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Analysis of Nicotine in Electronic Cigarettes Using Gas Chromatography-mass Spectrometry

Gloria Wink

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ANALYSIS OF NICOTINE IN ELECTRONIC CIGARETTES USING GAS
CHROMATOGRAPHY-MASS SPECTROMETRY

by
Gloria Wink

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science in Environmental Science

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July 23, 2015

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Abstract

Electronic cigarettes (ECs) have emerged in the marketplace in recent years and are gaining popularity, but with relatively little understanding of their health impact to consumers. To remedy the gap in knowledge about ECs and their emissions, we have developed a technique to measure their nicotine content, emission efficiency, and nicotine delivery using gas chromatography-mass spectrometry (GC-MS). Figures of merit for the GC-MS analytical method were determined. In addition to the analytical method development, we also studied the nicotine characteristics of disposable and re-fillable ECs (both in e-liquids and emissions) using our method. For the disposable ECs, prior to puffing, products were dissected; the nicotine containing solution was extracted with methanol and analyzed gravimetrically and by GC-MS to determine the contents of un-puffed devices. The aerosolized emissions of ECs were collected on filter pads using our in-house puffing machine, the contents of the filters were extracted, and subsequently analyzed gravimetrically and by GC-MS. Five popular brands of disposable ECs were studied and showed varying emission efficiencies under our puffing regime that corresponded to differing levels of nicotine delivery. Our results also show that there are discrepancies between the nicotine concentrations reported on disposable EC packaging by manufacturers and our analytical results. Three refillable EC devices were also tested for nicotine delivery using a variety of machine puffing parameters. Our results revealed the puffing parameters (puff duration, puff volume, and puff flow rate) play roles in the nicotine delivery of re-fillable ECs. In whole, this work contributes toward developing reliable analytical methods that will hopefully work toward a better understanding of the health impact of relatively new ECs on consumers and also to those in the indoor air environment that may passively consume EC emissions.
Introduction

The availability and use of ECs has increased significantly in the last few years. From 2010-2013, for example, the number of EC users in the US increased from 1.0% to 2.6% (King et al., 2014). Simultaneously, corporate marketing efforts have increased annually and a wide array of EC products is currently available to the consumer. ECs are often marketed as a safe alternative to tobacco cigarettes because they do not contain tobacco and nicotine is not delivered through combustion. Despite the increased popularity of ECs, there remains a need for the independent verification of manufacturer claims as to the actual nicotine content in disposable and refillable ECs, nicotine content of e-liquids, and the amount of nicotine the consumer receives from vaping.

Tobacco use in the US remains prevalent. The Centers for Disease Control and Prevention reports that approximately 480,000 Americans die due to tobacco cigarette smoking annually (Centers for Disease Control and Prevention, 2015). There are several main pollutant groups in tobacco cigarette smoke: nitrates, nicotine, tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), carbonyls, and glycols (Ding et al. 2006; McAuley et al. 2012; Tarrant, Mills, and Williard 2009; Zha, Qian, and Moldoveanu 2002), each of which presents its own health and safety concerns for the consumer. For example, incomplete combustion of tobacco during the smoking process forms PAHs, which are compounds known to cause cancer in living tissue (Ding et al. 2006). In one study, nitrate and PAH content was measured in tobacco cigarettes from different countries, using a variety of brands and it was shown that each brand can contain different concentrations of PAHs and nitrate (Ding et al. 2006). ECs reportedly contain lower concentrations of toxic
chemicals than tobacco cigarettes due to the absence of combustion of tobacco and the
supposed absence of toxic chemicals in the e-liquid. In ECs, heating of the nicotine-
containing e-liquid to make a nebulized aerosol is performed electronically and no plant
material is combusted (McAuley et al., 2012). The most common compounds found in
EC emissions are propylene glycol, glycerin, ethanol, and additional flavor additives that
may come in a variety of concentrations and be present in varying concentrations in
different types of ECs (Goniewicz et al., 2013).

ECs consist of five main components: the cartridge, the heating element, a
microprocessor, a sensor, and a LED diode (Goniewicz et al. 2013). There are varied
brands, flavors and types of ECs that contain different concentrations of nicotine and
varying amounts and types of flavor additives. An increasing number of flavors are
currently available to the consumer including: vanilla, chocolate, strawberry, apple, and
even gummy bear flavor (to name but a few). In addition to flavor variation, there are
choices in how much nicotine is delivered (dosage), with choices ranging from extra high
(36 mg/mL nicotine) to medium (16 mg/mL nicotine) to none (0 mg/mL nicotine). There
are generally two e-liquid supply techniques, a reservoir tank (no cotton) or a cotton
cartridge. These constructions may have different effects on nicotine delivery to the
consumer, but the differences between them have not been researched in depth (Schripp
et al. 2013).

ECs currently lack regulation or quality control in the US and a large number of
EC products come from China unregulated. EC popularity grew rapidly and can now be
found globally. Interestingly, most marketing of tobacco-related products is prohibited in
the US, yet there are no restrictions on EC advertising, especially marketing aimed at
young people (Saitta, Ferro, and Polosa, 2014). In fact, public opinion hints at a need for regulation. In an Australian study, it was shown that 66% of Australians believe that their country is in need of EC regulation, specifically that ECs should be only available for sale to individuals that are eighteen years old or older (Fraser et al., 2015). In the US, ECs are not yet regulated by the Food and Drug Administration (FDA).

In addition to the concern of unregulated ECs, recent studies reported that different puffing topographies can have a significant impact on the amount of nicotine in EC emissions and the amount that is delivered to the consumer (Trtchounian, Williams, and Talbot 2010; Williams and Talbot 2011). Changes to puffing topography can be a function of EC design or can, in some cases, may be manipulated directly by the consumer. In studies that test the effect of EC design on puffing topography, a puffer box that connects an EC to a U-shaped tube and a pump, is often used to puff the ECs, simulating real-world use. In one study, the results showed that the pump speed (revolutions per minute) required to produce EC aerosol and the aerosol density itself varies among the different EC brands and e-liquids (Williams and Talbot 2011). Another study showed that puff strength impacted the amount of aerosol produced from ECs, which can influence the amount of nicotine delivered to the consumer (Trtchounian, Williams, and Talbot 2010). There are other factors that also may play important roles in the delivery of nicotine from ECs, including the pH of e-liquids from disposable ECs and the bioavailability of free nicotine absorbed into the lungs through aerosol particles (Pagano et al. 2015), but these topics require more research to be fully understood.

The impact on human health from the use of ECs is poorly understood and current research is often conflicting. Some researchers have concluded that ECs pose no risk to
human health based on specific compounds they analyzed (McAuley et al. 2012), while other findings show that there are health hazards associated with inhalation of EC emissions (Schripp et al. 2013; Vardavas et al. 2012; Williams et al. 2013). Acute and passive EC vapers’ complete blood count does not seem to be affected compared to tobacco smokers’ whose white blood cell count was shown to increase for at least one hour after smoking (Flouris et al. 2012). Also, small particles were discovered in the environment and in human lungs from EC emissions (Schripp et al. 2013). The inhaled aerosol from EC emissions can change its size in the lungs, therefore it leads to a health hazard concern for the depositions of small particles in the lungs (Schripp et al. 2013). Volatile organic compounds (VOCs) were discovered in EC emission where a high amount of 1,2-propanediol, a small amount of 1,2,3-propanetriol, and traces of nicotine were detected in the smoker’s breath after consumption of an EC (Schripp et al. 2013). In addition, there was a small study on 30 people that used ECs for 5 minutes and showed increased resistance of air flow in the lungs and increased oxidative stress (Vardavas et al. 2012). The study of Vardavas et al. indicated only very short-term health effects from smoking for 5 minutes, so the long-term health impacts remain lesser understood (2012). One experiment studied ECs’ impact on indoor air quality, results showed no detectable amount of toxic and/or carcinogenic substances in the air (Romagna et al. 2012). Despite these few studies, very little is known about the impact of EC emissions on human health and indoor air quality, so further research is needed to better understand the impact to our health and environment (McAuley et al. 2012). Due to the lack of regulation, ECs often have inaccurate labeling of nicotine content, inconsistent nicotine delivery to the consumer, and could cause potential harm to as a result. In 2013, Goniewicz et al.
studied twenty different cartridges and 15 different refill solutions in relation to the labeled nicotine content (2013). The results indicated that 9 out of 20 cartridges analyzed in their study differed from the labeled nicotine concentration, some even exceeding a 20% difference from the label (Goniewicz et al. 2013). Further, in a recent study comparing the nicotine content of several brands, it was found that none of the three brands tested matched their labeled nicotine contents (Pagano et al., 2015). Additionally, some brands do not include a proper nicotine content on their labeling, making the comparison between the labeled and actual nicotine content impossible (Pagano et al., 2015). The issues regarding discrepancies between labeled and actual nicotine content call attention to the lack of a quality control program or regulation for ECs. Further evidence of the lack of regulation and quality control can be shown by differences between brands in terms of how much nicotine is delivered to the consumer (transfer efficiency).

