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Research Article

Evaluation of the Components within Electronic Cigarette Liquids and Drugs of Abuse Using Gas Chromatography-Mass Spectrometry

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Abstract

Introduction: As electronic cigarettes become more prevalent in society, their use as a delivery mechanism for drugs of abuse has increased. Electronic cigarette liquids present a complex matrix due to the lack of regulation in their manufacturing. Due to limited published data development of new analytical methods was deemed necessary.

Methods: A Gas Chromatography-Mass Spectrometry (GC/MS) method was developed to identify flavorants of the electronic cigarette liquids as well as identify and quantify nicotine and common drugs of abuse used with these devices. Five drugs of abuse were investigated: methamphetamine, heroin, cocaine and fentanyl. Electronic cigarette liquids from five manufacturers were sampled and five "flavors" of liquids from each were analyzed. Each liquid "flavor" was tested at the manufactures reported nicotine concentrations of 0 mg/mL, 12 mg/mL and 24 mg/mL (n=75). Liquid-liquid extraction was performed on all samples prior to analysis by GC/MS. Analysis was performed in replicates of five to identify the electronic cigarette liquid components as well as quantify nicotine and the four analytes of interest. For any electronic cigarette liquid labeled as containing 0 mg/mL of nicotine in which nicotine was identified the sample was analyzed by GC/MS to quantify the nicotine level. These concentrations were compared to the naturally occurring levels of nicotine found in certain food products.

Results/Conclusions: Identification of flavorants including those not approved for human inhalation, of the electronic cigarette liquids as well as the quantification of nicotine and the four commonly abused drugs was accomplished using GC/MS. Fourteen out of 25 e-liquids labeled by the manufacturer as containing 0 mg/mL of nicotine contained statistically significant levels of nicotine. Quantification of drugs of abuse were affected by matrix components and found to be dependent on both the specific e-liquid being used with the electronic cigarette as well as the analyte being investigated.

Keywords: Drugs of abuse; Electronic cigarettes; Electronic cigarette liquids; Nicotine

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Introduction

Electronic cigarettes, also known as Electronic Nicotine Delivery Systems (ENDS) or simply e-cigarettes are battery-operated inhalers that deliver nicotine to the user without the harmful combustion reactions of traditional tobacco cigarettes. There is no standard construction of e-cigarettes which allows different manufacturers to use different designs and components for their particular products [1].

One of the most variable components of e-cigarettes are the electronic cigarette liquids which are commonly known as "e-liquids". E-liquids contain propylene glycol, vegetable glycerin, nicotine and flavorants however the exact chemical make-up varies widely based on the manufacturer [2]. Manufacturers use different chemical combinations to produce specific flavors with ingredient lists often lacking all of the components if included at all with the user not knowing what they are inhaling. Although several of the chemicals used in the e-liquid flavorings have been approved for use in the food industry the effects of using these chemicals by heating and inhalation have not been studied at length [2].

Prior to 2016, e-cigarettes and e-liquids were not regulated by the FDA unless they were labeled as being for therapeutic use. However, in May 2016 the FDA published a rule that would regulate e-cigarettes and their components which includes e-liquids the same way that other tobacco products are regulated. The final rule went into effect on August 8, 2016. Some of the new regulations include prohibiting vending machine sales to minors prohibiting providing free samples to consumers and requiring packaging to carry warning labels [3].

As electronic cigarettes grow in popularity they are increasingly being used as drug delivery mechanisms by users and discussed on internet forum pages such as Drugs-Forum, Bluelight and Reddit [4-10]. Many users state that they use e-cigarettes to abuse various drugs because they are able to use these devices in public places without suspicion [4,8,9]. Users have also stated that they favor using e-cigarettes as a drug delivery mechanism because it provides them with a desirable high without the need to inject the drug they are abusing [7,8]. To date the drugs that users have reported success in abusing via e-cigarettes include methamphetamine, cocaine, fentanyl, heroin and synthetic cannabinoids [11-16].

The objective of this research was to determine the components of e-liquids obtained from several different manufacturers and to determine if drugs of abuse that have been added to e-liquids could be detected using Gas Chromatography-Mass Spectrometry (GC/MS). In addition the nicotine concentration reported by the manufacturers

was evaluated. Qualitative and quantitative methods have been developed for the analysis of these samples.

Experimental

Materials

Sodium hydroxide, sodium carbonate and High Performance Liquid Chromatography (HPLC) grade chloroform were purchased from Fisher Scientific Inc., (Pittsburgh, PA, USA). Certified reference material of fentanyl, fentanyl-d_5, nicotine, nicotine-d_4, methamphetamine, methamphetamine-d_5, heroin, heroin-d_9, cocaine and cocaine-d_3 were purchased from Cerilliant Corporation (Round Rock, TX, USA). Purified water was obtained by using a Direct-Q® 3 ultrapure water system from EMD Millipore/Merck KGaA Corporation (Darmstadt, Germany).

