Analytical evaluations of electronic cigarette e-liquids and aerosols for harmful and potentially harmful constituents

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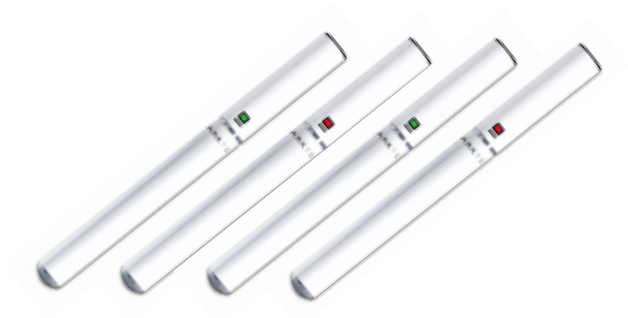
Presentation Overview

- Our initial analysis of e-cigarette e-liquids and aerosols
  - Devices with rechargeable batteries and disposable cartridges
- The development of methods specific to e-cigarette analysis
  - Carbonyls
  - Nicotine-Related Impurities and Degradation Products
  - Combustion-Related Compounds
- Constituents testing recommended by the US FDA for Electronic Nicotine Delivery Systems (ENDS)

Characterization of E-Cigarette Formulations and Aerosols

Initial Work, ~2014

- No Harmful or Potentially Harmful Constituent (HPHC) list specific to e-vapor products existed
- We used the cigarette filler and smoke HPHC abbreviated list published by the US FDA (2012)
- We also explored other potential e-cigarette impurities such as nicotine-related impurities and degradation products
- We investigated the 4 commercial NuMark LLC products, MarkTen®



Characterization of E-Cigarette Formulations and Aerosols

Formulations

Chemical class	Analyte
Metals*	Arsenic
	Cadmium
Tobacco specific nitrosamines (TSNAs)*	NNK
	NNN
Ammonia*	Ammonia
Nicotine-related impurities	Nicotine-N-oxides
	Cotinine
	Nornicotine
	Anatabine
	Myosmine
	Anabasine
	β-Nicotyrine

Aerosols

Chemical class	Analyte
Carbonyls*	Acetaldehyde
	Acrolein
	Crotonaldehyde
	Formaldehyde
Aromatic amines*	4-Aminobiphenyl
	1-Aminonaphthalene
	2-Aminonaphthalene
Volatile organic compounds (VOCs)*	Acrylonitrile
	Benzene
	1,3-Butadiene
	Isoprene
	Toluene
Tobacco specific nitrosamines (TSNAs) *	NNK
	NNN
Ammonia*	Ammonia
Polyaromatic hydrocarbons (PAHs)*	Benzo[a]pyrene
Carbon monoxide*	Carbon monoxide

*"Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke Under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act" (Guidance for the Industry, March 2012).

NNN = N-nitrosonornicotine
NNK = 4- (methylnitrosamino)-1-(3-pyridyl)-1-butanone

Initial Constituent Testing

- Most methods employed were adapted from traditional tobacco and cigarette methods developed and standardized by CORESTA
- CORESTA – Cooperation Centre for Scientific Research Relative to Tobacco
  - Association founded in 1956
  - Vision: “To be recognized by our members and relevant external bodies as an authoritative source of publically available, credible science and best practices related to tobacco and its derived products”
  - CORESTA Recommended Methods (CRMs) are available on CORESTA.org
- If standardized methods were not available, they were adapted from in-house tobacco and smoke methods

Aerosol Collection for E-Vapor Products

- Current standardized tobacco cigarette puffing regimes

Condition	Puff Volume (mL)	Duration (seconds)	Approx. Puff Count	Interval (seconds)	Ventilation blocking %	Puff Profile
ISO	35	2	5 – 10 / cig	60	0	Sine wave
HC	55	2	6 – 14 / cig	30	100	Sine wave

ISO = International Organization for Standardization
HC = Health Canada (or Canadian Intense, CI)

Aerosol Collection for E-Vapor Products

- Current standardized tobacco cigarette puffing regimes

Condition	Puff Volume (mL)	Duration (seconds)	Approx. Puff Count	Interval (seconds)	Ventilation blocking %	Puff Profile
ISO	35	2	5 – 10 / cig	60	0	Sine wave
HC	55	2	6 – 14 / cig	30	100	Sine wave
E-Cig	55	4	Analyte Specific*	30	NA	Square Wave

ISO = International Organization for Standardization
HC = Health Canada (or Canadian Intense, CI)

- For e-cigarette aerosol collection, we modified the HC regime

Cerulean 20-port linear

KC Automation 5-port linear

* Battery Exhaustion (up to ~100 puffs) except for Carbonyls (20 puffs) and CO (50 puffs)

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Summary: Published in Regulatory Toxicology and Pharmacology 2016 (online 2015)

<u>Formulation (n=3)</u>

- TSNA (NNK) – BLOQ
- Nicotine-Related Impurities – ND to 19 ug/g

<u>Aerosol (n=3)</u>

- Carbonyls
  - Acetaldehyde – BLOQ
  - Formaldehyde – 0.1 to 0.3 ug/puff
- TSNAs – BLOQ
- Ammonia - BLOQ


Regulatory Toxicology and Pharmacology 74 (2016) 1–11

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/yrtph

Characterization of potential impurities and degradation products in electronic cigarette formulations and aerosols

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BLOQ = below the limit of quantitation
ND = not detected
TSNA = Tobacco specific nitrosamines
NNK = 4- (methylnitrosamino)-1-(3-pyridyl)-1-butanone

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Summary: Published in Regulatory Toxicology and Pharmacology 2016 (online 2015)

- Most constituents investigated in this study were not detectable or were well below LOQs in the commercial MarkTen® products used in this study

- Standardized aerosol collection techniques and standardized analytical methodologies specific to e-cigarettes are urgently needed


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ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 18 November 2015
Accepted 19 November 2015
Available online 30 November 2015

Keywords:
E-cigarettes
E-vapor
Harmful and potentially harmful constituents (HPHC)
Formulation
Aerosol

ABSTRACT

E-cigarettes are gaining popularity in the U.S. as well as in other global markets. Currently, limited published analytical data characterizing e-cigarette formulations (e-liquids) and aerosols exist. While FDA has not published a harmful and potentially harmful constituent (HPHC) list for e-cigarettes, the HPHC list for currently regulated tobacco products may be useful to analytically characterize e-cigarette aerosols. For example, most e-cigarette formulations contain propylene glycol and glycerin, which may produce aldehydes when heated. In addition, nicotine-related chemicals have been previously reported as potential e-cigarette formulation impurities. This study determined e-liquid formulation impurities and potentially harmful chemicals in aerosols of select commercial MarkTen® e-cigarettes manufactured by NuMark LLC. The potential hazard of the identified formulation impurities and aerosol chemicals was also estimated. E-cigarettes were machine puffed (4-s duration, 55-mL volume, 30-s intervals) to battery exhaustion to maximize aerosol collection. Aerosols analyzed for carbonyls were collected in 20-puff increments to account for analyte instability. Tobacco specific nitrosamines were measured at levels observed in pharmaceutical grade nicotine. Nicotine-related impurities in the e-cigarette formulations were below the identification and qualification thresholds proposed in ICH Guideline Q3B(R2). Levels of potentially harmful chemicals detected in the aerosols were determined to be below published occupational exposure limits.

