Sensitive and Selective Method for Carbonyl Determination in E-Cigarette Aerosols

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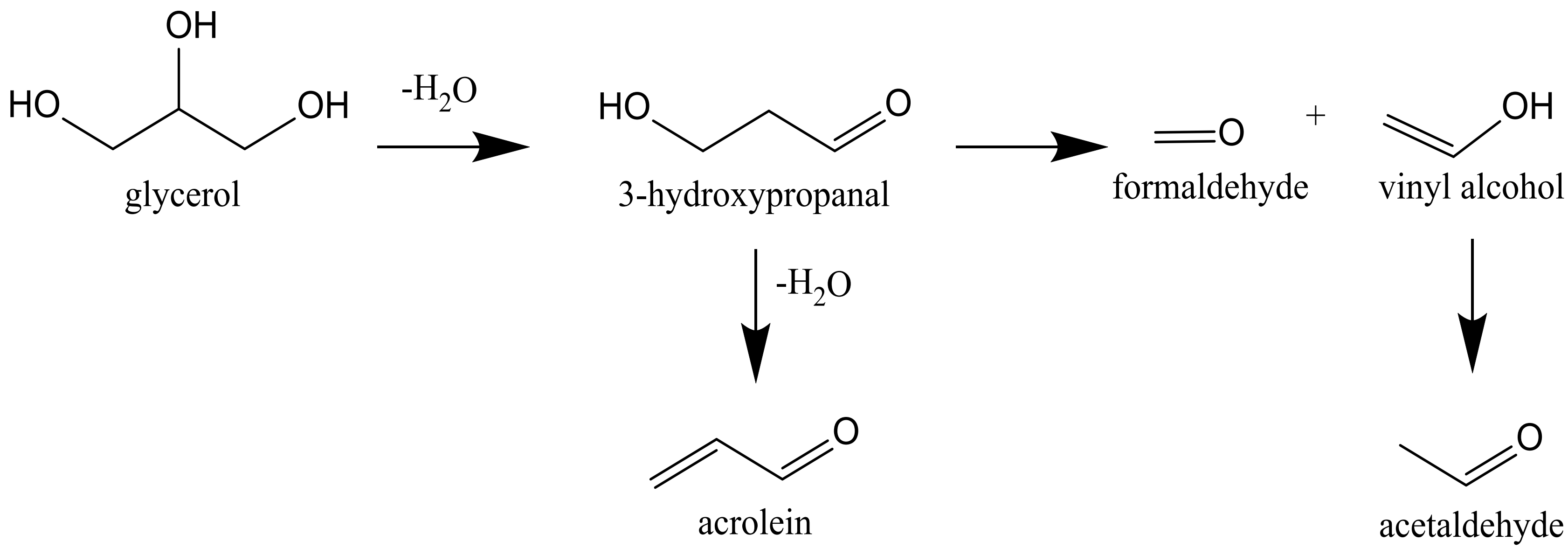
Carbonyls - Aerosol

- 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.

Background

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

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- 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. Anal. Sci 29, 1219-1222.
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- Cheng, T., 2014. Chemical evaluation of electronic cigarettes. Tob. Control 23 Suppl 2, ii11-ii17.
- Ohta K., Uchiyama S., Inaba Y., Nakagome H., Kunugita N. Determination of carbonyl compounds generated from the electronic cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine. Bunseki Kagaku. 2011;60:791–797

Characterization of E-Cigarette Formulations and Aerosols

- We previously investigated 4 commercially available MarkTen® e-cigarettes

- Formaldehyde was detected at 0.1 to 0.3 ug/puff and Acetaldehyde was found below the limit of quantitation
- Tobacco cigarette methodologies were adapted for the analysis

*US FDA: Abbreviated HPHC List**

Cigarette Smoke	Cigarette Filler
Acetaldehyde	Ammonia
Acrolein	Arsenic
Acrylonitrile	Cadmium
4-Aminobiphenyl	Nicotine (total)
1-Aminonapthalene	NNK
2-Aminonapthalene	NNN
Ammonia	
Benzene	
Benzo[a]pyrene	
1,3-Butadiene	
Carbon Monoxide	
Crotonaldehyde	
Formaldehyde	
Isoprene	
Nicotine (total)	
NNK	
NNN	
Toluene	

"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).