The e-liquid samples (Table 1) were purchased from VaporFi® (Miami Lakes, FL, USA), Mt. Baker Vapor© (Lynden, WA, USA), Crazy Vapors© (Augusta, GA, USA), Viking Vapor© (Ben Lomond, CA, USA) and ProVape© (Monroe, WA, USA). The e-liquid samples purchased for analysis were chosen to allow for as much consistency across manufacturers as possible. To facilitate this specific flavors of e-liquids chosen from each manufacturer fell into five different "flavor categories". These included tobacco, menthol, blueberry, vanilla, and chocolate. Three e-liquids from each flavor category were purchased from each manufacturer to evaluate a range of nicotine concentrations (0-24 mg/mL). Table 1 shows the specific e-liquids purchased from each manufacturer with a total of 75 different e-liquid matrices evaluated.

	0 mg/mL Nicotine Content	12 mg/mL Nicotine Content	24 mg/mL Nicotine Content
VaporFi®	Classic Tobacco	Classic Tobacco	Classic Tobacco
	Menthol Ice	Menthol Ice	Menthol Ice
	Blueberry Blast	Blueberry Blast	Blueberry Blast
	Very Vanilla	Very Vanilla	Very Vanilla
	Chocolate Delight	Chocolate Delight	Chocolate Delight
Mt. Baker Vapor©	East Coast Tobacco	East Coast Tobacco	East Coast Tobacco
	Menthol	Menthol	Menthol
	Blueberry	Blueberry	Blueberry
	French Vanilla	French Vanilla	French Vanilla
	Cookie Blaster	Cookie Blaster	Cookie Blaster
Crazy Vapors©	Cured Tobacco	Cured Tobacco	Cured Tobacco
	Menthol	Menthol	Menthol
	Blueberry	Blueberry	Blueberry
	French Vanilla	French Vanilla	French Vanilla
	Double Chocolate	Double Chocolate	Double Chocolate
Viking Vapors©	Tobacco	Tobacco	Tobacco
	Menthol Tobacco	Menthol Tobacco	Menthol Tobacco
	Blueberry	Blueberry	Blueberry
	Vanilla	Vanilla	Vanilla
	Chocolate	Chocolate	Chocolate
ProVape©	Ken's Tobacco	Ken's Tobacco	Ken's Tobacco
	Icy Menthol	Icy Menthol	Icy Menthol
	Frosted Blueberry	Frosted Blueberry	Frosted Blueberry
	Simply Vanilla	Simply Vanilla	Simply Vanilla
	Chocolate	Chocolate	Chocolate

Table 1: E-liquids samples analyzed.

All e-liquid samples were analyzed by GC/MS to determine their components. Samples in bold font were used for quantitative analysis by GC/MS.

GC/MS instrumentation/parameters

Gas chromatographic analysis was performed on an Agilent Technologies Inc., 7890A GC System (Santa Clara, CA, USA). Mass spectrometric analysis was performed on an Agilent Technologies Inc., 5975C inert Electron Impact/Chemical Ionization Mass Selective Detector (EI/CI MSD). Data analysis was performed using Agilent MSD Chem Station software (version E.02.02.1431).

The GC/MS parameters were optimized for the detection and quantitation of fentanyl, nicotine, methamphetamine, cocaine and heroin. A RestekRxi®-5HT (Bellefonte, PA, USA) column (30 m x 0.25 mm x 0.25 µm) was used for analysis. A split less injection was used with an injection volume of 1 µL an inlet temperature of 250°C and a flow rate of 1.3 mL/minute (min). The oven was held at an initial temperature of 60°C for two minutes, increased to 240°C at a rate of 30°C/min and then held for two minutes increased to 300°C at a rate of 15°C/min and held for one minute for a total run time of 15 minutes. The MS was operated in scan mode, using electron ionization, a solvent delay of four minutes, a MS quadrupole temperature of 230°C, and a MS source temperature of 150°C.

Extraction method

A Liquid-Liquid Extraction (LLE) was performed which allowed the analytes of interest as well as the e-liquid matrix components to separate into the organic phase for analysis. Two different LLEs were utilized. One was used to identify the e-liquid matrix components while a separate procedure was used to quantify any drugs of abuse. When the goal of the analysis was to identify matrix components 100 µL of e-liquid was pipetted into a disposable glass tube. This sample was then extracted by adding 1 mL of Double Deionized Water (DDW) 70 µL of 10% Sodium Hydroxide (NaOH) and 1.5 mL of chloroform. The sample was then vortexed for ten seconds and 1 mL of the chloroform layer was pipetted into an auto sampler vial for analysis by GC/MS.

When the goal of analysis was to quantify drugs of abuse 100µL of e-liquid matrix was pipetted into a disposable glass tube and then spiked with a working stock solution. The working stock solution was prepared from standards of fentanyl, methamphetamine, heroin and cocaine in methanol at a concentration of 100 µg/mL. This spiked sample was then extracted by adding 1 mL of DDW, 30 µL of 2% Sodium Carbonate (Na_2CO_3) and 1.5 mL of chloroform. This sample was then vortexed for ten seconds and 1 mL of the chloroform layer was pipetted into an auto sampler vial for analysis by GC/MS.