Nicotine delivery to consumers is especially important to monitor in ECs, as it is unregulated at this time and has been shown to vary depending on EC design and use (Pagano et al., 2015). Consumers may become frustrated if they are not receiving the dose of nicotine that they want, which can be a function of poor product control or mislabeling. In one study, the results revealed that ECs delivered much lower nicotine even though they were smoked more heavily than tobacco cigarettes (Norton, June, and O’Connor 2014). Another study that looked specifically at how much nicotine was delivered from ECs found that most of the brands they tested had a nicotine transfer efficiency of less than 30%, meaning that most of the nicotine remained in the EC after puffing (Pagano et al., 2015).
There exists an apparent need for standardization, quality control and regulation of ECs, particularly in regard to the best analytical methods and instrumentation to use when testing the nicotine content of ECs. Several researchers have analyzed the nicotine content of ECs (both in the unused liquid/cartridges and in the aerosol emission) with a variety of types of analytical instruments. Some instruments are best-suited to analyze tobacco-specific nitrosamines, metals, tobacco alkaloids, PAHs, VOCs, or nicotine specifically (Cheng 2014). In addition, each instrument and method has different accuracy, precision, and other figures of merit.

There are multiple methods to identify and quantify nicotine by using variety types of instruments. Goniewicz et al. 2013 used gas chromatography with a thermionic specific detector (GC-TSD). Other studies used high-performance liquid chromatography (HPLC) (reviewed in Cheng 2014). There is one study that used HPLC coupled with a diode array detector, which showed a large deviation from the labeled nicotine content, where actual nicotine content differed from the labeling by up to 105% (see Cheng 2014). Other researchers also used gas chromatography-mass spectrometry (GC-MS) mostly to identify nicotine (see Cheng 2014). Other studies used different types of instruments to quantify nicotine, such as LC/MS/MS, LC/MS/MS/trap, LC/TOF, NMR and GC/FID (see Cheng 2014). Another study showed a very reliable and low range of deviation from manufacturers’ nicotine label as such as -15 to 21% by variety of instruments, UHPLC/DAD, GC/FID and GC/MS, compared to about high range of -80 to -77% deviation from labels using GC (see Cheng 2014). There are multiple instruments that could analyze nicotine in ECs, yet the Centers for Disease Control and Prevention (CDC) standard nicotine method uses GC-FID for tobacco cigarettes. GC-FID was an
excellent fit to analyze nicotine in unflavored tobacco products, but now, with new flavoring compounds, interference with nicotine detection may occur due to poorer selectivity of the method (Stanfill et al. 2009). Stanfill et al. 2009 reported that GC-MS was able to measure nicotine quickly and accurately without chromatographic data interferences (2009) in smokeless tobacco products. Using GC-MS can greatly reduce the run time to analyze nicotine since it takes only about 3.7 minutes compared to 26.7 minutes for GC-FID (Stanfill et al. 2009). The instrument that seems to be the best fit for this research with multiple different types of flavored electronic cigarette brands is, therefore, GC-MS.

To date, there is no standard GC-MS method to analyze nicotine in ECs samples either directly from the refill liquid or in emissions. Further, EC nicotine studies to date have collected the emissions in solvent-filled impingers, which can be cumbersome, so in the current study we collected emissions on Cambridge filter pads. In this study, we propose a GC-MS method, with the appropriate analytical figures of merit, to be the standard method for the analysis of nicotine in e-liquids and emissions. To properly validate this procedure as a standard method, we determined an extraction/dilution method that is proper for measuring emission aerosols deposited on pads and also in e-liquids with a high recovery. Actual nicotine content in e-liquids was compared to manufacturer labels in disposable ECs using the developed GC-MS method to investigate the safe and reliable reporting of nicotine content. Additionally, the nicotine concentrations of EC emissions from different disposable EC brands at a constant puffing topography were determined. ECs’ transfer efficiency was also calculated under this constant puffing topography. Finally, we compared the impact of different puffing
parameters on nicotine emissions in refillable ECs. These studies on nicotine content were conducted to provide further evidence for the need for standardization/regulation in the use of ECs. And toward this goal, we believe that our validated GC-MS analytical procedure could be used as a standard method for testing nicotine in in EC products.

References


STUDY 1: DISPOSABLE ELECTRONIC CIGARETTES

Introduction

This chapter focuses on disposable (single-use) ECs that contain prefilled e-liquids and non-rechargeable batteries, designed to be disposed upon depletion. Several studies have been conducted on disposable ECs to quantify nicotine and other chemical components, but many of these studies have not been performed on many of the new brands, enhanced designs, and flavors/e-liquid make-ups that have come out in recent years. Studies have reported manufacturer labeling claims and actual measured results typically differ due to the lack of standards/regulations by the Food and Drug Administration (Goniewicz et al. 2013; Trehy et al. 2011; Pagano et al. 2015). Some of the studies that compared manufacturers’ claims and actual nicotine concentrations also investigated the amount of nicotine before and after puffing and number of puffs until depletion with large resulting differences (range of -100% to 100%) between claimed and actual results (Cheng 2014).

Transfer efficiency, or the portion of nicotine that is transferred from the EC to the user, is another important metric of ECs. Transfer efficiency may vary between brands due to the different engineering/manufacturing design of the products. In the real-world, EC users may display dissatisfaction with the products if they are not receiving the desired nicotine dose either due to inaccurate manufacturer labels or poor transfer efficiency. The current study focuses on the differences between manufacturer labels and actual results of nicotine concentration, as well as nicotine transfer efficiency, for a collection of disposable ECs using the GC-MS analytical method that we developed.
Materials and Methods

Product Selection and Sample Collection

Disposable EC brands were chosen based on popularity, locally and nationally, by researching online forums and interviewing smoke shop employees informally in Rochester, NY. We studied five disposable ECs brands: Blu, Criss Cross, Encore, Swisher and White Cloud. We analyzed five ECs from each the same manufacturing batch for each brand and conducted three trials for each EC. In the case of emissions testing, we puffed the ECs to completion. EC samples were collected using an in-house puffing machine, a full description of which is available from Pagano et al. (2015). In short, a vacuum chamber is used to puff ECs and the emissions are collected on a 44mm Cambridge filter pad while monitoring puffing parameters such as flow rate, puff volume and puff duration. To avoid overloading the pads, puffs were deposited onto several pads in succession by deposition only 10 puffs worth on each filter pad, before switching to a clean pad for the next 10 puffs.

Our internal standard for this study was quinoline due to its characteristics in providing a good chemical match to the nicotine analyte. Quinoline was further chosen because it does not interfere with the chromatography of nicotine, but behaves similarly throughout the analytical method. Nicotine and quinoline have different molecular weights (including different quantification and verification ion weights for the single ion monitoring mode in GC-MS) and different retention times.

To measure device transfer efficiency, we compared nicotine concentrations before and after ECs were puffed. We tested e-liquid from un-puffed ECs and the
emissions deposited on pads of puffed ECs to calculate the percentage of difference (or the ‘percent transfer efficiency’).

**Nicotine Standards for Un-puffed Electronic Cigarette Samples**

Five to nine nicotine (Pfaltz & Bauer, Nicotine 98%, CAS# 54-11-5) standards from 0.01mg/mL to 10 mg/mL were prepared in methanol to create calibration curves. Our internal standard was 50 mg/mL quinoline (ACROS Organics, quinoline, 99%, CAS# 91-22-5). The internal standard was prepared by adding 457 µL of quinoline to a 10 mL volumetric flask that was brought to volume and mixed with HPLC-grade methanol. All standards were combined with an internal standard in a GC-MS vial (1515 µL of prepared standard, 15 µL of prepared 50 mg/mL quinoline). All vials were capped and sonicated for 20 minutes and then vortexed for 10 seconds at 3000 rpm to ensure mixing. Five trials of each vial were run through the GC-MS, via the autosampler, to create calibration curves.

**Nicotine Standards for Puffed Disposable Electronic Cigarette Pad Samples**

Five to nine nicotine standards were created, ranging from 50 mg/mL to 0.5 mg/mL nicotine, in 10 mL volumetric flasks with methanol. 1 mL of each solution was aliquoted into empty 50 mL volumetric flasks and brought to volume with HPLC grade methanol. The flasks were inverted three times to ensure proper mixing. Each flask was poured directly into a labeled 4 oz. PTFE Lined Polypropylene closure amber glass jar (Quality Environmental Container, QEC) for each standard that contained three pads spiked with 495 µL (of 50 mg/mL) of the internal standard, quinoline. Final nicotine
standard solutions were filtered using syringe filtration (MicroSolv 0.45 \( \mu \)m regenerated cellulose membrane filters) and transferred directly into GC-MS vials, then run through the GC-MS to produce a calibration curve. The 50 mg/mL internal standard was prepared by adding 4 mL 570 \( \mu \)L of quinoline to a 100 mL volumetric flask and brought to volume with HPLC-grade methanol. Five trials of each standard were performed.

