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Brief report

Characterization of Nicotine Salts in 23 Electronic Cigarette Refill Liquids

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Abstract

Introduction: Many electronic cigarette manufacturers have begun offering liquids containing "nicotine salts," which are formed when an acid is mixed in a solution with free-base nicotine. Type of salt could play a significant role in the abuse liability of electronic cigarette liquids. As a first step to understanding nicotine salts, this study sought to identify the types of acids present in 23 commercially available electronic cigarette liquids.

Aims and Methods: Twenty-three electronic cigarette liquids advertised as containing nicotine salts were purchased for analysis. These liquids were tested for the presence of 11 different organic acids that were deemed likely to be used in a nicotine salt formulation. Liquids were analyzed using a combination of liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry methods, then compared to authentic acid standards for identification.

Results: Six of the 11 possible acids were identified in the liquids, from most to least common: lactic, benzoic, levulinic, salicyclic, malic, and tartaric acid. Acid(s) could not be identified in one of the liquids. Though most liquids contained only one type, three of the liquids contained multiple acids.

Conclusions: These data demonstrate that several types of salts/acids are currently being used in electronic cigarette liquids. The type and concentration of salt(s) used in these liquids may differentially alter sensations in the throat and upper airway, and overall pharmacology of the aerosols by altering liquid pH and from flavor and sensory characteristics of the acids themselves.

Implications: This study demonstrates that at least six different types of acids are being used to create the nicotine salts in electronic cigarette liquids, with the acids lactic, benzoic, and levulinic being the most frequently identified. Identification of these acids can serve as the foundation for future research to determine if type of nicotine salt alters pharmacological and toxicological effects of electronic cigarettes.

Introduction

Nicotine is an alkaloid that can be isolated as a free-base, but when combined with an acid becomes protonated and forms a salt[1] (Figure 1). Recently, many electronic cigarette companies have begun marketing liquids that contain salt forms of nicotine. For example, Juul describes incorporating salt forms of nicotine into electronic cigarette liquids in their patent.[2] In addition to Juul, other major manufacturers have begun to offer nicotine salt liquids (eg, RJ Reynolds' Vuse Alto or Imperial's MyBlu), as well as numerous smaller manufacturers. According to Juul's patent,[2] "certain nicotine salt formulations provide satisfaction in an individual superior to that of free-base nicotine" and "certain nicotine salt formulations provide greater satisfaction than other nicotine salt formulations."

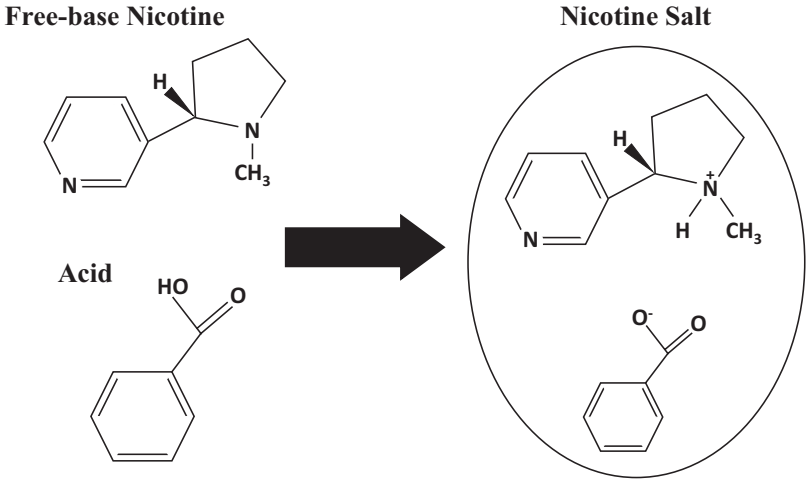

Figure 1. Formation of nicotine salt: Example of a benzoic acid ("Acid") molecule existing in a solution to form the salt *nicotine benzoate*. On the left side of the figure free-base nicotine is combined in a solution with benzoic acid ("Acid"). Once together in the same solution a hydrogen ion (proton) dissociates from the benzoic acid and bonds to the nitrogen group on the free-base nicotine molecule to form the monoprotonated form of nicotine. The mixture of the monoprotonated nicotine and deprotonated benzoic acid in the same solution is what comprises the "nicotine salt," which is nicotine benzoate in this example.