Carbonyls - Aerosol

- Carbonyls are typically present at trace levels in e-cigarette aerosols (e.g., 10 to 100 times lower than levels found in cigarette smoke)
- Carbonyls in e-cigarettes are often reported in the scientific literature as below the limits of detection (<LOD) or below the limits of quantitation (<LOQ) when using adapted tobacco cigarette methods such as CORESTA Recommended Method 74 (CRM 74)
- CRM 74 uses high performance liquid chromatography (HPLC) with ultraviolet (UV) detection
  - Limited sensitivity
  - Subject to interferences

Objective

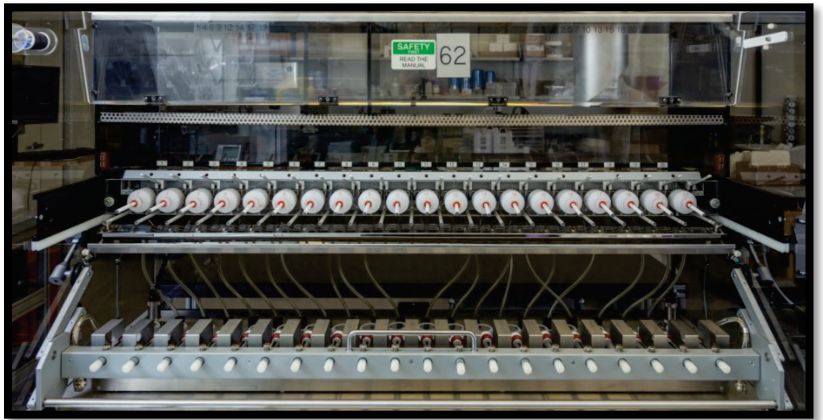
- To develop a more sensitive and selective method of measuring carbonyls in e-vapor products
  - Improved sensitivity
  - High selectivity
  - Using Ultra performance liquid chromatography with mass spectrometry (UPLC-MS)

Aerosol Collection

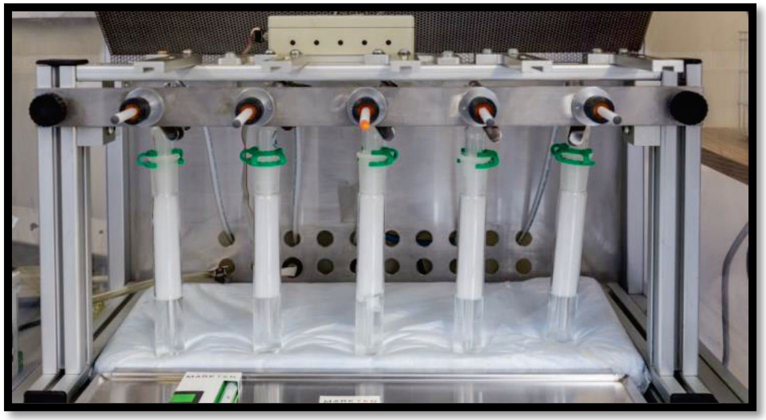
- Current standardized tobacco cigarette puffing regimes

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
MDPH	45	2	8 – 15 / cig	30	50	Sine wave
HC	55	2	6 – 14 / cig	30	100	Sine wave

- For e-cigarette aerosol collection, we modified the Health Canada (HC) regime and CRM 81



Cerulean 20-port linear



KC Automation 5-port linear

Aerosol Collection for Carbonyl Analysis

Puff Volume (mL)	Duration (seconds)	Collections	Interval (seconds)	Puff Profile
55	4	20 puff collections	30	Square wave

- Puff volume and interval were adapted from HC smoking regime
- Puff duration was the maximum for the 5 port linear machines when using impingers
- Collection in 20 puff increments was needed due to analyte instability
- Square wave puff profile was necessary to ensure puff sensors were activated at the beginning of the puff