**Un-Puffed Disposable Electronic Cigarette Liquid Samples**

Additional extraction steps were necessary to analyze the e-liquid in un-puffed disposable ECs. The liquid content from ECs was emptied, and the ECs were rinsed with methanol and put into a 50 mL volumetric flask and brought to volume with methanol. 1515 \( \mu \)L this solution was transferred into a GC-MS vial and 15 \( \mu \)L of internal standard (50 mg/mL quinoline) was added. The GC-MS vials and their contents were sonicated for 20 minutes and vortexed for 10 seconds. The e-liquids were analyzed in triplicate by GC-MS to quantify the nicotine content. Details of the extraction process can be found in Pagano et al. (2015).

**Puffed Disposable Electronic Cigarette Pad Samples**

For the analysis of puffed (used) ECs samples, the procedure was as follows. Each EC was puffed through a puffing machine that was set to a consistent strength of inhalation and duration of puff. Three cigarettes from each brand passed through the smoking machine, with their emissions being accumulated on filter pads (again, changing the pads throughout the runs so as to not overload them). The loaded filter pads were spiked with 495 \( \mu \)L (of the 50 mg/mL solution) of the internal standard, quinolone, and
then soaked in 50 mL of methanol and put on the orbital shaker for twenty-four hours. The jars were wrist-shaken for 15 minutes and then put on the orbital shaker again for another twenty-four hours. This procedure allowed for the complete breakdown of the Cambridge filter pads in the solvent. All jars were filtered through MicroSolv 0.45 μm regenerated cellulose membrane filters and inserted directly into GC-MS vials and analyzed by GC-MS via the autosampler injection.

**GC-MS settings**

The instrument and column used in this study are described in Pagano et al. (2015). The software that controlled the GC-MS is Perkin Elmer TurboMass (Version 5.4.2.1617). We used an autosampler with 5μL syringe attached to the GC. The injection volume was set to 1.0 μL within normal injection speed. The pre-injection solvent and sample washes were set to two washes each. The oven was set at 60 °C initially and increased 20 degrees per minute to 200 °C and then held at 200 °C for 3 minutes (hereafter referred to as ‘GC Method A’). The program run time for the oven was about 10 minutes. The carrier column was 30m long with a diameter of 250 μm (with vacuum compensation turned on). Carrier split control was set at 50:1 ratio. The oven split control was set at the flow of 50 mL/minute.

The MS detector was set up with two separate settings for selected ion recording (SIR) for nicotine and quinoline. A solvent delay was set to the first four minutes. The monitoring window started at 4 minutes and ended at 10 minutes. Three nicotine SIR ion masses were set on 84 mass to charge ratio (m/z) (confirmation, dwell time of 0.10 seconds), 133 m/z (quantification, dwell time of 0.10 seconds), and 162 m/z (confirmation, dwell time of 0.35 seconds); similar to (Gowadia, Oldham, and Dunn-
Rankin 2009; Stanfill et al. 2009). Three quinoline SIR ion masses were also set at 102 m/z (quantification, dwell time of 0.10 seconds), 129 m/z (confirmation, dwell time of 0.10 seconds), and 161 m/z (confirmation, dwell time of 0.35 seconds); similar to Stanfill et al. 2009. To quantify nicotine in samples and standards, we used the ratio of the chromatographic peak integrations of 133 ion to that of the 102 ion.

**Data analysis**

We determined the percent difference between advertised and actual nicotine content in ECs according to the following equation:

\[
\% \text{ Difference} = \left[\frac{(A - B)}{B}\right] \times 100 \quad \text{Eq.1}
\]

where \(A\) = Manufacturer reported strength (mg/cig. for disposable ECs) and \(B\) = Actual measurement of nicotine (mg/cig. for disposable ECs).

We also calculated the percent transfer efficiency before and after puffing ECs. The equation we used to calculate transfer efficiency was:

\[
\% \text{ Transfer Efficiency} = \left[\frac{(\text{Nicotine delivered to pad})}{(\text{Nicotine in unpuffed EC})}\right] \times 100 \quad \text{Eq. 2}
\]

**Results**

*Table 1* shows the percent difference between the manufacturers’ labels and actual nicotine concentration measured by GC-MS in our study. This table also includes battery capacity, the brand, manufacturers’ claimed number of puffs until depletion,
actual number of puffs achieved in this experiment, % difference, and lastly the
gravimetric measurement of e-liquid before the EC was puffed. Encore Summer Punch
had the highest battery capacity of 1.04 watt-hours (wH) and claimed number of puffs of
500 determined by the manufacturer. Our experiment revealed that all five brands were
used up to full consumption with lower number of puffs than claimed and that the
maximum number of puffs achieved using our experimental set-up was only 60% of that
claimed by the manufacturer. However, it should be noted that it is unclear what puffing
parameters the manufactures used to make their claims- so puffing to depletion results
can only be interpreted relative to, and using, our machine puffing set-up and parameters.

Table 1: Comparison between Manufacture’s Claims and Study Measurements for Number of Puffs

<table>
<thead>
<tr>
<th>Product</th>
<th>Claimed Number of Puffs</th>
<th>Actual Number of Puffs Achieved in this Study (SE)</th>
<th>% Puffs Achieved</th>
<th>E-liquid Extracted From Un-puffed E-Cig. [mg/E-Cig] (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criss Cross Regular</td>
<td>200</td>
<td>56 (1.7)</td>
<td>28</td>
<td>563.3 (10.0)</td>
</tr>
<tr>
<td>Swisher Natural</td>
<td>400</td>
<td>131.6 (14.4)</td>
<td>33</td>
<td>612.9 (46.6)</td>
</tr>
<tr>
<td>Blu Magnificent Menthol</td>
<td>400</td>
<td>184.6 (2.3)</td>
<td>46</td>
<td>1013.8 (7.7)</td>
</tr>
<tr>
<td>White Cloud Fling</td>
<td>400</td>
<td>239.6 (4.1)</td>
<td>60</td>
<td>1498.0 (9.3)</td>
</tr>
<tr>
<td>Encore Summer Punch</td>
<td>500</td>
<td>219.0 (6.9)</td>
<td>44</td>
<td>1570.1 (9.7)</td>
</tr>
</tbody>
</table>

*determined gravimetrically. This table was reproduced from Pagano et al. (2015).

Table 2 contains information related to the nicotine content of five different
disposable EC brands. It includes manufacturers’ labeled nicotine content, measured
nicotine content, amount of nicotine delivered to the filter pad (after puffing), and the
percent transfer efficiency. Manufacturer labeled nicotine content varies in the way it is presented, from basic description as such as “High” or “Full Strength, 2.4%”, to quantitative labeling, such as “16 or 24 mg/cig.” The third column shows the actual nicotine content of the un-puffed e-liquid based on our GC-MS analyses. These values range from 5.2 to 24.6 mg/cig. The fourth column shows the measured nicotine content delivered to the filter pads after puffing, which is naturally a subset (lower value) of the amount in corresponding un-puffed e-liquids. The fifth column shows the percent transfer efficiency for the devices, which compared the amount of nicotine that was delivered to filter pads as a function of the original amount of nicotine available in the un-puffed e-liquid. A higher transfer efficiency is related to superior delivery of nicotine content from the puffing process. The transfer efficiency of nicotine for these devices range from 17.6 to 57.5%. The last three columns represent a qualitative comparative rank of nicotine content in the e-liquid before puffing as well as that delivered to the filter pad from the puffing process- with “1” being the highest concentration and “5” the lowest. These rankings demonstrate how the different engineering characteristics of the different devices ultimately impact nicotine delivery.
Table 2: Comparison between Manufacture’s Claims and Study Measurements\(^\text{§}\) for Nicotine Content

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer Label</th>
<th>Measured Total Nicotine in Un-puffed E-Cig. [mg/E-Cig] (SE)</th>
<th>Delivered to Filter Pad(^\text{†}) [mg/E-Cig] (SE)</th>
<th>Nicotine Transfer Efficiency (%) (SE)</th>
<th>Comparative Rank of Nicotine Content (1=highest)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Based on Manufacturer Label(^\text{‡})</td>
</tr>
<tr>
<td>White Cloud Fling</td>
<td>Full Strength, 2.4%</td>
<td>24.6 (0.71)</td>
<td>5.41 (0.22)</td>
<td>22.0 (0.9)</td>
<td>unknown</td>
</tr>
<tr>
<td>Criss Cross Regular</td>
<td>High</td>
<td>6.2 (0.02)</td>
<td>1.09 (0.25)</td>
<td>17.6 (4.0)</td>
<td>unknown</td>
</tr>
<tr>
<td>Blu Magnificent Menthol</td>
<td>24</td>
<td>14.0 (0.11)</td>
<td>2.02 (0.10)</td>
<td>14.4 (0.7)</td>
<td>Highest</td>
</tr>
<tr>
<td>Swisher Natural</td>
<td>18</td>
<td>5.2 (0.67)</td>
<td>2.99 (0.52)</td>
<td>57.5 (10.2)</td>
<td>Middle</td>
</tr>
<tr>
<td>Encore Summer Punch</td>
<td>16</td>
<td>19.3 (0.40)</td>
<td>5.03 (0.16)</td>
<td>26.0 (0.8)</td>
<td>Lowest</td>
</tr>
</tbody>
</table>

\(^\text{§}\)From GC-MS analyses. \(^\text{*}\)total puffs used to make nicotine delivery measurements (as dictated by E-Cig depletion) are shown in Table 1, \(^\text{‡}\)for the 3 products that stated quantifiable nicotine content, and \(^\text{†}\)rankings based on averaged measurements of nicotine concentration. This table was reproduced from Pagano et al. (2015).