Tobacco industry documents also discuss how different types of nicotine salts (such as nicotine levulinate) have unique sensory effects and flavor profiles and influence nicotine exposure from cigarette smoke.[3] Thus, nicotine salts and the nature of the salt could potentially influence the abuse liability of electronic cigarette liquids.

Due to the rapidly increasing popularity of nicotine salts, and potential influence on the subjective and biological effects of electronic cigarettes, research on nicotine salts used in electronic cigarette liquids is warranted. A necessary first step is to identify the types of nicotine salts currently being used in electronic cigarette liquids. In this brief report, we describe an analytical method used for identification of acids present in 23 electronic cigarette liquids advertised as containing nicotine salts, as well as concentrations of nicotine, pH, and ratios of propylene glycol (PG) to vegetable glycerin (VG).

Methods

General Procedures

Twenty-three electronic cigarette liquids, advertised as containing nicotine salts and in refill containers typically used for second- or third-generation electronic cigarettes (N = 21) or disposable pods (N = 2), were purchased from online retailers. Sixteen of these liquids were selected by visiting a large online retailer of electronic cigarette liquids (https://www.electrictobacconist.com/), sorting by "top sellers" in the nicotine salts category, and selecting the top 16 liquids from unique brands. To examine major manufacturers' use of nicotine salts, two examples of disposable pod type liquid containers advertised as containing nicotine salts from RJ Reynolds' Vuse and Juul were purchased. Lastly, five unflavored formulations advertised online as containing nicotine salts were purchased for analysis.

Assessments

Salt/Acid Identification

Eleven organic acids were selected as analytical standards for identification. These were glycolic, pyruvic, lactic, levulinic, fumaric, succinic, benzoic, salicylic, malic, tartaric, and citric acids. These acids were selected because one is identified on the label of Juul's packaging (benzoic acid); others have been evaluated for inclusion in tobacco products such as cigarettes and electronic cigarette liquids (citric, malic, succinic, levulinic, and salicylic acids)[2,3]; and others are known to be present in foods (tartaric and glycolic acids), found in the body as metabolic intermediates (pyruvic and lactic acids), or are generally recognized as safe for human consumption (fumaric acid).

E-liquids were analyzed using liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS). Briefly, for LC-MS, samples of e-liquids were diluted 1:1000 in 95:5 water/methanol and injected onto a 150 mm × 3mm Imtakt Scherzo SM-C18 column. The analytes were eluted using a gradient of 0.3% formic acid in water (A) and 2% formic acid in methanol (B) from 60:40 A:B to 10:90 A:B over 7 minutes, at a flow rate of 0.4 mL/min. The mass spectrometer system, a Thermo Vantage (Thermo-Fisher Scientific), was operated in the negative ion heated electrospray ionization mode. Data were acquired in the full scan mode. For data analysis, the molecular ions (M-1) were extracted to generate ion chromatograms, allowing comparison of LC retention times and molecular masses with authentic standards for identification. For GC-MS, the acids were converted to their methyl esters. Ten microliters of e-liquids were combined with 200 µL aliquots of HCl/methanol in 13 × 100 mm glass culture tubes and heated at 75°C in a heating block for 30 minutes. The tubes were cooled, 2 mL of methyl tert-butyl ether was added followed by 0.5 mL of saturated aqueous sodium chloride. The tubes were vortexed for 5 minutes, centrifuged, and the methyl tert-butyl ether extracts (upper layers) were transferred to new tubes containing 0.5 mL each of saturated aqueous sodium bicarbonate and water. The tubes were vortexed for 5 minutes, centrifuged, and the methyl tert-butyl ether extracts (upper layers) were transferred to autosampler vials for GC-MS analysis using an Agilent 6890 GC with a 5973 mass selective detector. The injections (2 µL) were in the splitless mode, the column was an Agilent HP5 MS, 0.25 micron film thickness, 30 m length by 0.25 mm ID, and temperature programed from 40°C to 285°C. Ionization was isobutane CI, positive ion mode, and full scan from 60 to 475 amu. Identification was made by comparison of retention times and mass spectra to those of standards. In most cases, GC-MS confirmed the results of LC-MS. One exception was levulinic acid in which LC-MS did not work for this analyte. Levulinic acid was identified in three

