Sample 2: 101NXBY

The results from the gas chromatography analysis performed on sample 2 are shown below in Figures 4 and 5. Figure 4 displays the two compounds that were analyzed. The two compounds chosen for analysis were isopentanol, the retention time at 3.92 minutes, and 2-methyl-1-butanol, the retention time at 3.98 minutes, shown by the dashed pink line and dotted orange line, respectively.

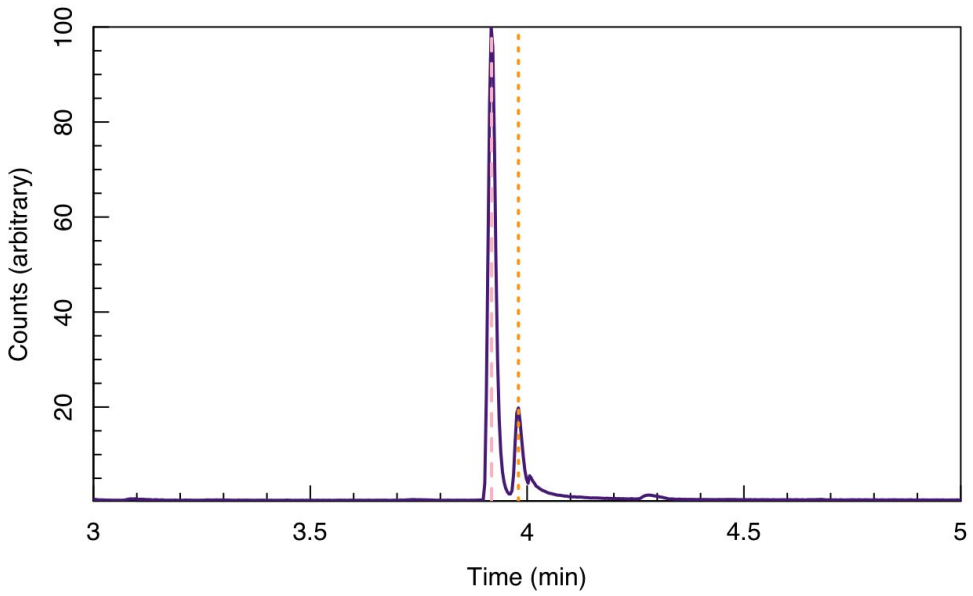

Figure 4: The gas chromatogram of Sample 2. The dashed pink line represents the location of isopentanol, whose mass spectrum is shown in Figure 5 below; the dotted green line represents 2-methyl-1-butanol, whose mass spectrum is shown in Figure 5 below.

The mass spectrum shown below in Figure 4A was determined by using MassBank of North America (MoNA) to be isopentanol. After further analysis of the mass spectrum, we were able to determine a reasonable molecular ion of 73.0 as can be seen in Figure 4B. The calculation for the numbers for carbons for isopentanol was calculated to be 7 and the number of rings or double bonds was calculated to be 0.

The mass spectrum shown below in Figure 4C was determined by using MassBank of North America (MoNA) to be 2-methyl-1-butanol. The most reasonable molecular ion is

located at 70.1 as can be seen in Figure 4D.. The number of carbons was determined to be 6 and the number of rings or double bonds was calculated to be 0.