Discussion

Our study revealed that all five disposable EC brands’ actual number of puffs to depletion differed from the manufacturers’ claimed number of puffs. The percentage of puffs achieved relative to the claimed number of puffs to reach depletion varied from 28% to 60%. Our experiment used specific puffing parameters to puff the disposable ECs that might be different than the manufacturers used, so again, these results should be interpreted in a relative manner. Each brand had a different battery capacity; therefore it showed that standards are not in place for manufacturers when it comes to the production and testing of ECs and their advertised claims.

Other studies have agreed with our results related to the range of disparity between manufacturers’ EC labeling and measured values of nicotine content in unpuffed ECs (Etter, Zäther, and Svensson 2013; Trehy et al. 2011; Goniewicz et al. 2013; Cameron et al. 2014). There were significant differences between labeled and measured nicotine...
quantities of nicotine content, which agrees well with the study done by Goniewicz et al. with their relative percent difference in nicotine concentration of measured results and manufacturers’ labeling (from 0 to -89% in their study) (2013). Another study on refillable ECs reported a smaller range of percent difference between labeled and actual nicotine content (from 85% to 121% in their study), but it is still a concern due to higher concentration disparity from the label, which means consumers will be getting more nicotine than they might be expecting (Etter, Zäther, and Svensson 2013). Trehy et al. also revealed that the battery and time between puffs impacts the total amount of nicotine delivered in EC emissions (2011). Without batteries, ECs would not be able to deliver nicotine due to the requirement for the process of electric heat (i.e. heating coil) to create emissions. Our results of the measured amount of nicotine in the disposable ECs were mostly lower than that of the labeled amount, agreeing with another study where 2 out of 7 brands ended up with significantly lower nicotine concentrations than reported by the manufacturer; for instance the highest concentration labeled (25-36 mg/mL) actually had an average of only 19.9 mg/mL nicotine based on three replicates (Cameron et al. 2014).

Table 2 contains additional depth of nicotine content comparison between manufacturers’ labeling and the actual measured results from our experiment. Unfortunately, EC manufacturers’ labels are often ambiguous (and do not give the units of measure of nicotine content, nor are the units standardized). For example, there are a few EC brands that labeled very generally, such as “High” or “Full Strength” in regard to their nicotine concentration (Table 2). The nicotine content of each brand was measured before and after puffing so that the transfer efficiency could be calculated. According to packaging labels, the Blu product was supposed to have among the highest nicotine
concentrations of the group, but it was actually measured to be lower than two brands that are labeled to have lower nicotine concentration (Table 2). The Swisher product was labeled to provide 18 mg nicotine/cig, yet yields about three times lower the concentration after puffing. The Encore product is interesting, where it is claimed to contain 16 mg nicotine/cig, but is measured to contain a higher nicotine concentration (19 mg nicotine/cig) in the un-puffed EC.

The data in Table 2 is shown visually in Figure 1. Nicotine content delivered to filter pads (open squares), and percentage nicotine transfer efficiency (thick “X,” right axis) of five E-Cig products is shown in the figure (symbols represent mean ± standard error). The swisher product has the highest percent transfer efficiency (almost at 60%), yet showed a low available nicotine concentration prior to puffing. The result of this is this is that the Swisher product has a relative closer difference between before puff and after puffing nicotine concentration due to the high transfer efficiency. Essentially, despite the low nicotine level starting point, more of the available nicotine is transferred in emissions during puffing compared to the other products. The White Cloud product has lower transfer efficiency (at about 20%) despite its higher concentration of nicotine in the e-liquid of the un-puffed EC. Therefore, the White Cloud product has a larger difference in the nicotine concentration measured before and after puffing the EC.
Unreliable or ambiguous package labeling could mislead consumers to unknowingly taking-in undesired levels of nicotine. In addition, due to non-uniform transfer efficiencies of products, the amount of nicotine that might enter the consumer’s system can further impact the dose of nicotine that they receive. In all, this can lead to confusion in product selection for the public. Quality control measures are needed for general EC products to ensure the accuracy of the manufacturers’ labeling and device engineering that takes transfer efficiency into consideration. Consumers receiving more nicotine than expected could experience health hazards due to the possibility of nicotine toxicity. Likewise, consumers who are getting less nicotine than expected might vape more ECs, supplement with tobacco cigarettes, or switch entirely to tobacco cigarettes due to product dissatisfaction.

Figure 1: Measured nicotine concentration in un-puffed electronic cigarettes.
**Conclusion/Future Directions**

This study demonstrates that consumers might be taking in either more or less nicotine than the manufacturing labeling claims due to either nicotine amounts in the e-liquid or to device transfer efficiency. On one extreme, low nicotine levels to the user could cause dissatisfaction by the consumer, while at the other extreme, vapers could be in danger of taking-in toxic levels of nicotine. The latter warrants need for standardization/regulation due to serious health implications, but both need addressing to ensure their proper use and the efficacy as a potential smoking cessation therapy. Agencies, such as the FDA, could regulate these products to ensure the mentioned consistencies.

A multitude of new EC products are hitting the market without regulation. Future research should analyze these new products (we have only tested 5 brands here, and even with all of the other studies to date, only a small number of the total products available have been tested). Also, contaminants that might reach the consumer, such as heavy metals and flavorants, as well as the sizes of the emitted particles, should be investigated. The flavors in ECs are becoming increasingly popular, which should be even more concerning to the public due to fact that little is known about how these flavorants behave chemically, interact with other chemicals in the sample matrix, and reactions that might occur when ECs are heated. Recently, the FDA Center for Tobacco Products had a public workshops, received public comments, and presented a proposed rule that specifically includes ECs, so the regulations on ECs may be established soon (FDA, 2015).
References


STUDY 2: REFILLABLE ELECTRONIC CIGARETTES

Introduction

This study focused on the effect(s) of varied puffing regimes on nicotine emissions from three different refillable EC products using a common e-liquid solution. Inasmuch as it is anticipated that each UC user will have a different puffing topography, users will likely receive different amounts of nicotine based on the way they draw vapor from an EC. The user may receive either more or less nicotine than they were expecting based on the manufacturers’ product labeling, the engineering characteristic of each device, and depending on how they draw aerosol from the EC (topography). There are few studies that directly account for the varied puffing topographies of different users in measuring the nicotine content of EC emissions that might be delivered to the body. This study represents preliminary work toward assessing the role of puffing parameters on the amount of nicotine that is found in EC emissions. Here, we varied the mechanical settings (puff duration, puff volume, and flow rate) of the puffing machine and collected EC emissions on filter pads for GC-MS analyses. This study is one of the first to measure the nicotine concentration in emissions from ECs that is aimed at understanding how parameters of the puffing machine impact nicotine delivery. The study reveals preliminary evidence of the importance of puffing parameters on nicotine concentration emitted and the need for regulations to enforce standards on the testing and production of nicotine in ECs and e-liquids. Though not discussed in this theses, our laboratory has already taken this work a step further by monitoring the real-world smoking topographies of users and setting-up the puffing machine to puff under those observed conditions, ultimately to determine how the real-world topographies impact nicotine levels delivered to the user.
Materials and Methods

Product Selection and Sample Collection

Selected brands of ECs were chosen based on popularity, locally and nationally, by researching online forums and conducting interviews with local smoke shop employees in Rochester, NY. For the refillable products, we studied three types of devices (iClearX.I, iClear30, and Halo) and collected 100 puffs worth for each trial of each product on filter pads. Fresh filter pads were constantly replenished during the trials so that they were not overloaded (typically about 10 puffs per pad). After deposition, the Cambridge filter pads were weighed to ensure that the loading did not exceed the pad’s capacity. Each brand’s 100-puff trial was run in triplicate. EC samples were collected using the in-house puffing machine described by Pagano et al. (2015). However, the puffing parameters like flow rate, puff duration, and puff volume were varied in this study to test the impact of these changing puffing parameters on nicotine emissions. For consistency, all trials (at all puffing machine settings and for all devices) were filled with the same e-liquid, Criss Cross brand Grape Pixie flavor at 24 mg/mL nicotine strength.