Method validation

A six point calibration curve was generated with a range of 20-300 µg/mL for the analytes nicotine, methamphetamine, cocaine, heroin and fentanyl in an e-liquid matrix containing 0 mg/mL of nicotine. "Unknown" samples were prepared in replicates of five by spiking a solution of methamphetamine, cocaine, heroin and fentanyl at a concentration of 150 µg/mL into e-liquid matrix samples with a nicotine concentration of 24 mg/mL (Table 1).

All samples were spiked with an internal standard solution of methamphetamine-d_5, cocaine-d_3, heroin-d_9, fentanyl-d_5, and

nicotine-d_4 at a concentration of 100 µg/mL. The Limit of Detection (LOD), Limit of Quantitation (LOQ), coefficient of determination (R^2) and the accuracy of the calculated "unknown" concentrations were determined using Chem Station integrator available in the MSD Chem Station software.

Results

Qualitative results

The e-liquid samples were evaluated by GC/MS to determine the components of the e-liquid matrix and over 40 different compounds were detected in addition to the manufacturer stated nicotine and propylene glycol. Tables 2 through 6 reflect the flavorants that were detected in each e-liquid sample by flavor category.

E-liquid Sample	Flavorant Compounds
VaporFi® Classic Tobacco	Cinnamic acid, methylester; β-damascone
Mt. Baker Vapor© East Coast Tobacco	Trimethylpyrazine; tetramethylpyrazine; menthol; β-citronellol; guaniol
Crazy Vapors© Cured Tobacco	Trimethylpyrazine; tetramethylpyrazine; gluethimide
Viking Vapor Tobacco	Piperonal
Pro Vape© Ken's Tobacco	Benzophenone; Tetramethylpyrazine; Menthol; Guaniol; Cinnamaldehyde

Table 2: Detected E-liquid Flavorants.

Flavorants detected in the tobacco flavor category e-liquid samples. Identification of flavorants were made by comparing the mass spectrum of the suspected flavorant to mass spectra from the National Institute of Standards and Technology library (NIST, Gaithersburg, MD, USA).

E-liquid Sample	Flavorant Compounds
VaporFi® Menthol Ice	Eucalyptol; Menthol
Mt. Baker Vapor© Menthol	Menthol
Crazy Vapors© Menthol	Menthol
Viking Vapors© Menthol Tobacco	Menthol; Piperonal; Benzyl Benzoate
Pro Vape© Icy Menthol	Benzyl alcohol; Menthol; Cinnamaldehyde; Benzophenone

Table 3: Detected E-liquid flavorants.

Flavorants detected in the menthol flavor category e-liquid samples. Identification of flavorants were made by comparing the mass spectrum of the suspected flavorant to mass spectra from the NIST library.

E-liquid Sample	Flavorant Compounds
VaporFi® Blueberry Blast	Benzyl alcohol; β-linalool; menthol; cis-geraniol; β-damascenone; davanone
Mt. Baker Vapor© Blueberry	Benzyl alcohol; tetra methyl pyrazine; β-linalool; menthol; benzophenone
Crazy Vapors© Blueberry	β-linalool; cis-jasmone; glutethimide
Viking Vapors© Blueberry	Benzyl alcohol; β-linalool
ProVape© Frosted Blueberry	Benzyl alcohol; β-linalool; benzophenone

Table 4: Detected E-liquid flavorants.

Flavorants detected in the blueberry flavor category e-liquid samples. Identification of flavorants were made by comparing the mass spectrum of the suspected flavorant to mass spectra from the NIST library.

Quantitative results

A six point calibration curve was generated over the range of 20 µg/mL-300 µg/mL and used to determine the LOD, LOQ and the R^2 value for each target analyte, methamphetamine, cocaine, nicotine, heroin and fentanyl. Figure 1 shows a combined calibration curve for the analytes nicotine, cocaine, heroin and fentanyl. Methamphetamine was unable to be quantified due to interference from the e-liquid matrix.

E-liquid Sample	Flavorant Compounds
VaporFi® Very Vanilla	Anethole; piperonal
Mt. Baker Vapor© French Vanilla	Piperonal
Crazy Vapors© French Vanilla	4-methoxy-benzaldehyde
Viking Vapors© Vanilla	Butanoic acid; 3-methyl-3-methylbutylester (apple oil); butyl butyryl lactate; vanillin
Pro Vape© Simply Vanilla	Anise alcohol; vanillin; benzophenone

Table 5: Detected E-liquid flavorants.

Flavorants detected in the vanilla flavor category e-liquid samples. Identification of flavorants were made by comparing the mass spectrum of the suspected flavorant to mass spectra from the NIST library.