1. Introduction

The U.S. Food and Drug Administration (FDA) recently established a list of harmful and potentially harmful constituents (HPHCs) for currently regulated tobacco products (e.g., cigarettes) (FDA, 2012a). FDA guidance defined harmful and potentially harmful constituents as "any chemical or chemical compound in a tobacco product or in tobacco smoke: a) that is or potentially is inhaled, ingested, or absorbed into the body; and b) that causes or has the potential to cause direct or indirect harm to users or non-users of tobacco products" (FDA, 2011). Subsequently, a list of more than 90 HPHCs was communicated (FDA, 2012a).

However, there is a lack of in-house laboratory capabilities, a potential for large and unmanageable testing volumes in contract laboratories, and a lack of validated analytical test methods for all of the HPHCs listed on the established list (FDA, 2012a). Therefore, FDA published an abbreviated HPHC list for initial regulatory reporting requirements (FDA, 2012b). The abbreviated list comprises constituents for which analytical methods were thought to be established and which represent several different chemical classes. Table 1 shows the abbreviated list of HPHCs that are initially required by FDA to be reported for cigarette smoke and cigarette filler (i.e., tobacco that is a component of a cigarette).

FDA requires reporting the quantities of all HPHCs on the abbreviated list for a regulated tobacco product. Currently, the regulated tobacco products that require HPHC reporting are cigarettes (smoke and tobacco filler), smokeless tobacco, and roll-your-own tobacco.

E-cigarettes, also referred to as electronic nicotine delivery systems or e-vapor products, are gaining popularity in the U.S and other global markets. FDA (2014) published a proposed deeming rule that would extend the agency's regulatory authority to include e-cigarettes; at this time in manuscript preparation (June 2015), the

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http://dx.doi.org/10.1016/j.yrtph.2015.11.009

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The Development of Methods Specific to E-Cigarette Analysis:

Carbonyls

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Background

- Low levels of thermal degradation products such as carbonyls have been reported in e-cigarette aerosols
  - Formaldehyde
  - Acetaldehyde
  - Acrolein

- Goniewicz, M. L., Knysak, J., Gawron, M., Kosmider, L. *et al. Tob Control* **2013**.
- Kosmider, L., Sobczak, A., Fik, M., Knysak, J., Zaciera, M., Kurek, J., Goniewicz, M.L., **2014**. Nicotine. Tob. Res 16, 1319-1326.
- Uchiyama, S., Ohta, K., Inaba, Y., Kunugita, N., **2013**. Anal. Sci 29, 1219-1222.
- Bekki, K., Uchiyama, S., Ohta, K., Inaba, Y., Nakagome, H., Kunugita, N., **2014**. Int. J. Envoron. Res. Public Health 11, 11192-11200.
- Cheng, T., **2014.** Tob. Control 23 Suppl 2, ii11-ii17.
- Ohta K., Uchiyama S., Inaba Y., Nakagome H., Kunugita N. Bunseki Kagaku. **2011**;60:791–797
- Flora, J.W., Meruva, N., Huang, C.B., Wilkinson, C.T. et al., **2016**, Reg Tox Pharm 74

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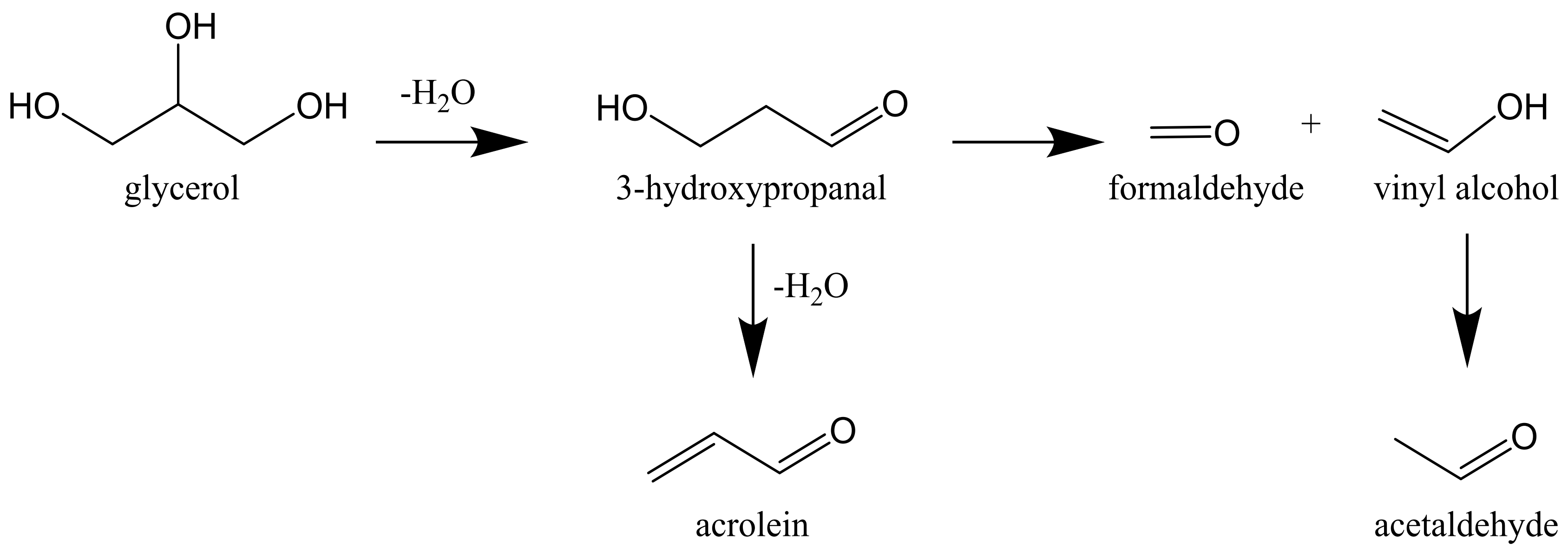
Carbonyls - Aerosol

- Glycerol and propylene glycol (PG) can form carbonyls under thermal conditions ((oxy)dehydration)*



*Uchiyama, S., Ohta, K., Inaba, Y., Kunugita, N., 2013. Determination of Carbonyl Compounds Generated from the E-cigarette Using Coupled Silica Cartridges Impregnated with Hydroquinone and 2,4-Dinitrophenylhydrazine, Followed by High-Performance Liquid Chromatography. *Analytical Sciences* 29, 1219-1222.
*Deleplanque, J., Dubois, J.L., Devaux, J.F., and Ueda, W., 2010. Production of acrolein and acrylic acid through dehydration and oxydehydration of glycerol with mixed oxide catalysts. *Catalysis Today* 157, 351-358.