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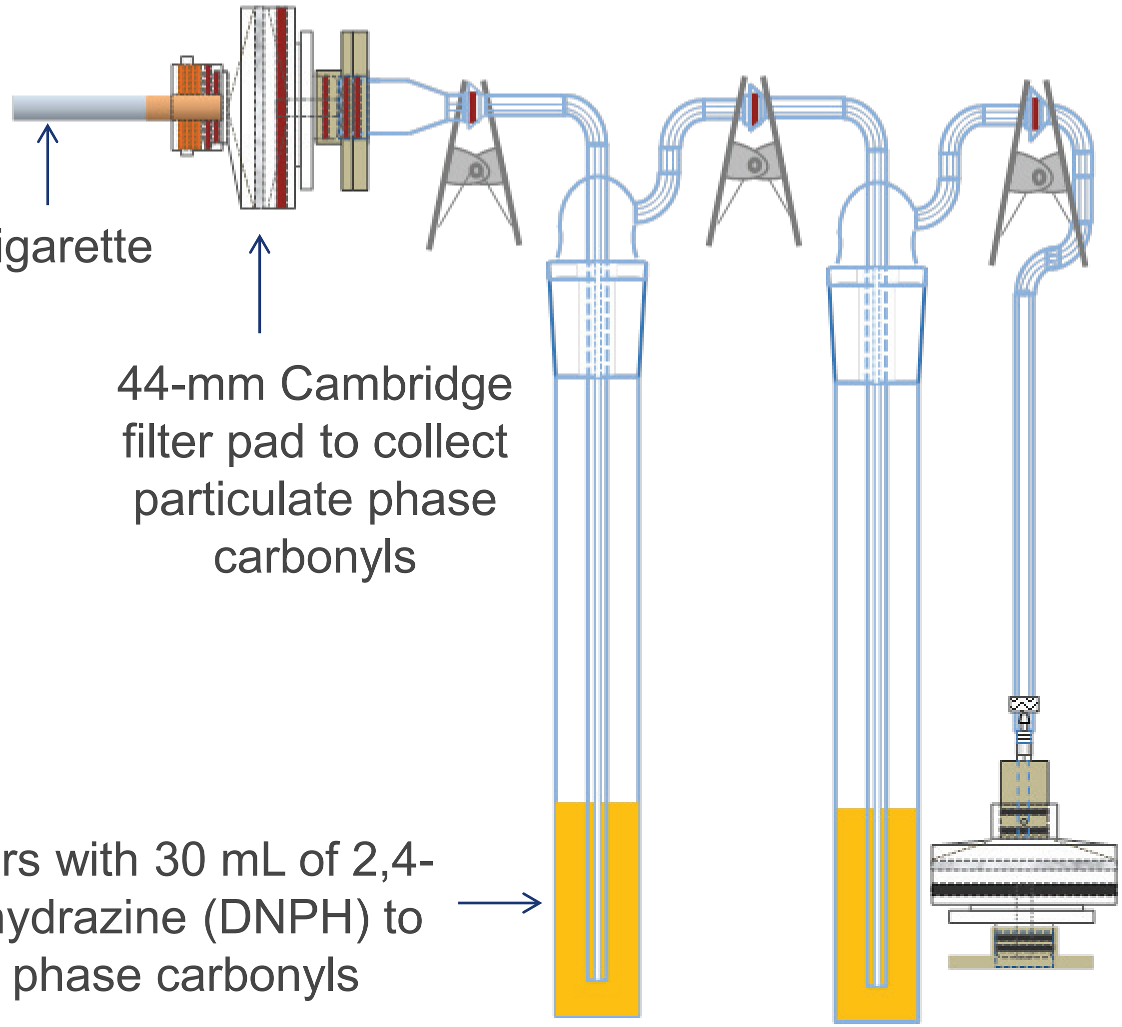
Trapping Efficiency Collection Configuration