E-liquid Sample	Flavorant Compounds
VaporFi® Chocolate Delight	Benzyl alcohol
Mt. Baker Vapor© Cookie Blaster	Menthol; piperonal; benzophenone
Crazy Vapors© Double Chocolate	Trimethylpyrazine; benzyl alcohol; tetramethylpyrazine; glutethimide
Viking Vapors© Chocolate	Trimethylpyrazine; tetramethylpyrazine
Pro Vape© Chocolate	Trimethylpyrazine; benzyl alcohol; tetramethylpyrazine; piperonal; benzyl benzoate

Table 6: Detected E-liquid flavorants.

Flavorants detected in the chocolate flavor category e-liquid samples. Identification of flavorants were made by comparing the mass spectrum of the suspected flavorant to mass spectra from the NIST library.

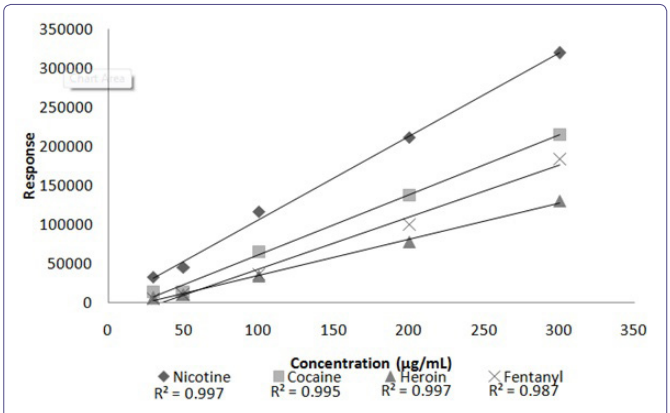

Figure 1: Calibration curve of nicotine, cocaine, heroin, and fentanyl.

Five of the six points generated were used in the calibration curve, with the lowest calibrator falling below the established LOQ. The R2 value for each analyte fell above the acceptable limit of 0.98.

It was determined that the LOD for this instrument and method was 1 µg/mL, and the LOQ was set at 30 µg/mL. The LOD was determined by analyzing a set of samples spiked at varying concentrations to determine the lowest concentration where the instrument was able to consistently detect the analytes of interest. The LOQ was set at 30 µg/mL due to the fact that the lowest calibration point initially used (20 µg/mL) was unable to be integrated by the software consistently and therefore unable to be quantified for all target analytes. The R^2 values for each of the analytes able to be quantified all fell above the acceptable value of 0.9800 with the R^2 value for nicotine being 0.9975

for cocaine being 0.9955 for heroin being 0.9971, and for fentanyl being 0.9879.

The accuracy of the "unknown" samples was determined by comparing the calculated concentration of each analyte to the target concentration of 150 µg/mL. The concentrated values determined to be greater than ±20% was considered to be inaccurate. The results of the accuracy calculations can be seen in table 7.

Twenty-five of the 75 e-liquid samples analyzed were stated by manufacturers to contain 0 mg/mL of nicotine. Of those 25 samples, only six contained no detectable nicotine five contained detectable levels of nicotine that fell below the LOQ and 14 contained detectable and quantifiable concentrations of nicotine. A t-test was conducted to determine if there was a statistical difference between the levels of nicotine in the 14 e-liquid sample compared to food products naturally containing nicotine (e.g. eggplant). A mean concentration of 0.1 µg/mL was found in eggplant samples and was used in the t-test calculations [17].

Discussion

Liquid-liquid extractions

At the time of this research much of the published literature on extracting e-liquids involved diluting the e-liquid in a solvent and analyzing the diluted mixture [18]. It was determined that a cleaner extraction technique would be beneficial when attempting to quantify the drugs of abuse added to the e-liquid samples so a liquid-liquid extraction technique was developed. Two different Liquid-Liquid Extractions (LLE) were performed for analysis by GC/MS.

In the LLE to determine the components of the e-liquid matrices, a 10% Sodium Hydroxide (NaOH) base was used. In the LLE to quantify the drugs of abuse added to the e-liquid matrices a 2% Sodium Carbonate (Na_2CO_3) base was used. Two extraction methods was necessary to prevent degradation of heroin due to an overly basic pH and therefore affecting quantitation. However this resulted in two different chromatograms of the same sample (Figure 2) due to the differences in the strength of the two bases. Although two distinct chromatograms were produced it did provide additional information regarding the e-liquid matrices. The LLE utilizing 10% NaOH provided more information regarding the components of the e-liquid matrix when compared to the 2% Na_2CO_3. It is possible that the use of a third base may provide an even greater amount of information regarding the components than either of these two methods.