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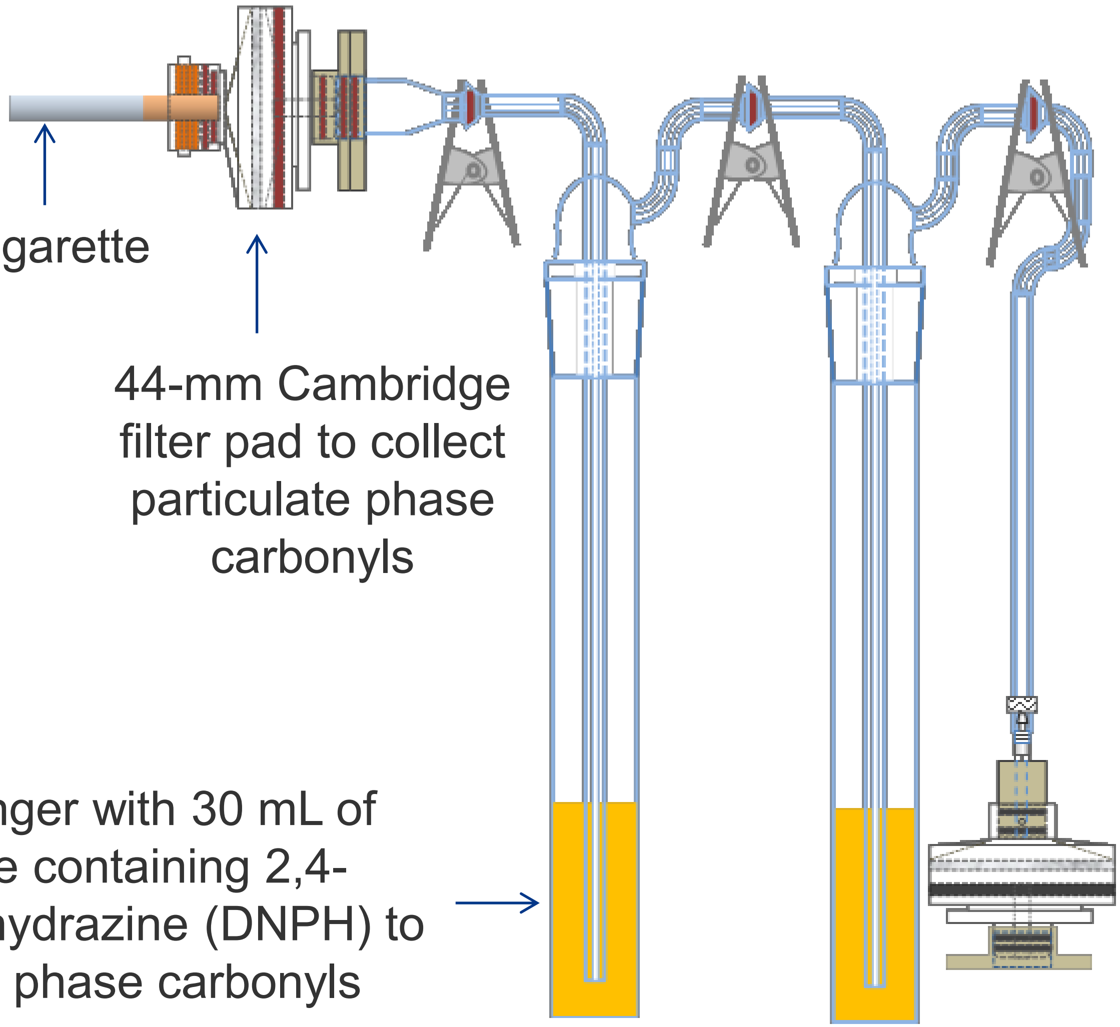
Trapping Efficiency Collection Configuration



E-cigarette

44-mm Cambridge filter pad to collect particulate phase carbonyls

Pad inside linear 5-port smoking machine (KC Automation)

Glass impinger with 30 mL of acetonitrile containing 2,4-dinitrophenylhydrazine (DNPH) to collect gas phase carbonyls

Puffing Regime: 55 mL Volume, 4 sec duration, 30 sec interval, square wave

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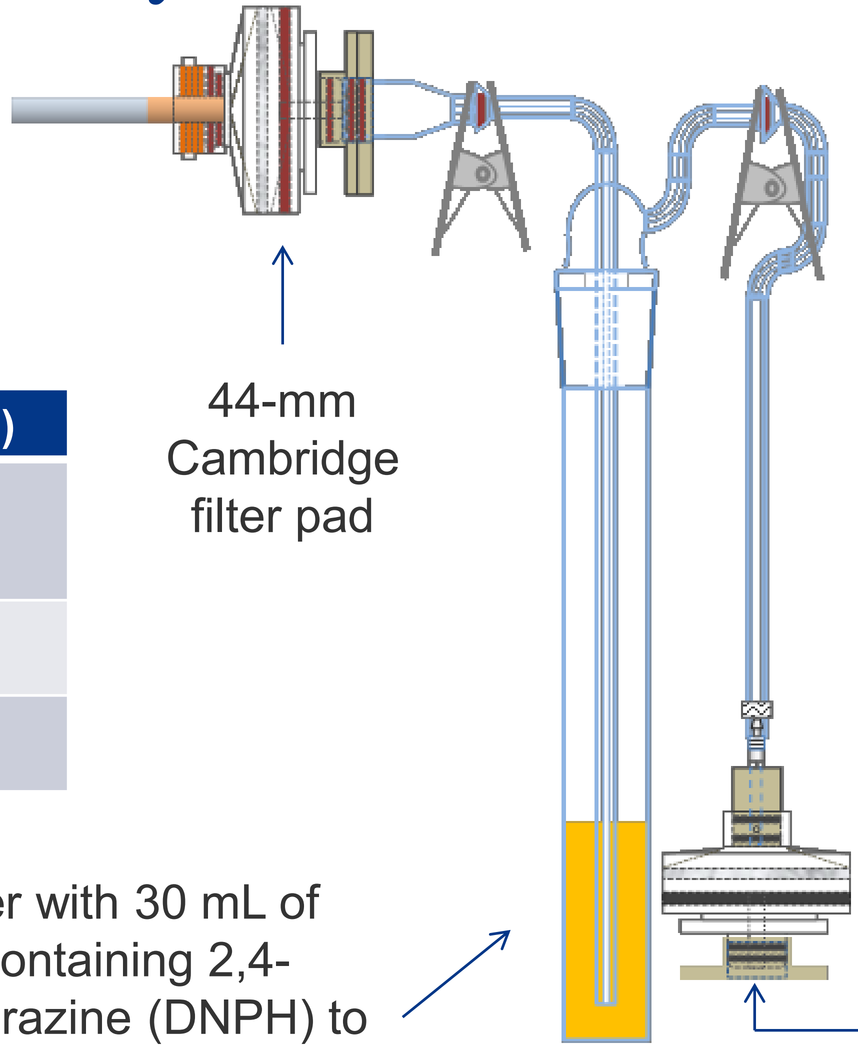
Optimized Carbonyl Collection



