Thermal Degradation Studies of Electronic Cigarette Liquids Part 1: A Novel Analytical Method to Study α-Dicarbonyl Formation

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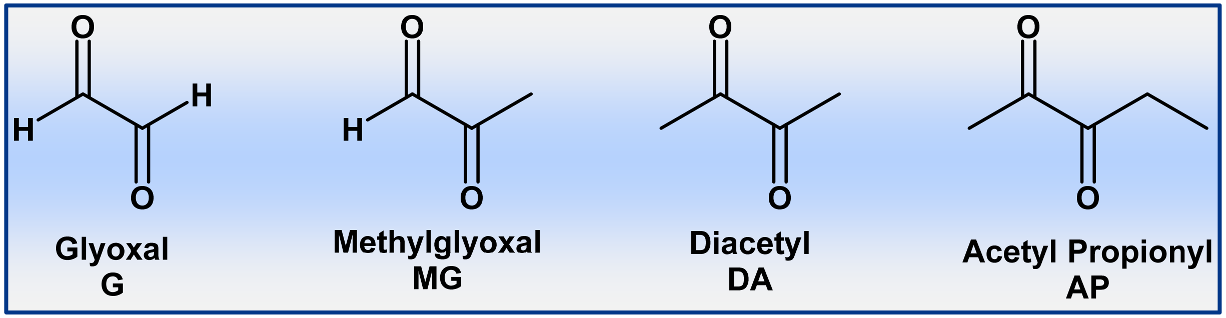
Formation of Diacetyl

- Internal preliminary data indicated possible diacetyl formation during aerosol generation
  - Diacetyl is not added to the e-liquid formulations
  - Higher levels in aerosol than in corresponding e-liquid
- Diacetyl and acetyl propionyl recommended for analysis by U.S. FDA in the 2016 Draft Guidance for Industry on ENDS products[1]
- How is diacetyl being formed during aerosol generation?
  - Need to elucidate reaction pathway

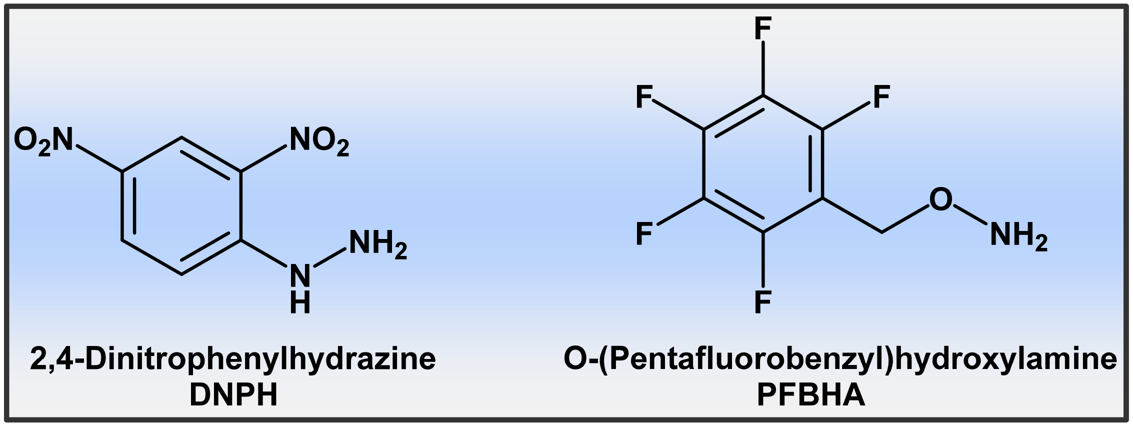
Analytical Method:

- α-Dicarbonyl Compounds of Interest



- Method Requirements:
  - Capable of supporting mechanistic studies
  - Selective for α-dicarbonyls

Are Available Methods Fit for Purpose?