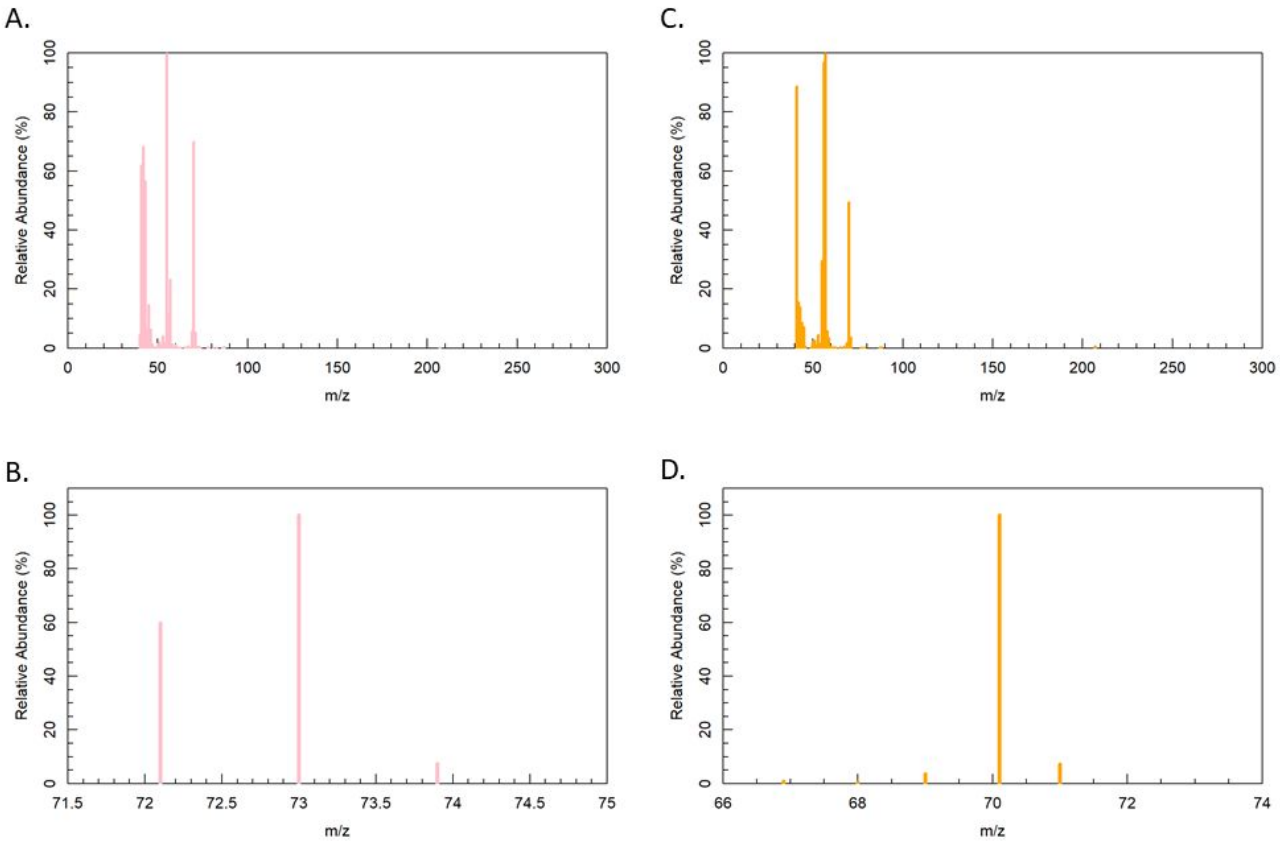

Figure 5: A. Mass spectrum of isopentanol. The peak for this compound is around 3.92 minutes in Figure 4, highlighted by the dashed pink line. B. This is the molecular ion region for ethyl 2-methylbutyrate. The y-axis has been rescaled to make M+. = 100%. C. Mass spectrum of 2-methyl-1-butanol. The peak for this compound is around 3.98 minutes in Figure 4, highlighted by the dotted orange line. D. This is the molecular ion region for 2-methyl-1-butanol. The y-axis has been rescaled to make M+. = 100%.

Flame AA

The results from the flame AA analysis are shown below in Figures 6 and 7. The purpose of this analysis was to determine if the zinc concentrations between the pre- and post-vape samples was significantly different. The first figure displays the standard curve of the five standards made which were the following concentrations: 0.1945, 0.3890, 0.5985, 0.798, and 1.064 mg/L. The linear regression value of the standard curve was 0.9997, which was an acceptable value.

The sample preparation for flame AA consisted of diluting the samples with ultrapure water before analysis. The diluted concentrations for the pre-vape and post-vape sample were -0.02856 ± 0.02731 mg/L and 0.06671 ± 0.02485 mg/L, respectively. The limit of quantitation was determined to be 0.05769 mg/L. The undiluted concentrations for the pre- and post-vape samples are -1.254 ± 1.199 mg/L and 0.6482 ± 0.2415 mg/L, respectively. These results are summarized in Figure 7. The pre-vape sample is below the limit of quantitation, meaning it cannot be distinguished from random noise. The confidence interval for the pre-vape sample almost reached zero, which could also indicate that the zinc concentration in that sample is insignificant.

The pre- and post-vape confidence intervals do not overlap, meaning there is a significant difference in zinc concentration in the pre- and post-vape samples. Outside studies have also reported a marked difference in the concentrations of various metals between unvaped e-cigarette liquid and aerosolized e-cigarette liquids (6). Even contact with the e-cigarette tank can contaminate the liquids. The results from this experiment match the results reported by the study.