Trapping Efficiency of Formaldehyde

	Average (n=3)
Cambridge Filter Pad	71.8%
Impinger #1	28.2%
Impinger #2	0.0%

Puffing Regime: 55 mL Volume, 4 sec duration, 30 sec interval, square wave

Sample Preparation

- Following 20 puff collections (55 mL Volume, 4 sec duration, 30 sec interval, square wave)
  - Remove Cambridge filter pad (CFP) from its holder and wipe holder with the pad
  - Insert CFP into the DNPH trapping solution within the impinger and vortexed for 5 seconds
  - Transfer 1 mL of aerosol extract to an amber auto sampler vial containing internal standard working solution and pyridine

Carbonyl	Internal Standard
Formaldehyde	Formaldehyde – d_2
Acetaldehyde	Acetaldehyde-d_3
Acrolein	Acetaldehyde-d_3
Crotonaldehyde	Acetaldehyde-d_3

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Data Collection

- Ultra Performance Liquid Chromatography (UPLC) with Mass Spectrometry (MS) detection – 4 minute run!
- Calibration range was 0.0107 µg/mL to 4.00 µg/mL or 0.016 µg/puff to 6.30 µg/puff based on a 20-puff collection
- LOQ was 0.0107 µg/mL or 0.016 µg/puff
- LOD was 0.002 µg/mL or 0.003 µg/puff

Considerably more sensitive than a standard cigarette smoke method!

- Fully validated based upon the 2005 International Conference on Harmonisation (ICH) guideline "Validation of Analytical Procedures: Text and Methodology Q2(R1)"

Method is "Fit-for-Purpose"

- Six commercial e-cigarettes (rechargeable batteries with disposable cartridges) were evaluated (n=5)

	Formaldehyde	Acetaldehyde	Acrolein	Crotonaldehyde
	µg/puff	µg/puff	µg/puff	µg/puff
Product A	0.19 to 14.1	0.05 to 13.61	<LOQ to 4.11	<LOD to 0.04
Product B	0.12 to 3.13	0.05 to 1.67	<LOQ to 0.69	<LOD to <LOQ
Product C	0.21 to 0.65	0.14 to 0.51	0.15 to 0.61	<LOD to <LOQ
Product D	0.10 to 0.22	0.29 to 0.51	0.03 to 0.10	<LOD to <LOQ
MarkTen® Classic	0.14 to 0.18	0.04 to 0.06	<LOQ to 0.02	<LOD
MarkTen® Menthol	0.07 to 0.14	0.03 to 0.06	<LOQ to 0.01	<LOD

Products selected based upon major percentage of convenience store sales (Wells Fargo Equity Research, 2014)

Summary: Published in the Journal of Chromatographic Sciences 2017 (online 2016)

- Cigarette smoke methodologies may not be sensitive enough to measure constituents in e-cigarette aerosols
- Methods specific to measuring constituents in e-vapor products are essential
- All commercial products tested (rechargeable batteries with disposable cartridges) in this study contained formaldehyde and acetaldehyde
- We also demonstrated with prototype devices that when e-cigarette heaters exceed ~350ºC, high levels of formaldehyde are observed

JCS

Journal of Chromatographic Science, 2017, Vol. 55, No. 2, 142–148
doi: 10.1093/chromsci/bmw157
Advance Access Publication Date: 7 October 2016
Article

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Article

Method for the Determination of Carbonyl Compounds in E-Cigarette Aerosols

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Received 1 April 2016; Revised 8 July 2016

Abstract

Low levels of thermal degradation products such as carbonyls (formaldehyde, acetaldehyde, acrolein, crotonaldehyde) have been reported in e-cigarette aerosols. The collection and analysis of e-cigarette aerosol carbonyls are often adapted from methods developed for tobacco cigarette smoke. These methodologies are often not sensitive enough to detect low carbonyl levels in e-cigarette aerosols. One objective of this work was to develop and validate a rapid, selective and sensitive ultra-performance liquid chromatography with mass spectrometry method optimized for analysis of carbonyls in e-cigarette aerosols. Aerosols were trapped in 20-puff collections, 4-s durations, 55-mL volumes, 30-s intervals, square wave puff profiles. Collection apparatus involved a linear smoking machine with Cambridge filter pad followed by a glass impinger containing acidified 2,4-dinitrophenylhydrazine. This method showed limits of quantitation and detection of 0.016 and 0.003 µg $puff^{-1}$, respectively, and run time of 4 min. Six e-cigarettes were evaluated (five devices each). All contained measurable levels of carbonyls. Levels were mostly well below those in conventional cigarettes. However, for some e-cigarettes, formaldehyde levels were above those for tobacco cigarettes (highest at 14.1 µg $puff^{-1}$). Temperatures related to carbonyl yields in e-cigarette aerosols were explored to better understand carbonyl formation: formation of formaldehyde is low at temperatures below 350°C.

Introduction

E-cigarettes, a type of e-vapor product, are battery-powered devices that contain a heating element and a cartridge filled with a liquid solution. The liquid solution in an e-cigarette, or e-liquid, typically contains tobacco-derived nicotine, water, propylene glycol, glycerin and flavors. The heating element vaporizes the e-liquid that then condenses into an aerosol. When inhaled by the user, the e-cigarette aerosol delivers flavor and nicotine. Because these products use tobacco-derived nicotine and do not burn tobacco, they do not generate or emit smoke; therefore, users inhale and exhale an aerosol and often describe it as "vaping".

Primarily, the aerosols generated from e-cigarettes are composed of fine particles of liquid and gas phases of the vaporized e-liquid. However, low levels of thermal degradation products such as carbonyls (e.g., formaldehyde, acetaldehyde, acrolein and crotonaldehyde) have been reported in e-cigarette aerosols (1–11). For example, in a study by Uchiyama *et al.* (2), 9 of the 13 commercial e-cigarette products tested showed detectable levels of carbonyls at variable levels. Goniewicz *et al.* (1) reported that, of the 15 e-cigarettes in their study, all products contained formaldehyde and acetaldehyde in the aerosols and acrolein was also detected in all but one (1).