E-liquid components

Manufacturers of e-liquids typically list only propylene glycol, vegetable glycerin and nicotine as ingredients and leave out all of the

	Nicotine (mg/mL)	Cocaine (µg/mL)	Heroin (µg/mL)	Fentanyl (µg/mL)
VaporFi® Classic Tobacco	29.38-30.26 (29.98)	134.2-142.1 (141.1)	133.9-138.8 (135.5)	156.0-168.1 (170.9)
VaporFi® Menthol Ice	26.46-29.92 (28.38)	119.9-138.3 (127.8)	84.25-99.69 (90.36)	153.2-178.8 (164.1)
VaporFi® Blueberry Blast	25.67-28.54 (26.45)	103.9-116.8 (111.2)	126.9-145.6 (136.3)	141.2-153.5 (149.4)
VaporFi® Very Vanilla	26.23-27.56 (27.09)	119.3-126.9 (124.1)	122.6-132.9 (127.8)	152.8-158.9 (155.1)
VaporFi® Chocolate Delight	25.20-25.92 (25.53)	103.9-118.5 (111.8)	113.8-136.3 (126.3)	147.7-154.9 (151.7)
Mt. Baker Vapor© East Coast Tobacco	26.05-29.54 (26.88)	113.8-135.2 (116.7)	129.3-151.2 (131.3)	151.9-165.2 (149.3)
Mt. Baker Vapor© Menthol	32.24-32.65 (32.39)	132.8-141.7 (137.5)	111.3-126.8 (120.1)	179.2-211.8 (199.7)
Mt. Baker Vapor© Blueberry	27.43-30.25 (29.09)	111.9-127.8 (118.8)	101.4-114.7 (106.3)	150.8-161.4 (156.2)
Mt. Baker Vapor© French Vanilla	24.07-27.61 (26.46)	94.46-118.6 (109.2)	105.5-133.7 (124.4)	120.2-151.0 (137.9)
Mt. Baker Vapor© Cookie Blaster	20.39-21.78 (21.14)	68.53-77.59 (73.33)	71.51-83.39 (77.65)	75.70-98.71 (87.07)
Crazy Vapors© Cured Tobacco	10.82-12.01 (11.58)	133.2-139.7 (135.1)	121.0-126.3 (122.4)	101.4-104.1 (102.5)
Crazy Vapors© Menthol	8.33-8.53 (-8.46)	102.3-112.7 (108.3)	124.1-131.6 (128.2)	84.58-92.57 (88.28)
Crazy Vapors© Blueberry	9.28-10.19 (9.59)	133.3-144.1 (138.9)	119.2-131.4 (125.8)	115.6-123.6 (119.2)
Crazy Vapors© French Vanilla	8.49-9.44 (-9.1)	155.5-167.2 (162.1)	177.8-184.7 (181.3)	167.9-174.9 (171.8)
Crazy Vapors© Double Chocolate	9.03-10.19 (9.65)	147.8-164.5 (156.5)	161.3-178.6 (170.5)	128.5-144.7 (142.9)
Viking Vapors© Tobacco	11.35-12.29 (11.72)	102.2-104.9 (103.9)	122.7-129.3 (126.4)	63.31-72.07 (68.19)
Viking Vapors© Menthol Tobacco	12.50-13.16 (12.81)	117.6-126.2 (120.5)	130.4-140.8 (136.6)	93.16-99.52 (96.51)
Viking Vapors© Blueberry	12.75-13.66 (13.21)	143.5-145.5 (143.6)	121.2-125.5 (123.3)	116.0-126.6 (123.3)
Viking Vapors© Vanilla	12.27-13.62 (12.77)	171.1-176.2 (174.3)	193.7-197.9 (195.7)	170.1-182.3 (177.1)
Viking Vapors© Chocolate	13.16-14.07 (13.54)	150.7-159.9 (154.5)	160.6-164.9 (162.6)	125.3-131.9 (128.3)
ProVape© Ken's Tobacco	17.61-18.20 (18.02)	150.7-156.6 (153.8)	138.1-145.0 (142.5)	134.4-141.3 (138.9)
ProVape© Icy Menthol	15.56-16.45 (15.95)	152.7-158.1 (154.9)	150.4-154.6 (151.8)	170.8-176.3 (173.4)
ProVape© Frosted Blueberry	16.39-18.15 (17.16)	138.6-146.9 (143.9)	117.8-136.9 (127.6)	117.9-124.8 (120.4)
ProVape© Simply Vanilla	14.86-15.18 (14.99)	169.9-177.8 (174.6)	177.1-192.7 (183.9)	188.3-199.3 (194.9)
ProVape© Chocolate	15.10-16.39 (15.63)	156.3-161.9 (160.1)	165.5-171.2 (168.1)	100.2-104.8 (102.6)

Table 7: Calculated concentrations of "Unknown" samples.