The expected QC concentration was 0.5985 mg/L, and the predicted QC concentration was 0.6032 mg/L. The percent difference was calculated to be 0.78%, which is considered passing.

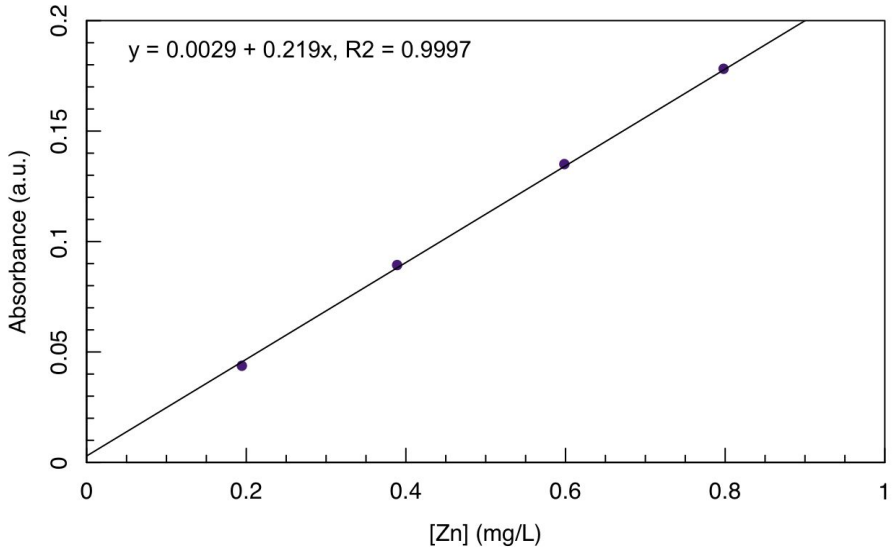

Figure 6: The standard curve of the five standards ranging from 0.1945 to 1.064 mg/L.

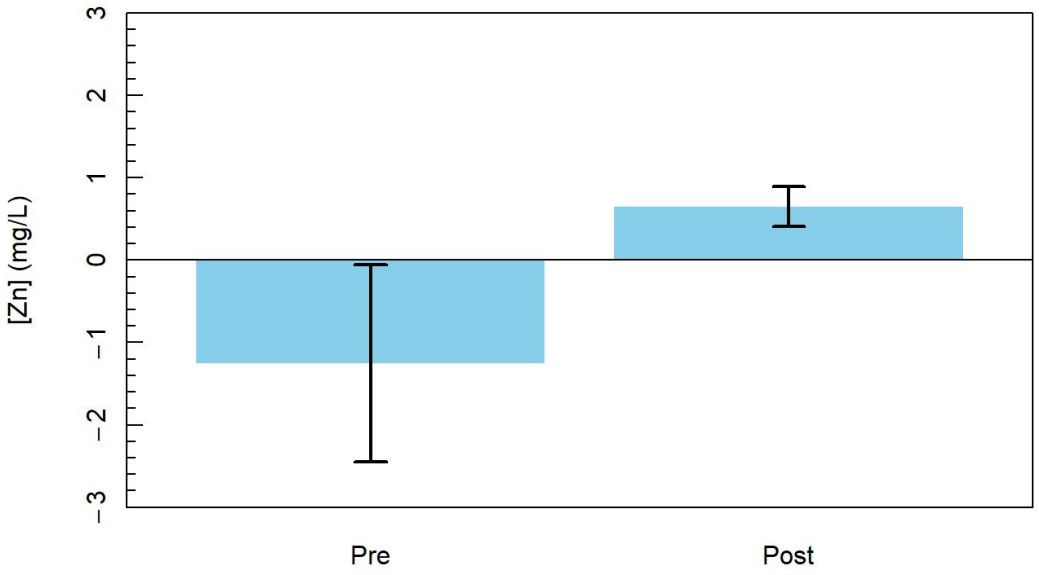

Figure 7: The bar plot above displays the concentration of the pre- and post-vape, along with the error bars that represent the 95% confidence limits.

HPLC

The purpose of this analysis was to determine the nicotine concentrations in each e-cigarette liquid sample. The standard curve plot can be seen in Figure 8, where the R^2 value is 0.9993; this is a passing R^2 value. The concentrations of the five standards made were 9.93 ppm, 39.72 ppm, 59.58 ppm, 79.44 ppm, and 99.3 ppm.