It is well known that carbonyls are relatively unstable due to volatility, thermal instability and sensitivity to acidic environments (12). Therefore, it is necessary to derivatize carbonyls as they are collected and minimize the number of puffs per collection (e.g., ≤20 puffs per collection) to minimize analyte loss. Carbonyls are also typically present at trace levels in e-vapor product aerosols, which puts considerable demands on the sensitivity of the analytical methodologies used for their quantitation. Carbonyl concentrations in e-cigarette aerosols are often measured by adapting analytical

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The Development of Methods Specific to E-Cigarette Analysis:

Nicotine-Related Impurities and Degradation Products

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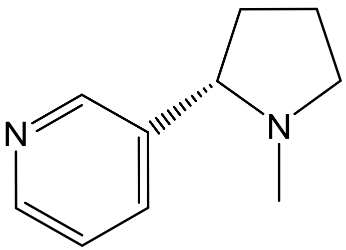
Example: Nicotine Impurities and Degradation Products

- The nicotine used in e-vapor products is extracted from tobacco, and the purity of the nicotine can vary depending upon manufacturer and grade (e.g., US Pharmacopeia grade)
- The US and European Pharmacopoeia make recommendations for the purity of nicotine intended for pharmaceutical products
- Recommendations are also made in the ICH Guidelines "Impurities in New Drug Products Q3B(R2)"*

Nicotine
3-[(2S)-1-methylpyrrolidin-2-yl]pyridine



**Q3B(R2): International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Impurities in New Drug Products. International Conference on Harmonisation. 2006.*

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Example: Nicotine Impurities and Degradation Products

- No official purity recommendation for the nicotine used in e-cigarettes has been made

- Only a few publications have evaluated the nicotine related impurities in e-cigarette fluids and none have evaluated these fluids during long-term storage

- Etter, J. F., Zather, E., Svensson, S., Analysis of refill liquids for electronic cigarettes. *Addiction* **2013**, 108 (9), 1671-1679.
- Trehy, M. L., Ye, W., Hadwiger, M. E., Moore, T. W. *et al.* Analysis of Electronic Cigarette Cartridges, Refill Solutions, and Smoke for Nicotine and Nicotine Related Impurities. *Journal of Liquid Chromatography & Related Technologies* **2011**, 34 (14), 1442-1459.
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- Westenberger, B., Evaluation of Johnson Creek Liquids for E-cigarette Fills. Washington, DC: US Food and Drug Administration, **2009**.
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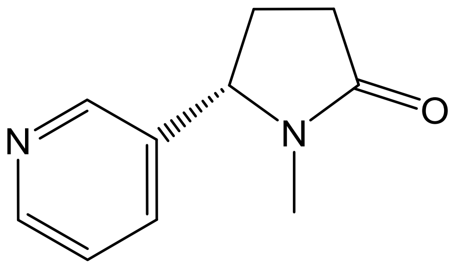
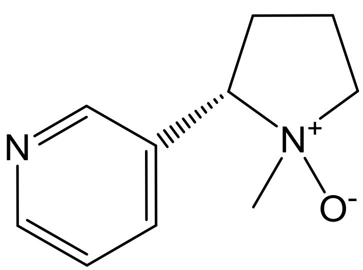
Specified Nicotine Impurities from European Pharmacopoeia (monograph 1452)

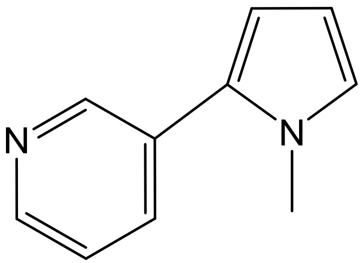
Cotinine
(5S)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one



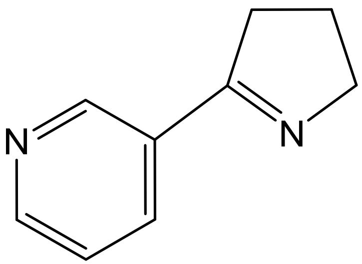
Nicotine-N'-Oxides
(1RS,2S)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (epimer at N+)



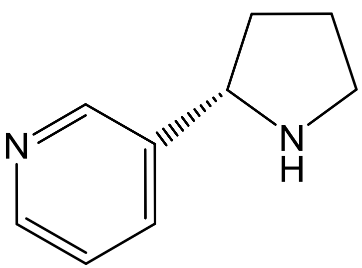
Beta-Nicotyrine
3-(1-methyl-1H-pyrrol-2-yl)pyridine



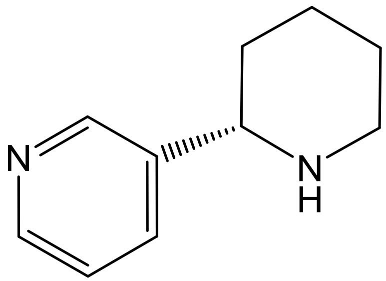
Myosmine
3-(4,5-dihydro-3H-pyrrol-2-yl)pyridine



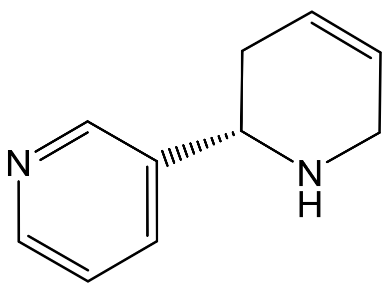
Nornicotine
3-[(2S)-pyrrolidin-2-yl]pyridine