The range of concentrations for each analyte in each matrix is shown with the average value being shown in parentheses (). Calculated concentrations that fell outside of the ±20% of 150 µg/mL range (cocaine, heroin, and fentanyl) and 24 mg/mL (nicotine) are in bold and italic font.

specific natural and artificial flavorants used. Analysis by GC/MS to determine the flavorants used in these e-liquids detected over 40 different compounds in addition to propylene glycol and nicotine. In the 75 e-liquids tested none of the drugs of abuse being investigated, aside from nicotine were detected in the unspiked samples. Vegetable glycerin was not detected in any of the samples, but was reportedly present in all samples according to the manufacturer's labels. The vegetable glycerin may not have been detected because its concentration was very low compared with the other components or this may have been a limitation of the method. Of the flavorants listed (Tables 2-6) 96% of them are approved by the FDA for use as additives in food or cosmetic products [19]. However one compound glutethimide which was detected in all of the Crazy Vapors© Double Chocolate, Crazy Vapors© Cured Tobacco and Crazy Vapors© Blueberry e-liquids is a Schedule II substance controlled by the DEA [19]. Identification of nicotine and flavorants used in the e-liquids was made by comparing the experimental mass spectra to the spectra found in the NIST library.

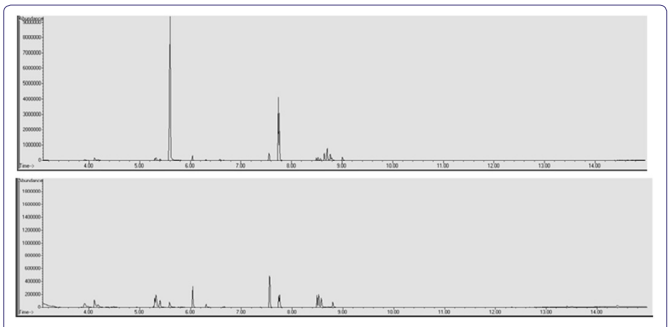

Figure 2: Differences in liquid-liquid extractions.

Top: Liquid-liquid extraction using 2% Na_2CO_3 as the base.

Bottom: Liquid-liquid extraction using 10% NaOH as the base. The 10% NaOH allows more information to be gathered about the e-liquid sample, however, the 2% Na_2CO_3 is necessary to use for successful quantitation of heroin.

While glutethimide was only detected in nine of the 75 e-liquid matrices, it was the fourth most abundant peak in those matrices and provided a spectra that showed the same ions present in the spectra found in the NIST library. Detection of this flavorant provides just one of several reasons why e-liquids needed stricter regulations. At the time of this research, manufacturers were not responsible for following any regulations in the preparation of their e-liquids [20]. One manufacturer, VaporFi® claims to use only FDA approved ingredients but this was not a requirement nor was common practice [21].

Even though there were no regulations surrounding e-liquids, some manufacturers had already removed certain flavorants that have been shown to cause damage to the airway when inhaled [22]. According to the Centers for Disease Control and Prevention (CDC) diacetyl, which is commonly used to give microwave popcorn its butter flavor has been shown to cause damage to the airways in animals during toxicology studies [22]. Diacetyl has been used in e-liquid flavors that have a creamy or buttery flavor; however, many manufacturers no longer use this flavorant and make a point to state so on their web pages.

The flavorants detected in the e-liquid matrices that are approved by the FDA still may not be safe to use as additives in e-liquids. Those flavorants were approved by the FDA for use as additives in food to be consumed orally and not necessarily by inhalation [19]. In e-cigarettes however those compounds are vaporized and inhaled and there is little published data on the effects that inhalation of those compounds has on the human body. Further research needs to be conducted involving these flavorants to determine their toxicity by vaporization and inhalation before they can be deemed safe to use in e-liquids.

In addition to detecting over 40 compounds in the e-liquid matrices tested, it was observed that there was little consistency between manufacturers. The greatest consistency between manufacturers was observed in the menthol flavor category. Menthol was detected in e-liquids from all five manufacturers in the menthol flavor category however, menthol was also detected by at least one manufacturer in the tobacco, blueberry and chocolate flavor categories (Tables 2-6). With the exception of menthol, the inconsistency in the flavorants used by different manufacturers within the same flavor category and even between different flavor categories within the same manufacturer is concerning. While complete consistency was not expected across manufacturers due to the fact that each manufacturer is attempting to produce their own unique product it further goes to show how little users know about these substances which they are inhaling.

There is also no discernable pattern for certain flavorants used by multiple manufacturers. For example, piperonal is used in four of the five different flavor categories. It is used by Viking Vapors© in their tobacco and menthol flavors but not in blueberry, vanilla or chocolate flavors. However, Mt. Baker Vapor© used piperonal in their vanilla and chocolate flavors but not in their tobacco, menthol or blueberry flavors. This lack of consistency within flavor categories and within manufacturers makes it impossible for users to determine what is actually in the product they are consuming.

Qualitative analysis of spiked samples

All of the spiked e-liquid samples were prepared in a matrix that was stated by the manufacturer to contain 24 mg/mL of nicotine. This resulted in a nicotine peak of such a high abundance that it masked nearly every other component of the e-liquid matrix, including the drugs of abuse. The nicotine peak was so abundant that the spiked e-liquid sample chromatogram was nearly identical to the unspiked e-liquid chromatogram. However, when the nicotine peak was ignored and the chromatogram was zoomed along the baseline, the peaks of the spiked drugs of abuse were observed (Figure 3).

