Published in final edited form as:

Drug Test Anal. 2017 October ; 9(10): 1637–1640. doi:10.1002/dta.2193.

Ethanol Concentration in 56 Refillable Electronic Cigarettes Liquid Formulations Determined by Headspace Gas Chromatography with Flame Ionization Detector (HS-GC-FID)

Justin L. Poklis, B.S.¹, Carl E Wolf II, Ph.D.^{2,3}, and Michelle R. Peace, Ph.D.³

¹Department of Pharmacology and Toxicology, Virginia Commonwealth University, Box 980613, Richmond, VA 23298-0613, USA

²Department of Pathology, Virginia Commonwealth University, Richmond, VA, USA

³Department of Forensic Science, Virginia Commonwealth Universities

Abstract

Personal battery powered vaporizers or electronic cigarettes were developed as an alternative to traditional cigarettes. The modern electronic cigarettes were patented in 2004 by Hon Lik in China. In May 2016, the US Food and Drug Administration (FDA) imposed regulatory statutes on e-cigarettes and their liquid formulations (e-liquids); prior to that, they were unregulated. E-liquids are typically composed of propylene glycol and/or glycerin, flavoring component(s), and active ingredient(s), such as nicotine. Fifty-six commercially available e-liquids, purchased from various sources, contained a variety of flavors and active ingredients. A headspace gas chromatography with flame ionization detector HS-GC-FID method was used to analyze these e-liquids for volatiles content. Only one of the e-liquids listed ethanol as a component. A headspace gas chromatography with flame ionization detector HS-GC-FID method was used to analyze the e-liquids. The chromatographic separation of volatiles was performed on a Restek BAC-1 column. A linear calibration was generated for ethanol with limits of detection and quantification (LOD/LOQ) of 0.05 mg/mL. Ethanol concentrations in the fifty-six e-liquids ranged from none detected to 206 mg/mL. The ethanol determined in these products may have been used in flavorants or a solvent; the reason for inclusion cannot be fully ascertained. The implications of vaporizing ethanol as an e-liquid component are unknown.

Keywords

Ethanol; electronic cigarettes; e-liquids; headspace-GC-FID

Introduction

Electronic cigarettes have become popular for those seeking an alternative to traditional tobacco products. While versions of electronic cigarettes were patented in 1930 and 1965, modern electronic cigarettes were patented in 2004 by Hon Lik in China as a safer

alternative to smoking after his father died of lung cancer (1,2,3). The modern electronic cigarette is constructed of a metal or plastic body to house a heating element, a cartridge, and a battery (4). The construction of the housing can vary such that some versions are capable of reuse and manipulation allowing the user to change the voltage, resistance of the heating element, and the e-liquid that is vaporized and inhaled (vaped). They work by either drawing negative pressure through the mouthpiece or depressing a button to activate a battery that heats a coil, through which is a wick saturated with liquid formulation known as the e-liquid. These e-liquids are typically composed of propylene glycol and/or glycerin as the primary solvent, as well as, flavoring component(s), and an active ingredient(s) such as a pharmaceutical agent and/or herbal remedy. When the electronic cigarette is activated, the e-liquid is vaporized, followed by rapid condensation into an aerosol that can be inhaled (5) creating an experience similar to that of traditional cigarettes.

Electronic cigarettes and their e-liquids were unregulated in the United States of America (USA) prior to May 2016 when the US Food and Drug Administration (FDA) announced the extension of their authority to regulate all tobacco products (6). A significant reason for the new regulations was to address the quality assurance of electronic cigarette products, from the devices themselves to the e-liquids.

Ethanol has been reported as an unlabeled ingredient in nicotine containing e-liquids available in the United States, although there are some regulations for reporting ingredients, including ethanol, in e-liquids in other countries (7). It has been suggested that ethanol is used as a solvent for flavorants and/or other additives in e-liquids (8). Herrington et al detected ethanol in four different popular brands of self-contained non-refillable electronic cigarettes (9) and Varlet et al. analyzed 42 commercial nicotine containing e-liquids and found 30 of them to contain ethanol (10).