Table 1. Electronic Cigarette Liquid Ingredients and Chemical Properties

Brand	Flavor	Type(s) of salt	Nicotine concentration (mg/mL)			PG:VG Concentration		pH
			Labeled	Analyzed	Difference (%)	Labeled	Analyzed	
Top sellers								
Aqua	Pure/Fruit	Lactic	50	41.2	−17.6	50:50	23.7:76.3	4.03
SaltNic Labs	Dry Tobacco	Benzoic	50	41.1	−17.8	45:55	41.3:58.7	6.63
Atomic Salts	Evermint Menthol	Benzoic	50	42.8	−14.4	50:50	46.8:53.2	6.22
Mad Hatter Juice	Spearmint Gum	Lactic	50	39.4	−21.2	50:50	43.7:56.3	4.02
Mr. Salt-E	Vanilla Custard	Benzoic, levulinic	45	32.9	−26.9	Unknown	33.3:66.7	4.87
Savage E-liquid	Bond	Lactic	50	44.1	−11.8	Unknown	72.8:27.2	4.01
The Milkman Salts	The Milkman	Lactic	40	28.9	−27.8	40:60	36.3:63.7	5.21
Glas Vapor	Fizzy Lemonade	Levulinic	50	30.5	−39.0	50:50	46.4:53.6	6.54
Naked 100	Brain Freeze	Lactic	50	35.8	−28.4	Unknown	44.9:55.1	3.98
California Grown	Napa Nectar	Lactic	50	36.7	−26.6	Unknown	40.1:59.9	3.98
Bad Drip Bad Salt	Cereal Trip	Lactic	45	30.3	−32.7	Unknown	49.2:50.8	4.01
Halo	Subzero	Benzoic	50	41.2	−17.6	50:50	48.9:51.1	6.25
Beard Vape Co	No. 32	Lactic	50	36.3	−27.4	Unknown	49.1:50.9	3.99
VGOD	Mango Bomb	Benzoic	50	41.6	−16.8	Unknown	40.2:59.8	5.66
Apollo	Tobacco Menthol	Lactic	50	41.7	−16.6	50:50	44.2:55.8	4.00
Dinner Lady	Lemon Tart	Salicylic	50	20.0	−60.0	Unknown	38.4:61.6	3.60
Major manufacturer								
Vuse	Alto Rich Tobacco	Lactic	50	51.6	3.2	Unknown	44.6:55.4	6.83
Juul	Mint	Benzoic	50	49.8	−0.4	Unknown	30.2:69.8	5.80
Unflavored								
Nude Nicotine	Smooth	Levulinic	100	88.6	−11.4	100:0	100:0	6.39
Nude Nicotine	Signature	Levulinic, benzoic, salicylic, malic, tartaric	100	74.0	−26.0	100:0	100:0	4.69
Nude Nicotine	Hit	Benzoic, malic	100	82.3	−17.7	100:0	100:0	4.97
MFS Nic Salt	Unflavored	Unknown†	48	38.8	−19.2	Unknown	49.1:50.9	3.45
Nicotine River	PureNic Smooth	Lactic	48	68.8	43.3	0:100	0:100	4.11

PG = propylene glycol; VG = vegetable glycerin.
†Did not contain measurable amounts of any of the 11 selected organic acids.

e-liquids by GC-MS; and because both GC-MS retention times and mass spectra were in excellent agreement with those of the standard, these identifications were considered solid. Tartaric acid was identified in one e-liquid by LC-MS; and because of the high polarity and low volatility of this analyte is not amenable to GC-MS analysis. Lack of interfering substances and lack of carryover between injections were verified during method development by analysis of blanks. The finding that one e-liquid did not contain any of the target analytes (no peaks at expected retention times) confirmed lack of interfering substances derived from sample preparation.

Nicotine Concentration

Nicotine concentrations were determined by GC with a nitrogen phosphorous detector[4] with modifications for capillary GC.[5] The limit of quantitation was 1 ng/mL.

Propylene Glycol and Vegetable Glycerin

PG and VG were quantified using a previously published method.[6]

pH

Liquids were diluted 1:10 with water and measured with a Starter 3100 pH Bench (OHAUS Corporation).

Statistical Analysis

Differences in labeled versus measured nicotine concentration were calculated by: [(labeled nicotine – analyzed nicotine)/labeled nicotine]. PG and VG ratios were calculated as relative concentrations of the two ingredients by weight.