These chromatograms demonstrate the importance of knowing the retention times of common drugs of abuse that may be added to e-liquids. For the method used in this analysis the retention time of cocaine is 10.451 minutes, heroin is 13.261 minutes and fentanyl is 13.701 minutes. While the peaks for the drugs of abuse spiked into the e-liquid sample are much lower in abundance when compared to the nicotine peak they were still able to be recognized and integrated by the Chem Station integrator. This demonstrates that if the retention times of common drugs of abuse are not known it is possible that they may be missed by an analyst in routine casework. The fragmentation pattern of the analytes of interest is also important in identifying the analyte in a complex e-liquid matrix. By extracting the ions of interest it is possible to separate the analyte of interest from the matrix as was seen with methamphetamine.

Another possible issue with the qualitative analysis of spiked e-liquid samples is interference from the e-liquid matrix masking drugs of abuse. In this study the retention time of methamphetamine (5.530 minutes) was very similar to both menthol (5.490 minutes) and ethyl maltol (5.587 minutes). This resulted in a lack of resolution of methamphetamine and detection until reaching a concentration of over 100 µg/mL. Figure 4 shows the methamphetamine peak in a spiked

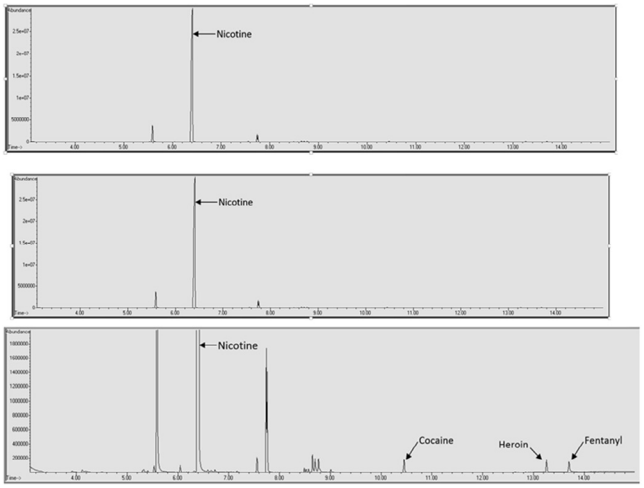

Figure 3: Chromatograms of the VaporFi® Very Vanilla E-liquid.

Top: unspiked VaporFi® Very Vanilla sample.

Middle: spiked VaporFi® Very Vanilla sample.

Bottom: spiked VaporFi® Very Vanilla sample zoomed in to show peaks of drugs of abuse used for quantitation.

VaporFi® Very Vanilla sample. In this chromatogram it can be seen that the methamphetamine peak is just barely resolved from the ethyl maltol peak and if the baseline is zoomed in it is possible to see that the two peaks are not completely resolved.

This interference issue indicates a need to further optimize the extraction technique and/or the GC/MS parameters to allow for better resolution of the peaks. This also indicates that it is possible for drugs of abuse to be present in e-liquid samples without being detected depending upon the concentration of the drug, what the drug and the e-liquid matrix being anlayzed.

Quantitative analysis of spiked samples

The accuracy of the calculated concentrations of the drugs being investigated varied widely based on e-liquid matrix as well as which analyte was being quantified. In general the calculated concentrations for cocaine and fentanyl were less accurate than those for heroin. With regard to e-liquid matrices, e-liquid samples that fell in the flavor categories of menthol and vanilla were less accurate than the e-liquid samples that fell in the flavor categories of tobacco, blueberry and chocolate (Table 7).

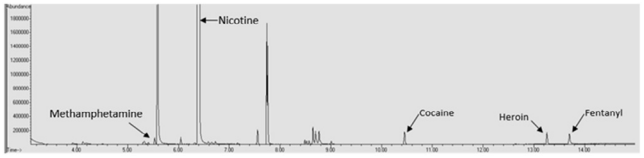

Figure 4: Chromatograms of the VaporFi® Very Vanilla e-liquid.

This chromatogram shows that the methamphetamine peak is unable to be completely resolved from the e-liquid component ethyl maltol.

The components of the e-liquid matrix generally eluted in under nine minutes while cocaine, heroin and fentanyl eluted in over nine minutes. This may have affected the calculated concentrations. For the VaporFi® and Mt. Baker Vapor© e-liquid samples the calculated concentrations of cocaine, heroin and fentanyl increased the further compound is eluted after the matrix components. However, for the Crazy Vapors©, Viking Vapors© and ProVape© samples the calculated concentrations of cocaine, heroin and fentanyl tended to decrease the further the compound eluted after the matrix components (Table 7).