Nicotine Standards for Un-puffed (e-Liquid) EC Samples

Five to nine nicotine standards (Pfaltz & Bauer, Nicotine 98%, CAS# 54-11-5), from 0.01 mg/mL to 10 mg/mL, were made to prepare a nicotine calibration curve. Our internal standard was 50 mg/mL quinoline (Acros Organics, Quinoline, 99%, CAS# 91-22-5). The internal standard was prepared by adding 457 µL of quinoline to a 10 mL volumetric flask that brought to volume using HPLC-grade methanol. All standards were
combined with the internal standard in GC-MS vials (1515 μL standard with 15 μL Quinoline). All vials were sonicated for twenty minutes and then vortexed for 10 seconds at 3000 rpm to ensure mixing. Five trials of each vial were analyzed using GC-MS. Our internal standard for this study was quinoline due to its characteristics in providing a good chemical match to the nicotine analyte. Quinoline was further chosen because it does not interfere with the chromatography of nicotine, but behaves similarly throughout the analytical method. Nicotine and quinoline have different molecular weights (including different quantification and verification ion weights for the single ion monitoring mode in GC-MS) and different retention times, making their separation distinct.

**Nicotine Standards for the EC Emissions Deposited onto Filter Pads Samples**

Five to nine nicotine stock standards were made, in concentrations from 0.25mg/mL to 25mg/mL, using 10mL volumetric flasks and a methanol (HPLC-grade) solvent. From the stock standards, working standards were created in concentrations from 0.01 mg/mL to 1 mg/mL nicotine by adding 1 mL of each stock standard to a 25 mL volumetric flask that was brought to volume with methanol. The flasks were inverted several times to ensure mixing. Each working standard was poured directly into a labeled, sealed 4 oz. Quality Environmental Container (QEC) PTFE-lined polypropylene closure amber glass jar that contained one blank filter pad spiked with 248 μL (of 50 mg/mL) quinoline, the internal standard. Standard loaded filter pads were taken through the same extraction procedure as the EC emission sample pads. The jars were placed on an orbital shaker for twenty-four hours and then wrist-shaken for 15 minutes, followed by
shaking for another twenty-four hours. This procedure allowed for the complete breakdown of the Cambridge filter pads in the solvent. The extracted nicotine standard solutions were filtered by syringe filtration through MicroSolv 0.45 µm regenerated cellulose membrane filters and transferred directly into GC-MS vials. The contents of successive vials were injected into the instrument via the autosampler and analyzed using GC-MS to produce a nicotine calibration curve. Each extracted standard was analyzed using five replicates.

**E-liquid Samples**

For e-liquid samples, we took 15 µL of the e-liquid and directly placed it into a GC-MS vial and added 1500 µL of methanol and 15 µL of our internal standard (50 mg/mL quinolone) to the same vial. All vials were sonicated for 20 minutes and then vortexed for 10 seconds at 3000 rpm to ensure mixing. Five trials of each vial were run through the GC-MS using the autosampler.

**EC Emissions onto Filter Pad Samples**

Each EC was passed through the puffing machine that was set to varying combinations of flow rate, puff volume, and puff duration. At each parameter combination of the puffing machine, three trials of each EC device were run, with a total of 100 puffs (whose deposition was spread over ten filter pads) for each device. The filter pads were spiked with 248 µL (of 50 mg/mL) quinolone, the internal standard, and placed in 25 mL of methanol into a labeled, sealed 4 oz. Quality Environmental
Container (QEC) PTFE-lined polypropylene closure amber glass jar. Each jar was placed on an orbital shaker for twenty-four hours. The jars were wrist-shaken for 15 minutes and then put on the orbital shaker again for another twenty-four hours. This procedure allowed for the complete breakdown of the Cambridge filter pads in the solvent. All jars were filtered through MicroSolv 0.45 µm regenerated cellulose membrane filters and inserted into GC-MS vials and analyzed by GC-MS via a programmable autosampler. Three replicates were run through the GC-MS for all of the samples.

**Data analysis**

We correlated different puffing settings with nicotine emissions to assess the impact of the variation of puffing parameters on amount of nicotine emitted. The relationships examined were: Nicotine (mg emitted per puff) vs. Puff Volume (mL), Nicotine (mg emitted per puff) vs. Puff Duration (seconds), Nicotine (mg/mL emitted per puff) vs. Puff Volume (mL), Nicotine (mg/mL emitted per puff) vs. Puff Duration (seconds), Nicotine (mg/s emitted) vs. Puff Volume (mL), and Nicotine (mg/s emitted) vs. Puff Duration (seconds). Further, we reported the overall relationship between the gravimetric method of analyzing total weight of aerosol deposition on filter pads and that of the GC-MS method for nicotine emission determination. As expected, the two methods correlated- as the quantity of nicotine deposited on the pad is a fairly constant subset of the total aerosol deposition.
Results and Discussion

The nicotine calibration curve specifically for e-liquid samples consisted of five standards in the range from 0.001 to 1 mg/mL nicotine (0.001, 0.05, 0.01, 0.5 and 1 mg/mL). Figure 2 shows the calibration curve for nicotine, where each point represents the average of triplicate analyses. The equation from the calibration curve was \( y = 1.6613x - 0.0067 \), where the “y” represents the peak GC-MS chromatographic peak area ratio (nicotine:quinoline) and “x” is the nicotine concentration (in mg/mL) of the nicotine standards. The coefficient of determination \( (R^2) \) was greater than 0.9999, demonstrating the strong linearity of the method over this range of nicotine concentrations.

![Figure 2: Nicotine calibration curve (0.001 – 1 mg/mL) used for determining nicotine concentrations of e-liquid samples.](image-url)
Figure 3 shows the nicotine calibration curve specifically for use in analyzing emissions deposited on filter pads, with standards ranging from 0.01 to 1 mg/mL (0.01, 0.05, 0.1, 0.3, 0.4, 0.6, 0.8 and 1 mg/mL). The calibration curve was generated using the average from five replicates for each standard. The coefficient of determination ($R^2$) was greater than 0.999, suggesting a high degree of linearity. The equation from this curve was $y = 1.4193x - 0.0246$, which was used to calculate the nicotine concentration from emissions deposited onto filter pad samples for refillable ECs.

![Nicotine Calibration Curve for EC Emissions](image)

*Figure 3: Nicotine calibration curve (0.01 – 1 mg/mL) for use on EC emissions captured on filter pads samples.*

Figure 4 shows the relationship between the weight measurements of EC devices as they change after puffing (weight of the EC device before puffing minus the weight of the EC device after puffing) and the corresponding nicotine concentrations from the emissions collected on filter pads during those same trials, but measured by our GC-MS.
method. Each data point represents an average of ten different measurements. The
weight change of the EC device after puffing had a strong positive correlation (with an $R^2$
of 0.9527) with the nicotine concentration measured by GC-MS. This dataset is based on
all types of EC brands and all of the different puffing machine settings combined. This
relationship between nicotine concentration and EC weight change is also significant
($p<0.0001$). This relationship is expected, as the quantity of nicotine deposited on the
filter pad is a fairly constant subset of the total aerosol deposition.

![Figure 4: Relationship of weight changes of EC devices during the puffing process and the nicotine concentration of emissions to filter pads measured by GC-MS.](image)

Similar to Figure 4, Figure 5 shows the relationship between the weight measurements of aerosols deposited on filter pads and the corresponding nicotine concentrations from the emission collected on the same filter pads, but measured by our
GC-MS method. Each data point represents an average of ten different measurements. The weight of aerosol deposited on pads during puffing had a strong positive correlation (with an \( R^2 \) of 0.9586) with nicotine emission concentration measured by GC-MS. This dataset included all types of EC brands and all of the different puffing machine settings combined. This relationship between nicotine concentration and pad weight change is also significant (\( p<0.0001 \)). Again, this relationship is expected, as the quantity of nicotine deposited on the filter pad is a fairly constant subset of the total aerosol deposition.

![Nicotine Concentration vs. Pads Weight Change](image)

**Figure 5:** Relationship of weights of aerosol deposited on filter pads during the puffing process and the nicotine concentration of emissions to the same filter pads measured by GC-MS.

For the following series of graphs that show relationships between nicotine emissions concentrations and different puffing machine parameters; linear, logarithmic,
and second-order polynomial fits of the data series were investigated. It was clear that in
some cases, logarithmic or second order polynomial trendlines fit the data better (either
through observed improvements in $R^2$ or p-values). A future direction of this study will
be to determine if there are scientific/engineering explanations for these different
functional fits.

Nicotine concentration (mg emitted per puff) of the refillable EC, iClear X.I,
showed a strong second-order polynomial relationship with puff volume (Figure 6). As
puff volume increased, mg of nicotine emitted per puff increased as well, but this
relationship did not appear to be linear. The polynomial relationship was applied to all
flow rates of 10, 30, and 50 mL/s. The flow rate of 10 mL/s showed a significant
correlation between puff volume and nicotine concentration, with a p-value of 0.014. The
data at a flow rate of 30 mL/s and 50 mL/s showed insignificant correlations between
puff volume and nicotine concentration, with a p-value of 0.08 and 0.9, respectively. For
this iClear X.I device, puff volume seemed to be an important factor in nicotine emitted
(mg emitted per puff).
Figure 6: Refillable EC - iClear X.I Relationship between Puff Volume (mL) and Nicotine Concentration (mg emitted per puff).