Results

Analytical results are presented in Table 1. The most frequently used organic acids identified were lactic (47.8%, $N = 11$), benzoic (34.8%, $N = 8$), and levulinic (17.4%, $N = 4$) acids. The other acids were salicylic, malic, and tartaric acids. One liquid contained five different salts. One of the liquids did not contain measurable amounts of any of the 11 selected organic acids. Measured nicotine concentrations averaged 45.2 (range 20.0–88.6, SD = 17.3) mg/mL, which was 18.7% (SD = 18.5%) lower than labeled values. The measured PG:VG ratios averaged 49:51 (range 0:100 to 100:0) and, of the 12 liquids that were labeled with a PG:VG ratio, measured PG values were 4.3% (SD = 7.4%) lower than labeled values on average—or vice versa for VG. The average pH of all liquids was 4.9 (range 3.5–6.8, SD = 1.1).

Discussion

Results of this study provide insight into the types of acids present in e-liquids marketed as containing nicotine salts, as well as an analytical methodology for their identification. E-liquid manufacturers are currently using at least six different acids to create nicotine salts. The utilization of nicotine salts in e-liquids is important in three aspects. First, the resultant acidification of the liquids

means the aerosols derived from them are not alkaline, potentially resulting in less throat and upper airway irritation.[1] Second, it has been demonstrated that nicotine transfers comparably from product to aerosols regardless of the salt type in tobacco[7] or electronic cigarette liquids.[8] Some salts (eg, nicotine citrate), however, generate aerosols with different ratios of free-base to monoprotonated nicotine salts—suggesting nicotine salt type could influence nicotine delivery.[8] This may be one mechanism to explain research cited in Juul's patent showing that different acids/salts alter the pharmacokinetic and subjective effects of electronic cigarette liquids.[2] Third, the acid component or thermal breakdown products of these formulations may result in toxicity and studies of thermal stability and identification of decomposition products are needed.

In liquids not advertised as containing nicotine salts the majority of nicotine (eg, >60%) is in the free-base form.[9] Compared to protonated nicotine, free-base nicotine is more volatile and more likely to impact nicotinic receptors in the mouth and upper airway, producing a stronger "throat hit."[1] The average pH of the liquids in this study (m = 4.9) is lower than most e-liquids not advertised as containing nicotine salts.[9–11] Though electronic cigarette liquids not advertised as containing nicotine salts can have pH levels comparable to the liquids measured in this study (eg, pH < 5), they typically have lower nicotine concentrations (ie, 24 mg/mL or less). Increased nicotine concentrations ordinarily would raise liquid pH (due to the basic pH of nicotine itself),[9] but the addition of acid counteracts this effect, allowing pH to remain low despite the higher nicotine concentrations used in the liquids analyzed here. The pH ranges of nicotine salt liquids resemble those of cigarette smoke, with typical smoke pH below 6.0.[12] As such, from a pharmacological perspective, the aerosol emitted by electronic cigarettes filled with nicotine salt type liquids more closely resembles cigarette smoke, while also containing a higher nicotine concentration than liquids not containing nicotine salts.

Our data also reproduce findings[11,13] that labeled nicotine concentrations on marketed products often differ from measured concentrations, and we find that the same is true for PG:VG ratios. Lower measured nicotine concentrations relative to labeling may be because PG and VG are hygroscopic and absorb water from the air, increasing the overall volume of the liquids while nicotine content remains constant, thereby reducing nicotine concentration. It is interesting to note that in all cases PG concentrations were lower than labeled. This may be because the PG used by manufacturers is not as pure as the analytical standards used in this study. These findings underscore the need to address issues with labeling accuracy, possibly by implementing regulatory measures.

Limitations

First, our analytical method measured 11 possible organic acids that we considered most likely to be used in e-liquids, and other acids could be present in these liquids. Second, when multiple formulations were offered for a liquid, the highest nicotine concentrations and the highest PG content available were purchased in order to facilitate ease of analysis. As such, the nicotine concentrations and PG:VG ratios should not be interpreted as representing typical nicotine salt liquids. Third, though the addition of water to electronic cigarette liquids is a common method for pH analysis, the addition of water may interact with the measurement sample to alter pH.[14,15] Thus, the pH values here are most translatable to other measurements where a similar dilution with water is used. Lastly, while top-selling liquids were selected in an attempt to represent those most generalizable to what is being used by the public, there are a great number of liquids being advertised as containing nicotine salts—making it difficult to ensure those in this study are a representative sample of what is being purchased by electronic cigarette users.

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Declaration of Interests

NLB is a consultant to Pfizer and Achieve Life Sciences, companies that market or are developing smoking cessation medications. He has also been a paid expert witness in litigation against tobacco companies. Other authors have no potential conflicts to disclose.

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