The complexity of the e-liquid matrix also appeared to have an effect on the calculated concentrations. Figure 5 shows a chromatogram of a spiked Mt. Baker Vapor© Cookie Blaster sample. The calculated concentrations for all three analytes in this matrix fell well below the actual spiked concentration of 150 µg/mL as can be seen in table 7. This is also a more complex e-liquid matrix compared to the VaporFi® Very Vanilla sample seen in figures 6 and 7 indicating that the complexity of the e-liquid matrix plays a role in the ability to quantify drugs of abuse added to e-liquids.

To calculate the amount of nicotine present in the e-liquid samples used for quantitative analysis the calibration curve needed to be extrapolated to reach the manufacturer stated 24 mg/mL to keep from saturating the column and to prevent carryover into subsequent samples. Of the 25 e-liquid samples used for quantitative analysis, 18 of the calculated nicotine concentrations fell outside of the ±20% acceptable accuracy range. Three of those 18 samples had calculated nicotine concentrations exceeding 24 mg/mL ±20% (27.43-32.65 mg/mL) and the remaining 15 samples had calculated nicotine concentrations lower than 24 mg/mL ±20% (8.33-18.20 mg/mL). The VaporFi® samples had the greatest number of matrices (four) where the calculated nicotine concentration fell within the acceptable range followed by Mt. Baker Vapor© which had three matrices fall within the acceptable range. None of the Crazy Vapors©, Viking Vapors© or ProVape© matrices fell within the acceptable accuracy range for nicotine concentration.

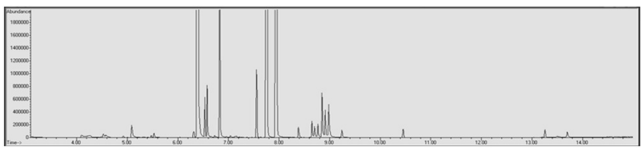

Figure 5: Chromatogram of Mt. Baker Vapor© Cookie Blaster sample zoomed in to show analytes of interest.

The calculated concentrations of cocaine, heroin, and fentanyl fell well below the target value which may be as a result of the overly complex e-liquid matrix when compared to other e-liquid samples, such as the ones seen in Figures 6 and 7.

The nicotine concentration was also calculated for e-liquid matrices that were labeled by the manufacturer as containing 0 mg/mL of nicotine where nicotine was detected. Of the 25 e-liquid matrices labeled as containing 0 mg/mL, 19 of those contained detectable levels of nicotine and 14 of those contained quantifiable levels of nicotine (31.92-415.58 µg/mL). The calculated nicotine concentrations (range: 31.92-415.58 µg/mL, mean: 113.50 µg/mL) were then compared to levels of nicotine found naturally in vegetables belonging to the nightshade family, specifically eggplant [17]. Nicotine levels in air pollution were also considered when conducting this comparison however, the levels of nicotine calculated from air pollution fell so far below the calibration range that it was excluded from further calculations [23].

Of the 14 e-liquid matrices that contained quantifiable levels of nicotine all had statistically higher levels of nicotine than those found naturally in eggplant (0.1 µg/mL). This is an important finding because consumers who use these products are under the impression that they are using a product that contains no nicotine. More research is needed to determine if the concentration of nicotine present would have a significant effect on a consumer. However, there is a potential that the nicotine present in these samples may keep a user who is

attempting to stop using nicotine products or quit smoking addicted to nicotine.

The quantitative results for nicotine in e-liquid matrices demonstrate the need for regulations of these products. In samples that were all labeled to contain 24 mg/mL of nicotine the range of actual concentrations was from 8.46-32.39 mg/mL. Also, detectable levels of nicotine were found in 76% of the matrices tested that were stated to contain 0 mg/mL of nicotine. Federal regulation of these products is necessary to protect consumers and create some consistency in what are now widely variable matrices.

Conclusion

Analysis by GC/MS also showed that a qualitative method for the detection of drugs of abuse in e-liquid samples is possible however certain drugs of abuse may be masked by e-liquid components of similar retention times. It may also be difficult to detect drugs of abuse in e-liquid samples containing high nicotine concentrations if the retention times or the identity of the drug(s) of abuse is not known for mass spectral library searches. A quantitative method is possible by GC/MS, however the accuracy of the quantitation depends on the e-liquid matrix that is used with the drugs of abuse. The accuracy of the quantitation may also depend upon the extraction technique that is used on the e-liquids and may potentially be further optimize.

Analysis by GC/MS showed that there is little to no consistency between components of e-liquids of similar flavors between different manufacturers and that it is possible that some e-liquid matrices may contain controlled or toxic substances. Component analysis also showed that a lack of regulation can lead to detectable and quantifiable levels of nicotine being present in e-liquids that manufacturers claim contain no nicotine, as well as inaccurate levels of nicotine compared to what is being reported by the manufacture on labels.

Future studies should include additional extraction techniques that may result in better separation between the e-liquid components and the analytes of interest and development of a different analytical method allowing more complete detection and quantification of drugs of abuse without interference from flavorants present in the sample. These two areas of focus will allow for better detection and quantification of analytes such as methamphetamine and others that may co-elute with matrix components.

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