Presented is the analysis of 56 e-liquids for ethanol content. Thirty-five of the 56 e-liquids, representing 20 different brand names, listed nicotine as the only active ingredient (Table 1). Four of these products were produced from dohka, an Arabian tobacco blend, consisting of dried and finely shredded tobacco mixed with leaves, bark and herbs. The remaining 21 products contained drugs other than nicotine (DOTN) (Table 2). Of these 21, three were labeled to contain nicotine as well as another active ingredient(s). The remaining 18 were labeled or identified to contain a wide range of compounds ranging from herbal or naturally occurring to a cannabinomimetic designer drug. Only one of the 56 e-liquids was labeled to contain ethyl alcohol. The ethanol content of these e-liquids was determined by headspace gas chromatography with flame ionization detector (HS-GC-FID) (11).

Methods

The 56 e-liquids were obtained over a four year period from a variety of sources via the internet and local “vape shops”. The commercial name and brand names of the e-liquids are listed in Tables 1 and 2. All samples were purchased in the United State. The samples purchased via the internet arrived from a United States post office address via the mail with the exception of the Blue Lotus Flower e-liquid which arrived from Thailand and Mountain Fresh samples which arrived from England. Only one example of each e-liquid was

purchased. All samples were stored at room temperature in the dark prior to analysis. HPLC grade methanol and de-ionized (DI) water, optima grade acetone, ethanol, methanol, n-propanol and isopropanol were purchased from Fisher Scientific (Hanover Park, IL, USA). The Ethanol 80 and Volatile Controls were purchased from UTAK[®] Laboratories, Inc. (Valencia, CA, USA). Nitrogen and helium gases were acquired from Praxair and Airgas (Richmond, VA, USA).

The ethanol content of the e-liquids was determined using a modified method routinely employed for the analysis of clinical and forensic samples for ethanol, acetone, isopropanol and methanol (9). Initially, 100 μ L of the e-liquid of each sample was diluted with 900 μ L deionized water. Then aliquots of 100 μ L of the e-liquid dilutions, calibrator (0.100, 0.157, 0.393, 0.984, 1.574, 3.148 and 8.000 mg/mL ethanol), and ethanol free controls consisting of DI water, UTAK controls, (Ethanol 80 and Volatile controls), were added to 900 μ L of internal standard consisting of 234 mg/L n-propanol in de-ionized water. Samples outside of the linear range of the assay were further diluted 1:10 or 1:100 with de-ionized water prior to aliquoting for re-analysis. Analysis was performed using a Tekmar HT3 Headspace sampler attached to a Shimadzu 2014 Gas Chromatograph (GC) with a flame ionization detector (FID). The chromatographic separation was performed on a RTX-BAC1, 30 m x 0.32 mm id x 1.80 μ M column (Restek Corp, Bellefonte, PA, USA). The headspace oven and transfer line temperatures were set to 160 °C with a standby flow rate of 200 mL/min. The sample platen (heating chamber) temperature was set to 80 °C with the mixer on. The sample equilibrium time was 3.5 min with a sample injection time of 0.5 min. The GC had an oven temperature of 50 °C (isothermal) and an injection temperature of 200 °C run in split mode with a 1:20 ratio. The column flow rate was 6.85 mL/min with purge flow of 0.5 mL/min. The detector temperature was 225 °C with both hydrogen and air set at 50 kPa. Ethanol concentrations were determined by linear regression. The calibration curve was constructed using peak ratio of the area counts of ethanol and the internal standard versus the calibrator concentrations. The limit of detection (LOD) and the limit of quantitation (LOQ) were administratively set at 0.05 mg/mL. The linear range of the assay was 0.05 – 8.0 mg/mL. LOQ/LOD samples prepared at 0.05 mg/mL (n=3) to verify that the LOQ/LOD was within $\pm$ 20% of the target value and had a response at least ten times greater than the signal to noise ratio of volatile free control. The coefficient of determination (r^2) was < 0.998 for all calibration curves. The bias for all the ethanol controls (n=10) ranged from 9.8 to 1.3% with Coefficient of Variations (CVs) < 9%. Sample carryover of the each volatile was evaluated by immediately injection of the highest calibrator followed by an ethanol free control. Lack of carryover was confirmed as no volatiles were detected in the negative control.