In the iClear X.I device, nicotine concentration (in mg emitted per puff) showed a strong positive linear relationship with puff duration (Figure 7). Each data point shows the average of ten trials from four different puff durations (1.8, 3, 4.5 and 7 seconds). The data from the flow rates of 30 and 50 mL/s shows similar slopes in the relationship. The trend from flow rate of 10 mL/s had a significant correlation between puff duration and nicotine concentration with a p-value of 0.012, but a less steep slope than the other flow rates.
In Figure 7, the relationship between puff duration (seconds) and nicotine concentration (mg emitted per puff) was examined for the iClear X.I device. The data for the three different flow rates (10, 30, and 50 mL/s) appeared to have a linear relationship with nicotine emission concentration in this device. The flow rate of 10 mL/s had a higher puff volume and nicotine concentration (mg/mL) initially, with a very steep slope compared to the other two flow rates (30 and 50 mL/s).

Figure 8 shows the relationship between nicotine (mg/mL emitted per puff) and puff volume for the iClear X.I device. This relationship was not as strong due to the coefficients of determination (R²) falling between 0.6829 and 0.7965. The data for the three different flow rates (10, 30, and 50 mL/s) appeared to have a logarithmic relationship with nicotine emission concentration in this device. The flow rate of 10 mL/s had a higher puff volume and nicotine concentration (mg/mL) initially, with a very steep slope compared to the other two flow rates (30 and 50 mL/s).
Figure 8: Refillable EC - iClear X.I Relationship between Puff Volume (mL) and Nicotine Concentration (mg/mL emitted per puff).

The data points in Figure 9 show an average of ten trials for nicotine concentrations as a function of puff duration, which ranged from 1.8 to 9 seconds. One fairly strong positive linear relationship ($R^2 = 0.9$, p-value = 0.05) between nicotine concentration emitted (mg/mL) and puff duration was observed for this data series - the emissions obtained at a flow rate of 30 mL/s (Figure 9). The data from the flow rates of 10 and 30 mL/s also had significant positive correlations between puff duration and nicotine concentration with p-values less than 0.05. Positive linear relationships between puff duration and nicotine concentration (mg/mL) were present for the data captured for all three different flow rates in the iClear X.I device.
Figure 9: Refillable EC - iClear X.I Relationship between Puff Duration (seconds) and Nicotine Concentration (mg/mL emitted per puff).

Figure 10 shows a logarithmic relationship between nicotine concentration (in mg/s) and puff volume (mL) iClear X.I device. The determination of coefficients for all flow rates were positive, but none were strong. The data from the flow rate of 10 mL/s showed points that were very close to each other, since the puff volume only increased to 100 mL. The other two flow rates of 30 and 50 mL/s went above 200 mL puffing and 0.05 mg/s nicotine were emitted. These relationships reveal that the refillable EC device, iClear X.I, emits higher nicotine (mg/s) with higher flow rates.
In the iClear X.I device, the linear relationship between puff mg/s of nicotine emitted and puff duration was fairly strong for a flow rate of 30 mL/s (Figure 11). The relationship became weaker as the flow rate was lowered (10 mL/s) or increased (50 mL/s). The data captured at flow rates of 30 and 50 mL/s emit higher mg/s of nicotine, to above a value of 0.06, compared to that from a flow rate of 10 mL/s (with below 0.05 mg/s nicotine). The flow rates of 10 and 30 mL/s had significant correlations between puff duration and nicotine concentration with a p-value below 0.05. The data collected at a flow rate of 50 mL/s had insignificant correlation between puff duration and nicotine concentration with a p-value of 0.12.
Nicotine concentration (mg emitted per puff) of the refillable EC device, iClear30, has very strong second-order polynomial relationship (greater than 0.96 for determination of correlation) with puff volume (Figure 12). Each data point was based on the average of ten trials. As puff volume increases, mg of nicotine emitted per puff increased as well. This relationship appeared to apply to all flow rates. For the iClear30 device, puff volume seems to be important in influencing nicotine concentration (mg emitted per puff) due to the high coefficient of determination.
Figure 12: Refillable EC - iClear30 Relationship between Puff Volume (mL) and Nicotine Concentration (mg emitted per puff).

With the iClear 30 device, nicotine concentration (in mg emitted per puff) had a strong positive linear relationship with puff duration (Figure 13). Each data point represents the average of ten trials for four different puff durations (1.8, 3, 4.5 and 7 seconds). At flow rates of 30 and 50 mL/s, the relationship produced similar slopes, both steeper than that of the trend at the 10 mL/s flow rate. The data from all flow rates showed significant relationships between nicotine concentration and puff duration, with a p-values below 0.05.
In Figure 14, the data produced from a flow rate of 30 mL/s showed the strongest relationship in the iClear 30 device, where the determination of coefficient was greater than 0.9, as it relates to nicotine emitted as a function of puff volume. At a flow rate of 10 mL/s, higher nicotine concentrations (mg/mL) were shown initially with a steep slope compared to the other two flow rates (30 and 50 mL/s).
Figure 14: Refillable EC - iClear30 Relationship between Puff Volume (mL) and Nicotine Concentration (mg/mL emitted per puff).

The data points shown in Figure 15 are based on the average of ten trials for puff durations ranging from 1.8 to 9 seconds. They are for the iClear 30 device. There were two fairly strong positive linear relationships ($R^2 = 0.82$ and $0.88$) between nicotine concentration (mg/mL) and puff duration in this figure at flow rates of 10 and 30 mL/s, respectively (Figure 15). The flow rates of 10 and 30 mL/s showed significant correlations between puff duration and nicotine concentration with p-values below 0.05. A weak, yet positive linear relationship between puff duration and nicotine concentration (mg/mL) was shown at the highest flow rate (50 mL/s) in the iClear30 device.
Figure 15: Refillable EC – iClear30 Relationship between Puff Duration (seconds) and Nicotine Concentration (mg/mL emitted per puff).

Figure 16 shows a logarithmic relationship between nicotine concentration in mg/s and puff volume (mL) for the iClear 30 device. The data for the coefficients of determination for all flow rates were positive, but not strong. The lowest flow rate, 10 mL/s, had the shallowest slope and the data points were very close to each other since the puff volume only went up to 100 mL. The other two flow rates of 30 and 50 mL/s went above 250mL of puff volume, corresponding to 0.05 mg/s nicotine emitted.
Figure 16: Refillable EC – iClear30 Relationship between Puff Volume (mL) and Nicotine Concentration (mg/s emitted).

With the iClear30 device, the relationship between mg/second of nicotine emitted and puff duration were fairly strong when it had a flow rate of 10 and 30 mL/s (Figure 17). The relationship became weaker as the flow rate got higher (50 mL/s). The flow rate of 10 and 30 mL/s had significant correlations between puff duration and nicotine concentration, with a p-values below 0.05. The flow rate of 30 and 50 mL/s trials emit higher mg/s of nicotine at the highest puff volumes (to above 0.05 compared to flow rate of 10 mL/s with below 0.4 mg/s nicotine).
Figure 17: Refillable EC – iClear30 Relationship between Puff Duration (seconds) and nicotine concentration (mg/s emitted).

Nicotine concentration (mg emitted per puff) for the refillable EC Halo device had a strong second-order polynomial relationship ($R^2 > 0.99$) with puff volume (Figure 18). Each data point is based on an average of ten trials for nicotine concentration and puff volume. As puff volume increased, mg of nicotine emitted per puff naturally increased as well. This was true for the data at all flow rates (10, 30 and 50 mL/s), yet the data collected at a flow rate of 10 mL/s seemed to increase more rapidly than those of the higher flow rates. For the Halo device, puff volume seems to have an important role in nicotine concentration (mg emitted per puff) due to the high coefficient of determination ($R^2 > 0.99$). All flow rates had significant correlations between nicotine concentration emitted and puff duration, with p-values less than 0.05.
Figure 18: Refillable EC – Halo Relationship between Puff Volume (mL) and nicotine concentration (mg emitted per puff).

With the Halo device, nicotine concentration (in mg emitted per puff) had strong positive linear relationships with puff duration. Each data point is based on the average of ten trials of four different puff durations (1.8, 3, 4.5 and 7 seconds). All flow rates showed similar slopes. The data collected at all flow rates (10, 30 and 50 mL/s) have significant correlations between nicotine emission concentration and puff duration, with p-value of 0.003, 0.003 and 0.02, respectively.
Figure 19: Refillable EC – Halo Relationship between Puff Duration (seconds) and Nicotine Concentration (mg emitted per puff).

In Figure 20, a couple of correlations between nicotine (mg/mL emitted per puff) and puff volume were not strong due to low coefficients of determination for the Halo product. For the data at a flow rate of 10 mL/s, the coefficient of determination was 0.85, indicating a fairly strong relationship. The data at a flow rate of 10 mL/s had a higher nicotine concentration (mg/mL) initially, with a steep slope, compared to the other two flow rates (30 and 50 mL/s).
In Figure 21, the data points are based on nicotine emission from an average of ten trials for different puff duration, which ranged from 1.8 to 9 seconds, for the Halo device. There was one strong positive linear correlation ($R^2 = 0.90$) between nicotine emission concentration (mg/mL) and puff duration- that at a flow rate of 10 mL/s. There was a weak, positive linear relationship between puff duration and nicotine concentration (mg/mL) for the medium flow rate (30 mL/s) and fairly weak for the 50 mL/s flow rate for the studies relating to the Halo device. The flow rate of 10 mL/s had significant correlations between puff duration and nicotine concentration with a p-value of 0.048. The data at flow rates of 30 and 50 mL/s did not show a significant relationship, with p-values of 0.28 and 0.14, respectively.
Figure 21: Refillable EC – Halo Relationship between Puff Duration (seconds) and Nicotine Concentration (mg/mL emitted per puff).