The sample preparation for this analysis involved diluting the samples with ultrapure water. The diluted concentrations for sample 1 and sample 2 were 117.7 ± 3.9 ppm and 260.7 ± 10.38 ppm, respectively. The undiluted concentrations were determined to be 5290 ± 175 ppm for sample 1 and 12110 ± 483 ppm for sample 2. The two samples can be seen graphed against the 79.44 ppm standard in Figure 9. The QC concentration was determined to be 59.65 ± 3.77 ppm. The known QC concentration is 59.58 ppm. The QC passes with a percent difference of 0.12%. The limit of detection was determined to be 65.7 ppm and the limit of quantitation was determined to be 219 ppm. The concentrations of both samples were above the limit of detection and the limit of quantitation. This means they can be counted as actual data, and can both be kept.

The expected nicotine concentration for both samples is 6000 ppm, meaning the difference between the expected and measured concentrations is significant. Comparing these results to those of other studies, the difference was not expected. The results from other studies found the concentrations to be relatively accurate (7).

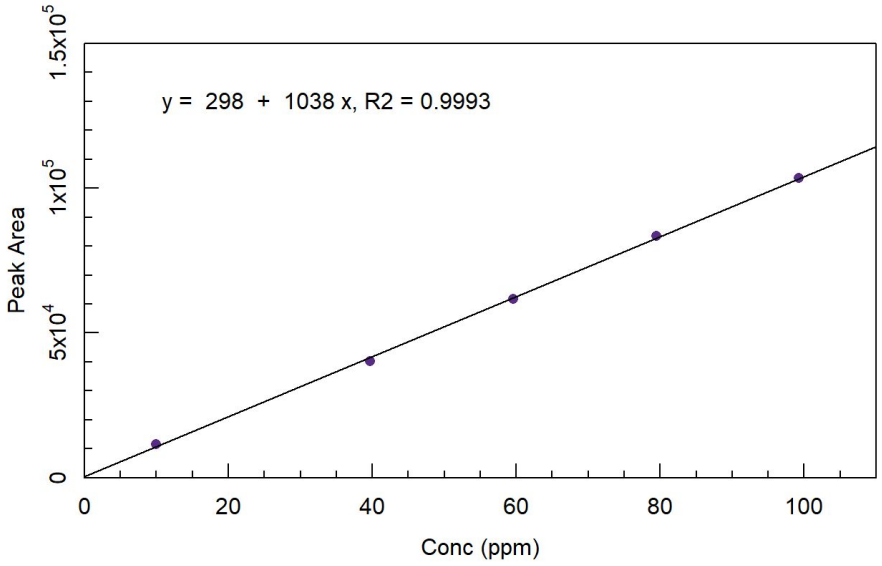

Figure 8: The standard curve of the five standards ranging from 9.93 to 99.3 ppm.

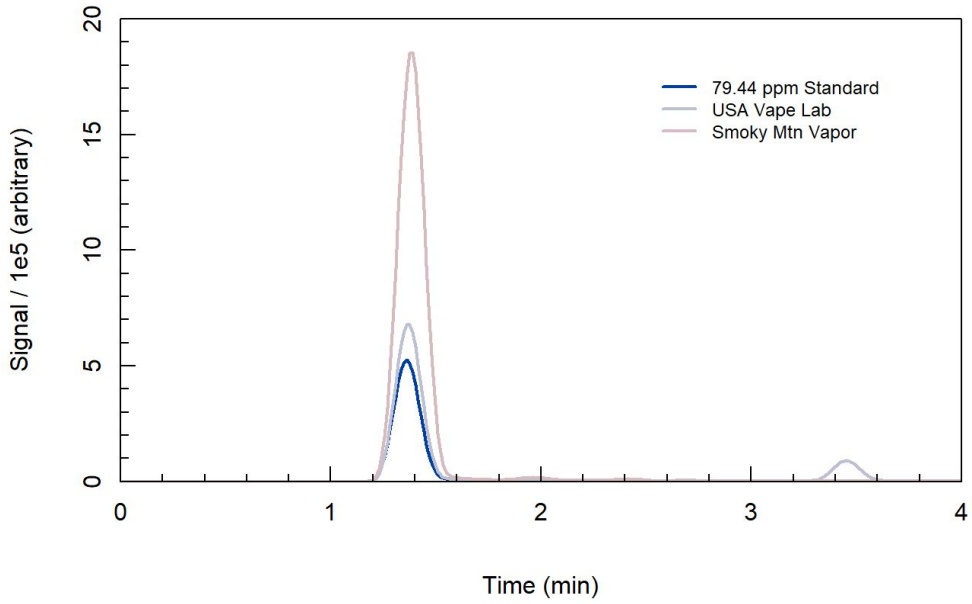

Figure 9: The chromatogram of the two samples graphed against the 79.44 ppm standard.