Results

Ethanol was determined in thirty-three of the thirty-five nicotine (only) containing e-liquid. The concentrations ranged from 0.07 to 206 mg/mL ethanol. Two e-liquids samples from a single manufacturer, Top Vapor, contained ethanol concentrations >100 mg/mL,. Five samples from five different manufactures contain between 10 and 100 mg/mL ethanol. Seventeen samples contained between 0.05 and 1.0 mg/mL. The three dohka containing products from Nirvana Super E-Liquid contained ethanol at 1.80, 3.17 and 13.0 mg/mL.

Cheery by VapeWell was the only e-liquid labeled to contain ethyl alcohol, and it was determined to contain 56.0 mg/mL ethanol.

Three of the 21 products containing DOTN were labeled as containing nicotine. Twenty of these products had ethanol concentrations ranging from 0.10 to 194 mg/mL. Three of the four samples unlabeled as to the active ingredients or drug contained >100 mg/mL ethanol. These three samples contained the designer drug methyl (2S)-2-[[1-[(4-fluorophenyl)methyl]indazole-3-carbonyl]amino]-3,3-dimethylbutanoate (MDMB-Fubinaca), a cannabimimetic. The Blue Lotus Flower Extract was the only DOTN containing sample that was ethanol free. This sample contained psychoactive alkaloids, apomorphine and nuciferine, which are found in the Blue Lotus flower.

Discussion

E-liquids typically used in modern electronic cigarettes are often labeled to contain propylene glycol and/or glycerin, flavoring component(s), and an active ingredient(s) such as a pharmaceutical agent and/or herbal remedy. In the presented study, Vape Well's Cherry was the only e-liquid that included ethanol as a labeled component. Ethanol was determined in 53 of the 56 e-liquid. Varlet et al reported ethanol concentrations in 42 brand name e-liquids from 14 manufactures purchased in 2013(8). All but five of these samples were reported to contain nicotine as the active drug, and 30 of the 42 e-liquid samples were reported as positive for ethanol with concentrations > 3.70 mg/mL. In this present study, the e-liquids were purchased between 2012 and 2016 and contained a wider range of labeled active ingredient(s). Ethanol concentrations found in 12 of the nicotine labeled samples and 5 of the DOTN were determined to be greater than 3.7 mg/mL as reported by Varlet et al (11) and some significantly greater than 3.70 mg/mL with the upper end of the range being 206 mg/mL.

Ethanol is a common component found in e-liquid regardless of the labeled active ingredient(s). The ethanol detected in these products may be used in flavorant or a solvent, however, the reason for inclusion cannot be fully ascertained. The implications of vaporizing ethanol as an e-liquid component are largely uninvestigated.

Acknowledgments

This project was supported by National Institute on Health (P30DA033934) and by the National Institute of Justice Office of Justice Programs, U.S. Department of Justice (2014-R2-CX-K010 and 2016-DN-BX-0150). The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

References

1. Lik, H., inventor. Flameless Atomizing Electronic Cigarette. WO. 2004080216. 2004. Available from: <http://patentscope.wipo.int/search/en/WO2004080216>
2. Robinson, J., inventor. Electronic Vaporizer. US. 1775947. 1930.
3. Gilbert, HA, inventor. Smokeless Non-Tobacco Cigarette. US3200819. 1965.
4. Breland A, Soule E, Lopez A, Ramôa C, El-Hellani A, Eissenberg T. Electronic cigarettes: what are they, and what do they do? *Ann N Y Acad Sci*. 2016;1–26.

5. Etter JF, Zäther E, Svensson S. Analysis of refill liquids for electronic cigarettes. *Addiction*. 2013; 108:1671–1679. [PubMed: 23701634]
6. U.S. Food and Drug Administration [FDA]. [accessed May 12, 2016] FDA Takes Significant Steps to Protect Americans From Dangers of Tobacco Through New Regulation. 2016. <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm499234.htm>
7. The European Parliament and the Council of the European Union. European Union Tobacco Products Directive. 2014. 2014/40/EU
8. Valentine GW, Jatlow PI, Coffman M, Nadim H, Gueorguieva R, Sofuoglu M. The effects of alcohol-containing e-cigarettes on young adult smokers. *Drug Alcohol Depend*. 2016; 159:272–276. [PubMed: 26778759]
9. Herrington JS, Myers C. Electronic cigarette solutions and resultant aerosol profiles. *J Chromatogr A*. 2015; 30(1418):192–199.
10. Varlet V, Farsalinos K, Augsburger M, Thomas A, Etter JF. Toxicity assessment of refill liquids for electronic cigarettes. *Int J Environ Res Public Health*. 2015; 12:4796–4815. [PubMed: 25941845]
11. O'Neal CL, Wolf CE, Levine B, Kunsman G, Poklis A. Gas chromatographic procedures for determination of ethanol in postmortem blood using t-butanol and methyl ethyl ketone as internal standards. *Forensic Sci Int*. 1996; 83:31–38. [PubMed: 8939011]