Figure 22 reveals a logarithmic relationship between nicotine concentration (in mg/second) and puff volume (mL) for the Halo device. The slope of the data from all flow rates were positive, but not as strong as other model, as indicated by the lower R² values. The data at a flow rate of 10 mL/s showed the shallowest slope and the data points were very close to each other since the puff volume only went up to 100 mL. The other two flow rates of 30 and 50 mL/s went above 250 mL of puff volume. All flow rates barely emit over 0.04 mg/s of nicotine at the highest puff volume.
Figure 22: Refillable EC – Halo Relationship between Puff Volume (mL) and Nicotine Concentration (mg/s emitted).

With the Halo device, the relationship between mg/second of nicotine emitted and puff duration were fairly strong when it had flow rates of 10 and 50 mL/s (Figure 22). The relationship became weaker as the flow rate increased to 30 mL/s. Higher flow rates with longer puff durations showed increased nicotine concentrations. The flow rate of 50 mL/s emitted higher mg/s of nicotine to above 0.04 compared to flow rates of 10 and 30 mL/s, which were below 0.4 mg/s nicotine. The flow rate of 10 mL/s had a significant correlation between puff duration and nicotine concentration with a p-value of 0.048. The data at flow rates of 30 and 50 mL/s have insignificant correlations in regard to the relationship of nicotine emission concentration and puff duration, with p-values of 0.28 and 0.14, respectively.
Conclusion/Future Directions

Our results demonstrate that gravimetric measurements of the emissions studies (both in weight changes measured on EC devices and filter pads throughout the puffing process) correlate well with their corresponding nicotine concentrations measured using our GC-MS method. This was indicated by strong and significant relationships between nicotine concentration from emissions on filter pads and weight change (gravimetric) with the coefficient of determination at 0.95 (Figures 3 and 4). In essence, the gravimetric method can act as an approximation for showing relationships of nicotine emissions as a function of varying machine puffing parameters. This makes sense
because it assumed that nicotine will be a fairly consistent subset of the total aerosol deposition during emissions studies. As stated in prior chapters, more data on the matrix effects of e-liquid solutions and nicotine partitioning is needed to fully understand nicotine emissions in ECs. As of now, the best metric for nicotine concentration determinations is through direct measurements using GC-MS or other analytical methods.

This study represents a preliminary and descriptive attempt at observing nicotine emissions using varying machine puffing regimes/parameters. This first study looks at three refillable EC products, all with the same e-liquid solutions, puffed at a set of strategically chosen puffing machine settings. Linear, logarithmic, and second-order polynomial fits were superficially explored to see which function best explained (by comparing R2 and p-values for the different fits) the data series for nicotine emission concentrations versus different puffing machine parameters best. Future work will examine the different scientific/engineering rationale that might cause some of these fits to either be linear, logarithmic, or polynomial.

Based on all three EC devices used in this study, iClear X.I, iClear30 and Halo, the trends in the correlations between nicotine concentration and puff volume show that data collected at a flow rate of 10 mL/s tended to have a very steep slope with low puff volume and high nicotine concentration. Data collected at flow rates of 30 and 50 mL/s tended to behave similarly when it comes to the correlation between nicotine emission concentration and puff duration. Each refillable EC device differed slightly (or sometimes more substantially) in regard to its nicotine emissions with varying puffing parameters. These results could bridge to an understanding of how the different puffing topographies of EC users could impact the nicotine levels that are delivered to their bodies.
Some of the differences in the emissions results might be attributable to the design and function of the different refillable EC devices. The iClear X.I product has a bottom coil clearomizer, double coil, 3 mL capacity tank, and is mounted on an itaste MVP 2.0 controller with 2600 mAh battery. The iClear 30 product has a top coil clearomizer, double coil, 3 mL capacity tank, and is mounted on an itaste MVP 2.0 controller with 2600 mAh battery. The Halo product has a top coil clearomizer, single coil, 2.4 mL capacity tank, and is mounted on a 650 mAh battery and controller.

In conclusion, ECs seemed to be more likely to deliver higher nicotine concentrations when the consumer takes a longer puff and higher puff volume, though the equations that describe these two relationships take different forms and vary between the different devices tested. EC emission are produce in part by electric heat, so it requires time to heat up and give out nicotine in the emissions, which may account for the increased concentrations. This study is preliminary and more work will be done to get at the exact impact of the different puffing parameters. The next step of this study will be measuring real-world user puffing topographies to test for nicotine concentrations in EC emissions. Our laboratory has already begun to measure real-world puffing topographies of consumers and collected emissions at those topographies to assess the role of individual topographies on nicotine delivery.

References

STUDY 3: METHOD VALIDATION AND DETERMINATION OF FIGURES OF MERIT

Introduction

Our goal in developing a GC-MS standard method was to establish a protocol that is sensitive, accurate, precise, and relatively fast and easy to run. Part of this process entailed finding the optimal instrumental settings; including injection and oven temperature programs, carrier gas flow rates, and MS detector settings, for the optimal determination of nicotine in ECs (both in e-liquids and emissions to filter pads). We also wanted to validate the developed procedure by determining its figures of merit including: Limit of Detection (LoD), Limit of Quantitation (LoQ), accuracy (as % recovery), and precision.

The methods for determining the LoD and LoQ for this procedure were modified from several studies (Armbruster, Tillman, and Hubbs 1994; Conference et al. 2005; Needleman and Romberg 1990; Underwood, Kananen, and Armitage 1997). Briefly, we chose the validation procedure whereby we measured 10 replicates of low concentration nicotine samples and calculated the standard deviation of these responses (Armbruster, Tillman, and Hubbs 1994; Conference et al. 2005). The LoD was determined as 3.3 times the standard deviation of the response divided by the slope of calibration curve of the analyte (Conference et al. 2005). Likewise, LoQ was determined as 10 times the standard deviation of the response divided by the slope of calibration curve of the analyte (Conference et al. 2005). We further determined the LoD and LoQ based on the Signal-to-Noise (S:N) response of the detector using the analyte concentration that yielded S:N values of 3.3 and 10, respectively (Conference et al. 2005).
The accuracy and precision of this procedure was determined by analysis of a minimum of five determinations per concentration level of the analyte (Shah et al. 1991). The accuracy and % recovery were calculated by three replicates of a minimum of 3 concentrations (representing low, intermediate, and high concentrations within our working range)) that cover the entire range of our nicotine standard concentrations (Conference et al. 2005). We used methods modified from prior studies (Tarrant, Mills, and Williard 2009; Zha, Qian, and Moldoveanu 2002) for use on our ECs. Sufficient numbers of trials and standards were used to maximize the power of the testing.

Materials and Methods

GC-MS settings

The instrument and column used in this study are described in Pagano et al. (2015). The software that controlled the GC-MS is Perkin Elmer TurboMass (Version 5.4.2.1617). We used an autosampler with a 5uL syringe attached to the GC. The injection volume was set to 1.0 μL with a ‘normal’ injection speed. The pre-injection solvent and sample washes were set to two washes each. The oven was set at 60 °C initially and increased 20 degrees per minute to 200 °C and then held at 200 °C for 3 minutes (hereafter referred to as “GC Method A”). The total run time for this method was about 10 minutes. The GC column was 30m long with an inner diameter of 250 μm (with vacuum compensation turned on). The carrier split control was set at a 50:1 ratio. The oven split control was set at a flow rate of 50 mL/min

The MS detector was set up with two separate settings for selected ion recording (SIR) mode for nicotine and quinoline. A solvent delay was set to the first four minutes. Three nicotine SIR ion masses were set on 84 mass to charge ratio (m/z) (confirmation,
dwell time of 0.10 seconds), 133 m/z (quantification, dwell time of 0.10 seconds), and 162 m/z (confirmation, dwell time of 0.35 seconds); similar to Gowadia, Oldham, and Dunn-Rankin 2009; Stanfill et al. 2009). Three quinoline SIR ion masses were also set at 102 m/z (quantification, dwell time of 0.10 seconds), 129 m/z (confirmation, dwell time of 0.10 seconds), and 161 m/z (confirmation, dwell time of 0.35 seconds); similar to Stanfill et al. 2009). To quantify nicotine in samples and standards, we used the ratios of the chromatographic peak integrations of m/z=133 ion relative to the m/z102 ion.

To improve our early method, we changed GC-MS settings to higher initial temperature of 80 °C, quicker ramp rate of 25 °C per minute up to 245 °C and a hold at 245 °C for 4.50 minutes. This modified procedure will hereafter be referred to as “GC Method B”.