Conclusion

E-cigarette or vaping associated lung injury has led to hospitalizations and in some cases, death. It is an epidemic that leads to adverse health effects specifically affecting the lungs. Patients with this illness often undergo oxygen and steroid therapies to combat the illness. Part of the reason for this epidemic is the presence of possible toxicants in e-cigarette fluid, as its manufacturing is not yet strictly controlled.

To determine the composition and possible toxicant contamination of two e-cigarette liquids, they were analyzed through various instrumentation methods. Through flame AA, the zinc concentration for pre- and post-vaped e-cigarette juice was compared. The zinc concentration for the post-vape sample was higher than the pre-vape sample, suggesting that the aerosolization of the e-cigarette liquid has a significant effect. This difference in zinc concentration could be due to the metal coils and tanks that are used in e-cigarettes, and their contamination of the e-cigarette liquids.

FT-IR and the HQI were used to determine the purity of the samples. Sample 1 had a HQI of 0.9996 and sample 2 had a HQI of 0.9962. With these values, both of the samples are considered a match to the reference, meaning there is no adulteration present.

Through GC-MS, the two most abundant flavoring compounds in each sample were analyzed. From Sample 1 (101PTYR), two flavoring compounds were analyzed, ethyl 2-methyl butyrate and butyl pentanoate. The ethyl 2-methylbutyrate compound had a retention time of 5.6 minutes. The molecular ion is located at m/z = 130.1, with 7 carbons and 1 ring or double bond. The actual molecular weight of this compound is 130.18 g/mol. The molecular ion closely matches this value. The structure of this compound has one double bond, which corresponds to the calculated value. This compound is a colorless oily liquid that is insoluble in water (8). The butyl pentanoate compound had a retention time of 7.7 minutes. The most reasonable molecular ion was located at m/z = 103.1, with 10 carbons and 1 ring or double bond. The actual molecular weight of this compound is 158.24 g/mol. The structure of this compound has one double bond, which corresponds to the calculated value. This compound is a liquid with an apple-raspberry odor (9). It is possible that this

compound is not actually the compound the MassBank said it was, based on the actual weight.

From Sample 2 (101NXBY), the compounds isopentanol and 2-methyl-1-butanol were analyzed. The isopentanol compound had a retention time of 3.92 minutes. The most reasonable molecular ion was located at m/z = 73.0, with 7 carbons and no rings or double bonds. This compound is a colorless liquid with a mild choking alcohol odor. It is known to produce an irritating vapor (10). It is possible that this compound is not actually the compound the MassBank said it was, due to the actual molecular weight of isopentanol (88.15 g/mol). The 2-methyl-1-butanol compound had a retention time of 3.98 minutes. The most reasonable molecular ion was located at m/z = 70.1, with 6 carbons and no rings or double bonds. This compound is an alcohol that can cause symptoms such as coughing, dizziness, sore throat, and drowsiness if inhaled (11). It is possible that this compound is not actually the compound the Mass Bank said it was, due to the actual weight of 2-methyl-1-butanol (88.15 g/mol). In summary, it seems that the majority of obtained flavoring compounds did not closely match the compounds predicted by the MassBank. This could have been a result from contamination of the sample or the instrument.

HPLC was used to determine the nicotine concentration of both samples. It was found that the nicotine concentration of Sample 1 was significantly lower than the expected concentration. The nicotine concentration for Sample 2 was almost double the expected concentration. Results from previous research found that this difference was abnormal, as their concentrations were not significantly different in nicotine concentration from what was expected (7). The disparity in nicotine concentrations could potentially be harmful to consumers of these products.

Future improvements include repeating the methods used in this report using different types of e-cigarette devices to determine if the quality or price of these devices has any effect on the toxicants it releases. A wider range of e-cigarette liquids could be analyzed to determine if the results obtained from this study are reproducible. The analysis of these e-cigarette liquids through these instrumentation methods identified potential toxicants and disparities in quality control. The results from this study could be used as a

basis for new standards and safety regulations for the production of e-cigarette liquids. The information gathered from these analyses also provides insight into the possible cause of EVALI and the lung injury epidemic seen among those who use e-cigarettes.

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