Table 1

E-liquids analyzed for ethanol content that contained nicotine as the only active drug

Manufacturer	Brand Name	Label	Ethanol (mg/mL)
AVAIL Vapor	Blueberry Cupcake	10 mL, 1.2% Nicotine, PG, VG, Water, Flavoring.	0.62
AVAIL Vapor	Captain's Cut	10 mL, 2.4% Nicotine	7.00
AVAIL Vapor	Continental Breakfast	10 mL, 2.4% Nicotine, PG, VG, Water, Flavoring.	37.2
AVAIL Vapor	Midnight Splash	10 mL, 2.4% Nicotine, PG, VG, Water, Flavoring.	7.02
AVAIL Vapor	Persian Winter	10 mL, 2.4% Nicotine, PG, VG, Water, Flavoring.	0.25
AVAIL Vapor	Queen's Cup	10 mL bottle, 1.2% Nicotine, PG, VG, Water, Flavoring.	1.76
AVAIL Vapor	Sapphire Morning	15 mL, 1.8% Nicotine, PG, VG, Water, Flavoring.	0.41
AVAIL Vapor	Seduction	15 mL, 1.2% Nicotine, PG, VG, Water, Flavoring.	0.15
FennetHIGH	Janty	No Ingredients on Label	6.01
Five Pawns Signature Vapor Liquid	Gambit	Name: Gambit, Bottle number: Sample, Lot number 27, Nicotine Strength: 6mg, Blend % 50 PG/ 50 VG, 4mL	0.23
Gremlin Juice	Berry Custard	12 mg, 50 PG/ 50 VG	0.10
Gremlin Juice	Chai Latte	No Ingredients on Label	0.08
Gremlin Juice	Eggnog	No Ingredients on Label	0.56
Grand Traverse Vapor	Blueberry Lemonade	10 mg/12mL, 50/50 PG/VG	48.0
Juice Mafia	Peach Tobacco	12 mg, 50/50 PG/VG Nicotine, Natural and Artificial Flavors.	7.33
Lightening Vapes	Lightening Loops Max VG	6 mg, MAX VG, Ingredients: May Contain Nicotine, Diacetyl Acetoin, Acetyl Propionyl, Distilled Water, PG, VG, Artificial and Natural Flavors.	ND
Mount Baker Vapor	Gorilla Pirate	12 mg Nicotine, PG 20% VG 80%, Extra Shots: 0, Ingredients: PG, VG, Natural and/or Artificial Flavoring.	1.4
Mount Baker Vapor	Ecto Cooler	12 mg Nicotine, PG 20% VG 80%, Extra Shots: 0, Ingredients: PG, VG, Natural and/or Artificial Flavoring.	7.87
Nirvana Super E-Liquid*	Skull Control	16 mg Nicotine, 30 mL, Ingredients: PG, Kosher VG	3.17
Nirvana Super E-Liquid*	Headrush	16 mg Nicotine, 30 mL, Ingredients: PG, Kosher VG	13.0
Nirvana Super E-Liquid*	Citrus O.D.	16 mg Nicotine, 30 mL, Ingredients: PG, Kosher VG	1.8
PALM	Green Tea	8 mg, 10 mL, Natural Product, Composition: PG, VG, Natural Essence	0.49
PALM	Juicy Peach	12 mg, 10 mL, Natural Product, Composition: PG, VG, Natural Essence	0.67