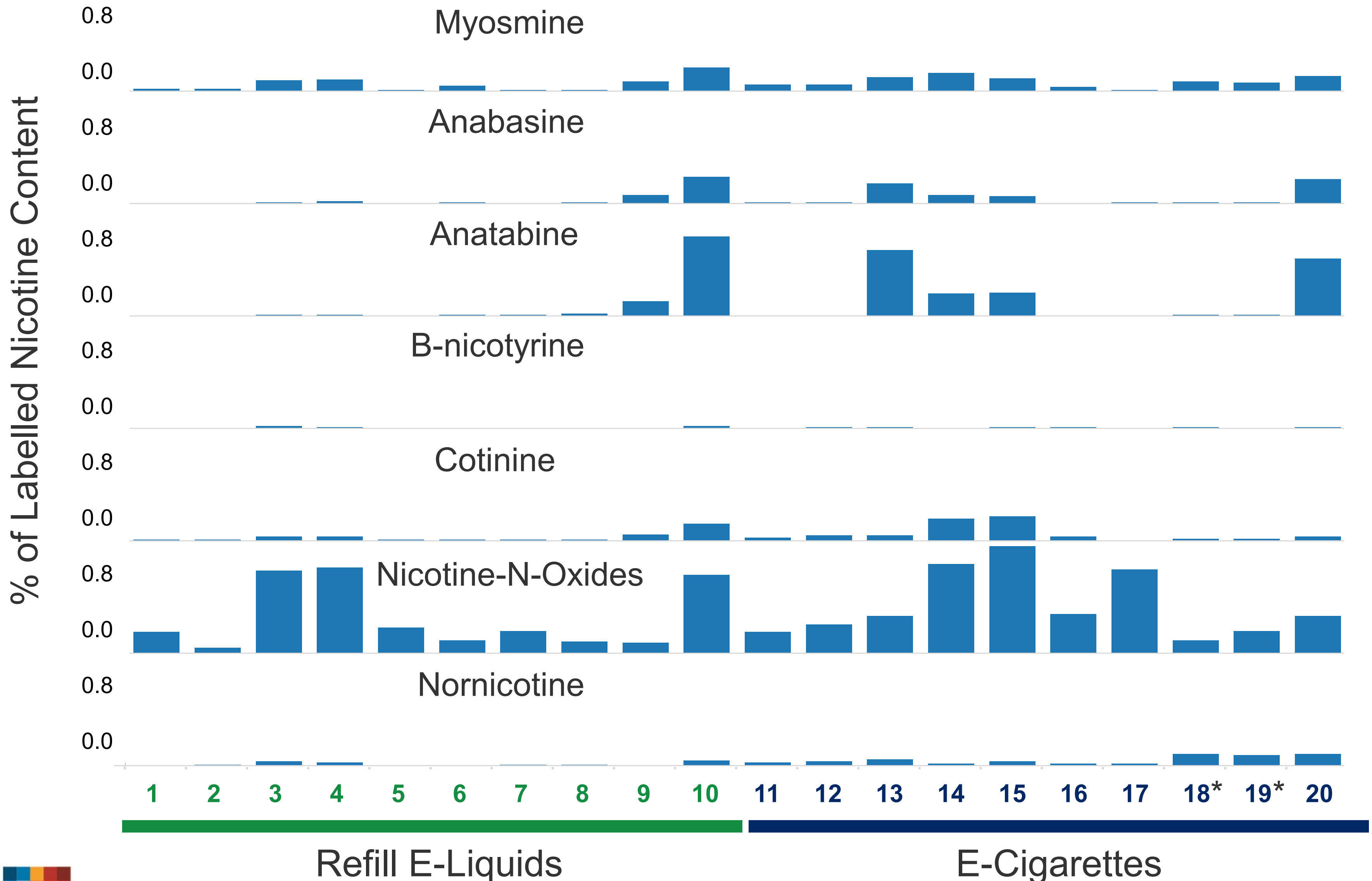
Anabasine
3-[(2S)-piperidin-2-yl]pyridine



Anatabine
(2S)-1,2,3,6-tetrahydro-2,3'-bipyridyl



Nicotine-Related Impurities (n=3)



*18 and 19 are MarkTen® e-cigarettes manufactured by NuMark LLC

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Nicotine-Related Impurities During Shelf-life

- To quantify changes in these impurities during long-term storage of commercial e-cigarettes

- MarkTen® XL e-cigarettes were evaluated during long-term storage (manufactured by NuMark LLC)

Storage Description	Storage Conditions
Long-term (Q1A(R2))*	25^0C ± 2^0C / 60% RH ± 5%

- Tested at 1, 2, 4 and 6 months

Classic Menthol

* ICH Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products

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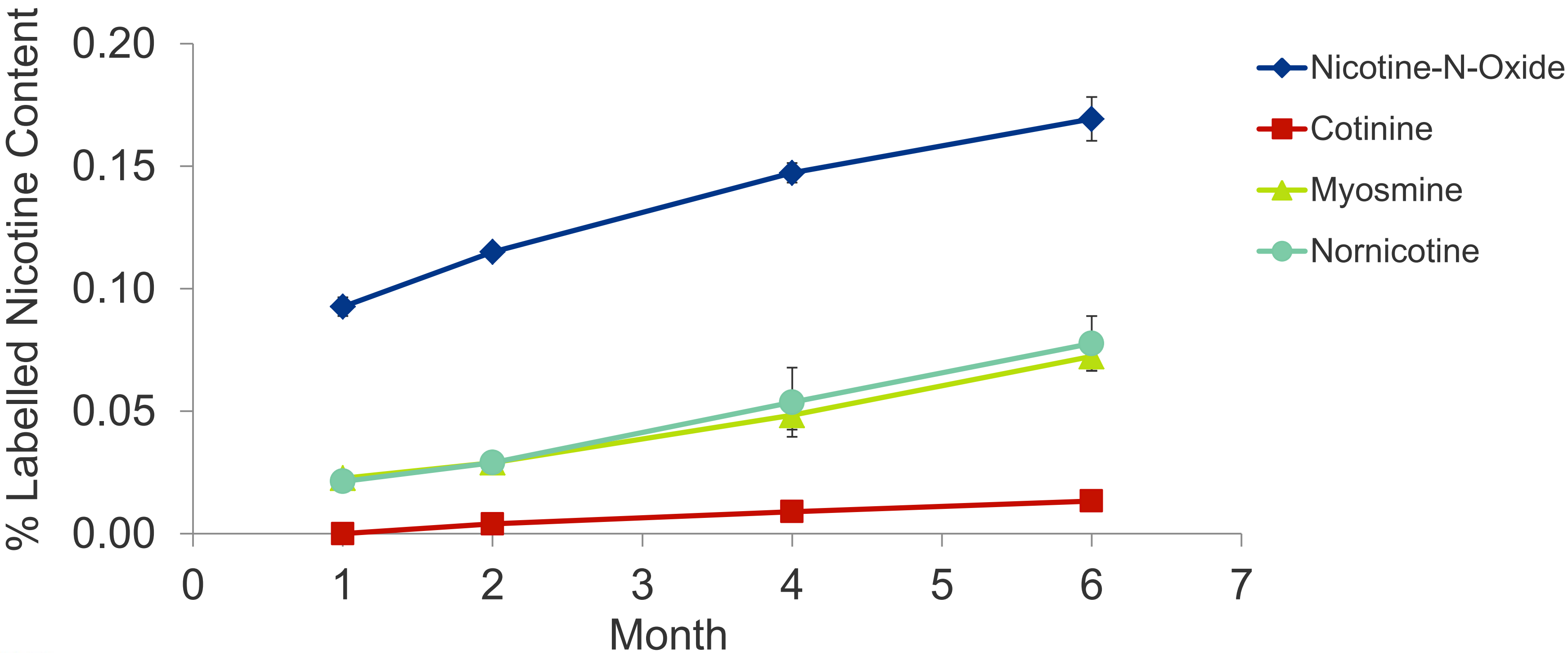
Nicotine-Related Impurities During Shelf-life (n=3)

- All specified impurities are <0.2% of the total nicotine concentration after 6 months at Long-Term conditions
- No measurable increase was observed for anabasine, β-nicotyrine, and anatabine