Results

Using our GC-MS method, we selected a sample with a low known nicotine concentration (0.001 mg/mL), ran ten replicates, and then calculated the standard deviation of the responses. Using the computed standard deviation, we also used the slope from the nicotine calibration curve to calculate the LoD and LoQ for our method according to the definitions above. Results show that our GC-MS method has a LoD and LoQ for nicotine of about 200 ng/mL and 600 ng/mL, respectively. Data used to make these determinations are shown in Table 3.

Table 3: LOD/LOQ of Nicotine in Electronic Cigarettes in GC-MS
<table>
<thead>
<tr>
<th>Trial</th>
<th>Nicotine Concentration (mg/mL)</th>
<th>Peak Area Ratio ($m/z_{133} / m/z_{102}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.135E-03</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>8.711E-04</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>8.973E-04</td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
<td>9.027E-04</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>9.073E-04</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>8.844E-04</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>8.019E-04</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>9.371E-04</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>7.801E-04</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>8.088E-04</td>
</tr>
</tbody>
</table>

**Standard Deviation**: 9.960E-05

**Slope from Calibration Curve**: 1.611

<table>
<thead>
<tr>
<th>LoD ($\mu$g/mL)</th>
<th>0.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoQ ($\mu$g/mL)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

The accuracy of the method was assessed analyzing direct instrument-injected nicotine standard solutions and comparing them to the sample solutions after they have been run through our extraction process. We used three different concentrations that covered low, medium and high nicotine concentrations (0.05, 0.3 and 0.8 mg/mL, respectively) of our studies and each run with three replicates for both the straight instrument-injected nicotine standards and their corresponding solutions after they have been taken through our extraction process. Results are reported as percent recovery. Our mean accuracy (as percent recovery) was about 97%, validating that our method did well to recover our analyte. The date that was used to make the accuracy determinations is reported in Table 4.

**Table 4: Accuracy (% Recovery) of the Method**
To assess the precision of our method, we ran six replicates of the same 1.0 mg/mL nicotine standard and calculated the average and standard deviation of the replicate responses. The precision was determined based on the relative standard deviation, which we computed to be 2.51%. The date that went into this determination is shown in Table 5.

<table>
<thead>
<tr>
<th>Nicotine Concentration (mg/mL)</th>
<th>Trial 1 (S.D.)</th>
<th>Trial 2 (S.D.)</th>
<th>Trial 3 (S.D.)</th>
<th>Average % Recovery (S.D.)</th>
<th>Overall Average % Recovery (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>93.69 (3.56)</td>
<td>100.88 (0.58)</td>
<td>91.87 (4.54)</td>
<td>97.80 (3.56)</td>
<td>97.25 (0.58)</td>
</tr>
<tr>
<td>0.30</td>
<td>99.87 (4.54)</td>
<td>91.86 (0.58)</td>
<td>101.33 (4.54)</td>
<td>96.65 (4.54)</td>
<td></td>
</tr>
<tr>
<td>0.80</td>
<td>99.84 (4.89)</td>
<td>97.22 (0.58)</td>
<td>98.74 (0.58)</td>
<td>97.31 (4.89)</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Precision (%) of the Method

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Nicotine Concentration (mg/mL)</th>
<th>m/z_{133}/m/z_{102}</th>
<th>y-intercept</th>
<th>slope</th>
<th>Nicotine Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0000</td>
<td>1.4968</td>
<td>-0.0067</td>
<td>1.6113</td>
<td>0.9331</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1.5578</td>
<td>-0.0067</td>
<td>1.6113</td>
<td>0.9709</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1.5852</td>
<td>-0.0067</td>
<td>1.6113</td>
<td>0.9879</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>1.5182</td>
<td>-0.0067</td>
<td>1.6113</td>
<td>0.9464</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>1.4934</td>
<td>-0.0067</td>
<td>1.6113</td>
<td>0.9310</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>1.4963</td>
<td>-0.0067</td>
<td>1.6113</td>
<td>0.9328</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>0.9504</td>
</tr>
<tr>
<td></td>
<td>St. dev.</td>
<td></td>
<td></td>
<td></td>
<td>0.0238</td>
</tr>
<tr>
<td></td>
<td>Precision (%)</td>
<td></td>
<td></td>
<td></td>
<td>2.51</td>
</tr>
</tbody>
</table>

Again, we wanted to optimize our original GC-MS method (Method A). Figure 24 shows a calibration curve using optimized Method B, created with same nicotine standards used throughout for Method A. The coefficient of determination for the optimized method was also very strong ($R^2 > 0.9999$). This calibration curve also has $p < 0.001$, supporting its significance.
Figure 24: Nicotine Calibration Curve for E-Liquids with Improved GC-MS Method (Method B).

Discussion

GC-MS Method A has a precision of about 2.5% in the quantification of nicotine. Our method also has a LoD of 0.2 µg/mL and a LoQ of 0.6 µg/mL Nicotine. In comparison to the method (involving GC-TSD) used by Goniewicz et al., they report a LoQ of 0.05 mg/mL. It is expected that a GC-MS method run in selected ion monitoring mode would have greater sensitivity than an GC-TSD method. However, GC-TSD appears to be well within the range of sensitivity needed for similar EC studies and can be more cost-effective. The real utility of GC-MS in selected ion monitoring mode can be seen in its improved selectivity- where other compounds present in the samples, like flavorants, do not interfere with the chromatographic signals for nicotine. Our developed method can detect much lower nicotine concentration accurately than most of studies in range of 20 to 50,000 ng/mL (Cameron et al. 2014; Goniewicz et al. 2013; Trehy et al. 2011). Another study report a method with a LoD of 0.01 – 0.03 µg/mL for certain
nicotine-related substances in refill solutions, which is similar to (if not a bit more sensitive than) our results (Etter, Zäther, and Svensson 2013). Cobb et al. studied nicotine in EC cartridges, and they report their LoD to be between 3.23 and 4.07 mg/cartridge (Cheng 2014).

Our Method A has a 97.25% recovery in measurements of nicotine as a way to assess its accuracy, which is fairly close to the average % recovery determined in other studies (which range from 80 to 102% (Cheah et al. 2012; Goniewicz et al. 2013) and also one measuring polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke average (% recovery of 98.6% reported) (Tarrant et al. 2009). Our GC-MS method seems to be reliable and sensitive, with good precision and accuracy (a relatively high % recovery).

GC-MS Method B was slightly modified from the original method to optimize the results and functionality of the instrument. Nicotine and quinolone were processed through the column quicker (shorter retention time). The improved method also allowed for longer times at higher oven temperatures to ensure that all compounds from the ECs were removed from the column at the end of each injection. This helped to improve instrument performance and lengthen the lifetime of the GC column and MS filaments.

It is important to address the use of the Cambridge filter pads in collecting the EC emissions throughout our studies. Most EC nicotine studies to date have collected the emissions in solvent-filled impingers, which can be cumbersome, require copious amounts of volatile solvents, and produce extra organic waste. We believe that the filter pad method is collecting much of the nicotine (Pagano et al., 2015). The filter pad method ultimately measures emissions that were captured in the liquid phase, but could underestimate nicotine emissions if some is in the gas-phase form (Pagano et al., 2015).
More research on the liquid-gas partitioning of EC emissions is greatly needed in the field and could give support to the use of filter pads or impingers in measuring EC emission, and specifically, nicotine content (Pagano et al., 2015). Better understanding of the pKa of nicotine in the e-liquid matrix and the temperatures of the emissions at different points in the EC devices (and as they leave the EC) would be of great benefit to the field (Pagano et al., 2015).

**Conclusion**

In this study, we were able to validate our GC-MS method. Due to its strong sensitivity and selectivity (avoids interferences from other molecules in the sample matrix), GC-MS with selected ion monitoring is a strong method for the quantification of nicotine in EC e-liquids and emissions. Further, based on the comparison of the figures of merit to other methods (summarized in Cheng 2014), our method also appeared to function well. We believe that our procedure could be used as a standard method for the determination of nicotine in the e-liquids and emissions of ECs.

**References**


THESIS CONCLUSION

Many new EC products are being introduced to the market without any form of regulation in the United States. Perhaps due in part to the lack of regulation or quality control, this work revealed that consumers could get either more or less nicotine from disposable ECs than the manufacturers’ labeling. Machine puffing parameters played important roles in delivering different amounts of measured nicotine in refillable ECs. Inasmuch as consumers puff ECs differently, the issue of puff topography needs to be taken into serious consideration by regulating agencies in studying the health impact of ECs. Future research should analyze many more products (as so few of the plethora of product entering the market daily have been analyzed) and other contaminants, such as heavy metals and added flavorants, as well as the size of emitted particle, should among the foci of future research in the field. Our laboratory has already begun work on measuring real-world user puffing topographies, and using the obtained topographies/patterns to test emissions qualitatively and quantitatively. Since our work has validated that our GC-MS method for nicotine quantitation in e-liquids and EC emissions is robust, reliable, accurate, and precise, we believe that the method could be adopted by other researchers in the field.