Manufacturer	Brand Name	Label	Ethanol (mg/mL)
PALM	Mint Bubble Gum	12 mg, 10 mL, Natural Product, Composition: PG, VG, Natural Essence	0.50
PALM	Strawberry	12 mg, 10 mL, Natural Product, Composition: PG, VG, Natural Essence	0.61
S&S MODS	Grumpy's Hooch	mg: 12, mL: 30, PG: 50, VG: 50, BOD: 1/14, Expires: 1/15	61.0
Seduce Juice	Jango	Ingredients: Vegetable Glycerin, Propylene Glycol, Nicotine, Natural and Artificial Flavors.	0.30
STLVapor	Spearmint	22 mg. cont. Propylene Glycol/Vegetable Glycerin & Flavoring	ND
Supreme Nicotine	Rally Squirrel	16 mg/mL	0.07
The Vapor Company	Shark Juice Imperial Apple Brandy	10 mg Nicotine	0.63
The Vapor Company	Shark Juice Watermelon Smash	10 mg Nicotine	0.09
Top Vapor	Apple Cider	30 mL 6mg, Ingredients: VG, PG Organic, and/or Natural Flavors	139
Top Vapor	Honeydew	30 mL 6 mg, Ingredients: VG, PG Organic, and/or Natural Flavors	206
Vape Dudes Gourmet eJuice	Vanilla	16 mg, 30 PG/70 VG, 10 mL	0.24
VapeWell	Cheery	High 18mg, 30 mL, Ingredients: Nicotine, Propylene Glycol, Vegetable glycerin, Ethyl Alcohol , Artificial & Natural Flavoring.	56.0

Table 2

E-liquid analyzed for ethanol content that contained drugs other than nicotine

Manufacturer	Brand Name	Label	DOTN	Ethanol (mg/mL)
Best Ecig	Vitamin (No Nicotine)	PG, VG, Flavors: Vitamin	None Identified	0.10
Best Ecig	Vitamin (With Nicotine)	PG, VG, Flavors: Vitamin, High Nicotine	None Identified	0.19
Buzzy Bee Sales	Blueberry B Juice	No Ingredients on Labeled	MDMB-FUBINACA	139
Buzzy Bee Sales	Regular B Juice	No Ingredients on Labeled	MDMB-FUBINACA	194
Buzzy Bee Sales	Vampire B Juice	No Ingredients on Labeled	MDMB-FUBINACA	160
Energy Shisha. PHARMA	Energy Shisha Caffeine	PG, VG, Caffeine 0.6mL, Flavorings, Distilled Water	Caffeine	142
Good As Gold	Purple Haze	PG, VG, Natural and Artificial Flavoring	Mitragynine	1.20
Good As Gold	Purple Haze	PG, VG, 12 mg Nicotine, Natural and/or Artificial Flavoring	Mitragynine	1.41
High Voltage	Lights Out	PG, VG, Melatonin, Natural and Artificial Flavors	Melatonin	4.12
High Voltage	Shock Treatment	PG, VG, Natural and Artificial Flavors, Caffeine and Vitamin Blend	Caffeine	0.13
High Voltage	Tesla	PG, VG, Natural and Artificial Flavors, Caffeine and Vitamin Blend	Caffeine	3.77
King Kratom	King Kratom	VG, Natural Kratom Extract, Natural and/or Artificial Flavorings	Mitragynine	0.23
King Kratom	King Kratom	VG, Natural Kratom Extract, 12 mg Nicotine, Natural and/or Artificial Flavoring	Mitragynine	0.20
Lotus-extracts	Blue Lotus Flower	PG, VG, 4% Blue Lotus (Nymphaea caerulea)	Nuciferine, Apomorphine	ND
Mountain Fresh	Catnip	VG, Nepeta cataria	None Identified	1.72
Mountain Fresh	Catuaba Bark	VG, Erythroxylum catuaba	None Identified	1.59
Mountain Fresh	Damiana	VG, Tumeria diffusa	None Identified	3.15
Mountain Fresh	Maca Root	VG, Lepidium meyens	None Identified	0.24
Mountain Fresh	Passion Flower	VG, Passiflora incarnata, 100%	None Identified	1.83
Mountain Fresh	Poppy	VG, Anapola Papaver	None Identified	1.49
Mountain Fresh	Wormwood	VG, Artemisia absinthium	None Identified	1.10