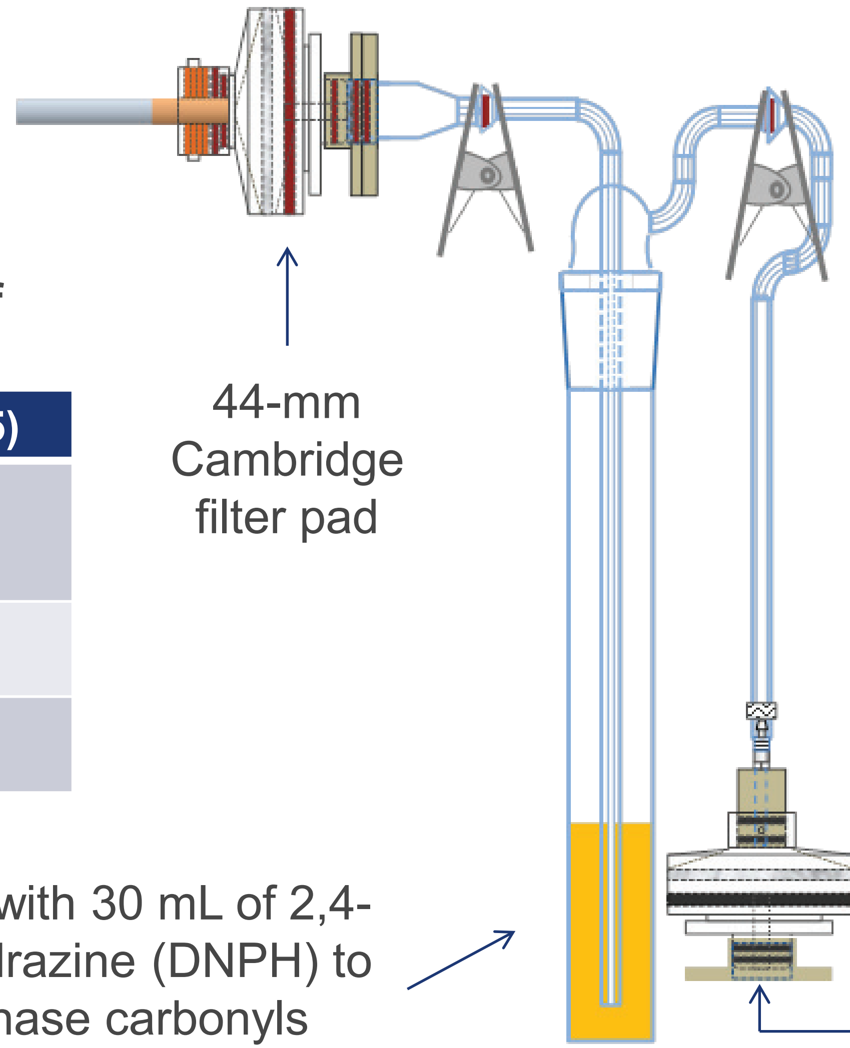
E-cigarette

44-mm Cambridge filter pad to collect particulate phase carbonyls

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

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

Optimized Carbonyl Collection



Trapping Efficiency of Formaldehyde

	Average (n=5)
Cambridge Filter Pad	77%
Impinger #1	23%
Impinger #2	0%

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Sample Preparation

- Following 20 puff collections
  - 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 autosampler 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

Ultra Performance Liquid Chromatography

- Mobile phase A is 98:2 10mM Ammonium Acetate:Methanol
- Mobile phase B is 90:10 Acetonitrile:1-Propanol
- Waters Acquity BEH C18, 2.1 x 50 mm, 1.7 um, with a 0.2 μm stainless steel frit
- Injection volume: 1μL
- Mobile phase gradient – 4 minute run!

Time (min)	Flow (mL/min)	A (%)	B (%)	Curve
0.0	0.5	65	35	Initial
2.0	0.5	40	60	6
2.5	0.5	40	60	6
2.7	0.5	65	35	6

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Mass Spectrometry

- Electrospray ionization in negative ion mode (selected ion monitoring)

Analyte	m/z
Formaldehyde - DNPH	209.1
Acetaldehyde - DNPH	223.1
Acrolein - DNPH	235.1
Crotonaldehyde - DNPH	249.2
Formaldehyde – d_2	211.1
Acetaldehyde-d_3	223.1

- Calibration range was 0.0107 µg/mL to 4.00 µg/mL
- Corresponds to 0.016 µg/puff to 6.30 µg/puff based on a 20-puff collection

Results and Discussion

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

Carbonyl	Recovery
Formaldehyde	90.7% to 106%
Acetaldehyde	92.4% to 111%
Acrolein	60.5% to 70.7%
Crotonaldehyde	90.7% to 108%

- limit of quantitation (LOQ) was 0.0107 µg/mL or 0.016 µg/puff
- limit of detection (LOD) was 0.002 µg/mL or 0.003 µg/puff

Method is "Fit-for-Purpose"

- Six commercial e-cigarettes 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 convenient store sales (Wells Fargo Equity Research, 2014)

Conclusions

- In most cases, the HPHCs found in conventional tobacco cigarettes are not observed in e-cigarette aerosols
- Cigarette smoke methodologies may not be sensitive enough to measure constituents in e-cigarette aerosols
- A new sensitive and selective method for carbonyl analysis in e-cigarette aerosols has been developed and validated
- All commercial products tested in this study contained formaldehyde and acetaldehyde

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