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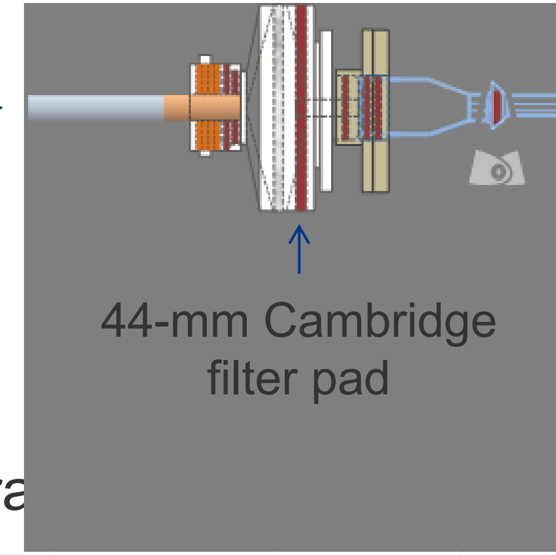
Nicotine-Related Impurities: Estimated Transfer Efficiency



- Estimate the % tra e aerosol (n=6)

Transfer % in aerosol	Myosmine	Nornicotine	Cotinine	Anabasine	Nicotine-N-Oxide	Anatabine	B-nicotyrine
MarkTen Classic	97	95	103	80	5.4	89	141
MarkTen Menthol	97	110	103	91	4.9	96	164

Non-validated method

- GC-MS analysis of nicotine-N-oxides showed primary thermal decomposition pathways were to nicotine and β-nicotyrine

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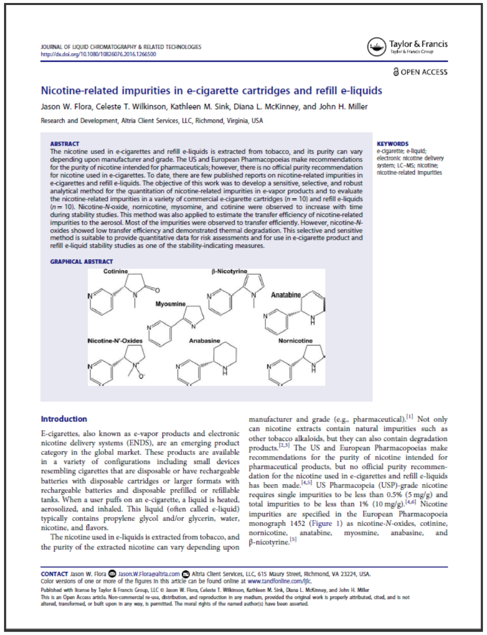
Summary: Published in the Journal of Liquid Chromatography and Related Technologies 2017

(online 2016)

- Shelf-life information on e-cigarettes and refill e-liquids should be established through rigorous stability testing measuring a variety of constituents appropriate for the products to ensure quality and safety
- Nicotine-N-oxides, nornicotine, mysomine, and cotinine have been observed to increase with respect to time during stability studies*
- Transfer efficiency of nicotine-N-oxides is low (<6%) due to thermal degradation during the aerosol formation process



JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
http://dx.doi.org/10.1080/10826076.2016.1266500

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Nicotine-related impurities in e-cigarette cartridges and refill e-liquids

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ABSTRACT

The nicotine used in e-cigarettes and refill e-liquids is extracted from tobacco, and its purity can vary depending upon manufacturer and grade. The US and European Pharmacopoeias make recommendations for the purity of nicotine intended for pharmaceuticals; however, there is no official purity recommendation for nicotine used in e-cigarettes. To date, there are few published reports on nicotine-related impurities in e-cigarettes and refill e-liquids. The objective of this work was to develop a sensitive, selective, and robust analytical method for the quantitation of nicotine-related impurities in e-vapor products and to evaluate the nicotine-related impurities in a variety of commercial e-cigarette cartridges ($n = 10$) and refill e-liquids ($n = 10$). Nicotine-*N*-oxide, nornicotine, mysomine, and cotinine were observed to increase with time during stability studies. This method was also applied to estimate the transfer efficiency of nicotine-related impurities to the aerosol. Most of the impurities were observed to transfer efficiently. However, nicotine-*N*-oxides showed low transfer efficiency and demonstrated thermal degradation. This selective and sensitive method is suitable to provide quantitative data for risk assessments and for use in e-cigarette product and refill e-liquid stability studies as one of the stability-indicating measures.

KEYWORDS
e-cigarette; e-liquid; electronic nicotine delivery system; LC–MS; nicotine; nicotine-related impurities

GRAPHICAL ABSTRACT

Cotinine
β-Nicotyrine
Anatabine
Myosmine
Nicotine-N'-Oxides
Anabasine
Nornicotine

Introduction

E-cigarettes, also known as e-vapor products and electronic nicotine delivery systems (ENDS), are an emerging product category in the global market. These products are available in a variety of configurations including small devices resembling cigarettes that are disposable or have rechargeable batteries with disposable cartridges or larger formats with rechargeable batteries and disposable prefilled or refillable tanks. When a user puffs on an e-cigarette, a liquid is heated, aerosolized, and inhaled. This liquid (often called e-liquid) typically contains propylene glycol and/or glycerin, water, nicotine, and flavors.

The nicotine used in e-liquids is extracted from tobacco, and the purity of the extracted nicotine can vary depending upon manufacturer and grade (e.g., pharmaceutical).[1] Not only can nicotine extracts contain natural impurities such as other tobacco alkaloids, but they can also contain degradation products.[2,3] The US and European Pharmacopoeias make recommendations for the purity of nicotine intended for pharmaceutical products, but no official purity recommendation for the nicotine used in e-cigarettes and refill e-liquids has been made.[4,5] US Pharmacopeia (USP)-grade nicotine requires single impurities to be less than 0.5% (5 mg/g) and total impurities to be less than 1% (10 mg/g).[4,6] Nicotine impurities are specified in the European Pharmacopoeia monograph 1452 (Figure 1) as nicotine-*N*-oxides, cotinine, nornicotine, anatabine, myosmine, anabasine, and β-nicotyrine.[5]

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Flora, J.W. et al, 2017 *J Liq Chrom Related Tech*

* ICH Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products

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The Development of Methods Specific to E-Cigarette Analysis:

Combustion-Related Constituents

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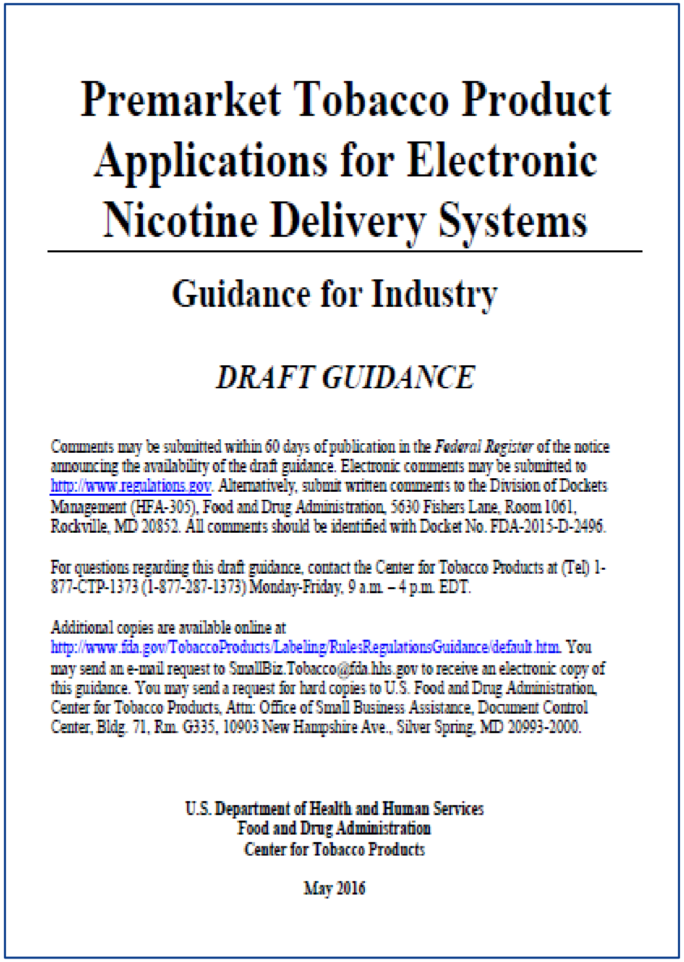
US FDA 2016 Deeming - HPHCs

- "FDA recommends that you consider the following constituents for analysis in e-liquids and aerosols, as appropriate, for your product:."
- "These constituents are constituents that, to FDA's current thinking, potentially could cause health hazards depending on the level, absorption, or interaction with other constituents."



Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems

Guidance for Industry

DRAFT GUIDANCE

Comments may be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Electronic comments may be submitted to http://www.regulations.gov. Alternatively, submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with Docket No. FDA-2015-D-2496.

For questions regarding this draft guidance, contact the Center for Tobacco Products at (Tel) 1-877-CTP-1373 (1-877-287-1373) Monday-Friday, 9 a.m. – 4 p.m. EDT.

Additional copies are available online at http://www.fda.gov/TobaccoProducts/Labeling/RulesRegulationsGuidance/default.htm. You may send an e-mail request to SmallBiz.Tobacco@fda.hhs.gov to receive an electronic copy of this guidance. You may send a request for hard copies to U.S. Food and Drug Administration, Center for Tobacco Products, Attn: Office of Small Business Assistance, Document Control Center, Bldg. 71, Rm. G335, 10903 New Hampshire Ave., Silver Spring, MD 20993-2000.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Tobacco Products

May 2016

Guidance for Industry, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems, May 2016

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HPHCs in E-liquids and Aerosols [1]

Origin	Compounds
Base formulation	nicotine, glycerol, propylene glycol
Impurities and flavors	diethylene glycol, ethylene glycol
	menthol, diacetyl, acetyl propionyl, ammonia
Nicotine related impurities	anabasine
	NNK, NNN
Leachables	cadmium, chromium, lead, nickel
Thermal degradation products	formaldehyde, acetaldehyde, acrolein, crotonaldehyde
Combustion related compounds	benzo[a]pyrene
	1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl
	acrylonitrile, benzene, 1,3-butadiene, isoprene, toluene

1. Guidance for Industry, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems, May 2016

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Note: RED constituents were not listed on the Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke Under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act" (Guidance for the Industry, March 2012).

Combustion Related HPHCs

- B[a]P, aromatic amines, and VOCs are products of incomplete combustion[1-4]
- E-cigarettes have low operating temperatures relative to tobacco cigarettes (i.e. ~ <350ºC vs 900ºC)[2,5,6]
- The mechanism of aerosol formation for e-cigarettes does not involve combustion

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Combustion Related HPHCs: Overview

- Develop methods for the analysis of combustion related HPHCs in e-liquids and e-cigarette aerosols
  - B[a]P
  - Aromatic amines
  - VOCs
- Analyze commercial refill e-liquids and the aerosols from rechargeable e-cigarettes with disposable cartridges for combustion related HPHCs
- Investigate the formation and transfer efficiency of combustion related HPHCs using reference e-liquids (not discussed)

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Aerosol Collection

- Puffing regime
  - 55 mL volume, 5 sec puff duration, and 30 sec puff interval, square wave puff
- Sample trapping technique
  - B[a]P and aromatic amines: Cambridge filter pad
  - VOCs: Cambridge filter pad followed by a course-fritted impinger containing 20 mL of methanol (-70° C)
- Sample collection (n=5)
  - 25 puffs (~ 100-150 mg of aerosol)
- All Aerosol collections were conducted on smoking machines that have never been used to collect smoke from combustion cigarettes and blanks were always tested

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Sample Preparation and Analysis

B[a]P (GC-MS, EI)	Aromatic Amines (GC-MS, NCI)	VOCs (GC-MS, EI)
Fortify sample with B[a]P-d12	Fortify sample with deuterated internal std.	Fortify impinger solution or e-liquid with benzene-d6, toluene-d8
Extract CFP or 0.3 g e-liquid with 2 mL water and 10 mL toluene	Extract CFP or 0.3g e-liquid with 2 mL of water and 10 ml of toluene	Dilute e-liquid in 10 mL methanol
Shake 20 minutes	Add TMA[1] and derivatize with HFBA[2]	Analyze impinger solution or diluted e-liquid
Remove the top layer for analysis	Load onto florisil SPE and elute with DCM	

1. TMA = trimethylamine
2. HFBA = Heptafluorobutyric acid

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Summary: Combustion-Related Constituents in Commercial E-Cigarette e-Liquids and Aerosols – Submitted for Publication 1/2017

- 13 commercial refill e-liquids covering a range of nicotine concentrations (0.6%-1.2%) and propylene glycol / glycerin ratios
  - Sweet, fruit, coffee/tea extract, tobacco
  - None of the combustion related HPHCs were detected in any of the commercial refill e-liquids (n=3)
- 6 of the top-selling commercial e-cigarettes including MarkTen® XL Classic and MarkTen® XL Menthol (Nu Mark LLC)
  - None of the combustion related HPHCs were detected in the aerosol from commercial e-cigarettes tested (rechargeable batteries with disposable cartridges) (n=5)

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Overall Summary

- Constituent testing of e-cigarette e-liquids and aerosols continues to evolve
- Cigarette filler and smoke methodologies are typically not suitable for e-cigarette analysis (e.g., lack the necessary sensitivity)
- Standardized aerosol collection and testing methodologies are essential to accurately measure "What's in Your E-Cigarette"
- Measures must be taken to prevent contamination/carry-over from combustion cigarettes
- Most of the Harmful and Potentially Harmful Constituents (HPHCs) found in cigarette filler and smoke are not detectable or are at considerably lower levels in e-cigarette e-liquids and aerosols[1,2]

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