- Available methods: DNPH, PFBHA, and direct analysis
- Issues specific to study (purpose):
  - Geometric Isomer Distribution:
    - Cis/trans distribution of mono- and bis-derivatized products
  - Inclusion of analytes of interest:
    - Glyoxal and methylglyoxal not amenable to direct analysis

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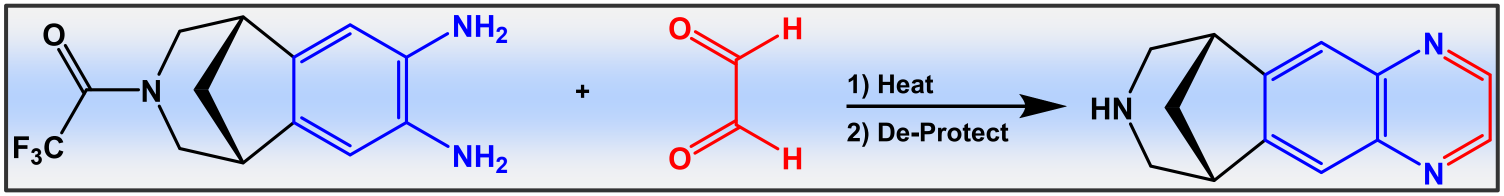
Alternate Derivatizing Agent

- o-Phenylenediamine (OPD) condensation with glyoxal critical step in varenicline(CHANTIX®) synthesis[2]



- Develop analysis of α-diketones as quinoxaline derivatives
  - Provides single, aromatic product
  - Inherent stability of aromatic compounds allows for use in mechanistic/^{13}C-labeling studies
- OPD used for analysis of analytes of interest in beer, wine, cheese[3]
- Evaluated for cigarette smoke analysis[4]

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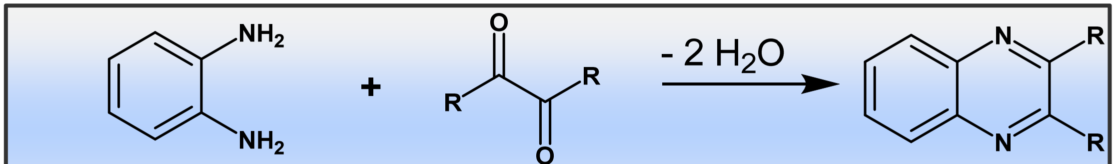
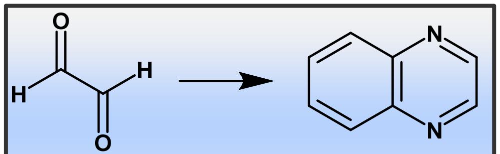
Generic and Target Analyte Reactions



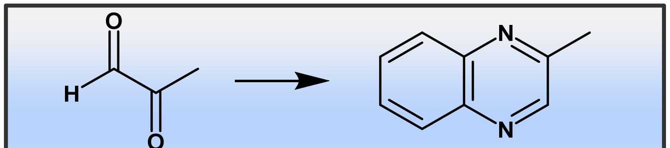
o-Phenylenediamine
OPD

Quinoxaline



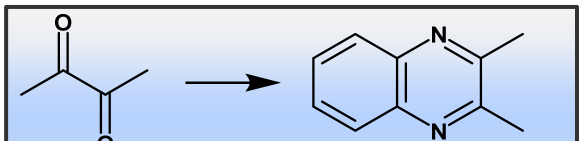
Glyoxal
G

Quinoxaline
G-Q



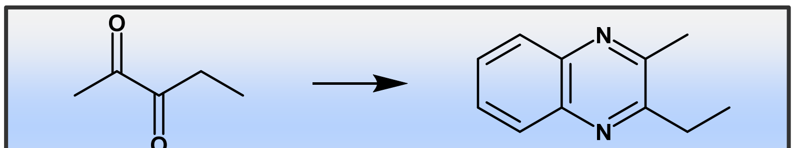
Methylglyoxal
MG

Methylquinoxaline
MG-Q



Diacetyl
DA

Dimethylquinoxaline
DA-Q



Acetyl Propionyl
AP

Methylethylquinoxaline
AP-Q

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Materials

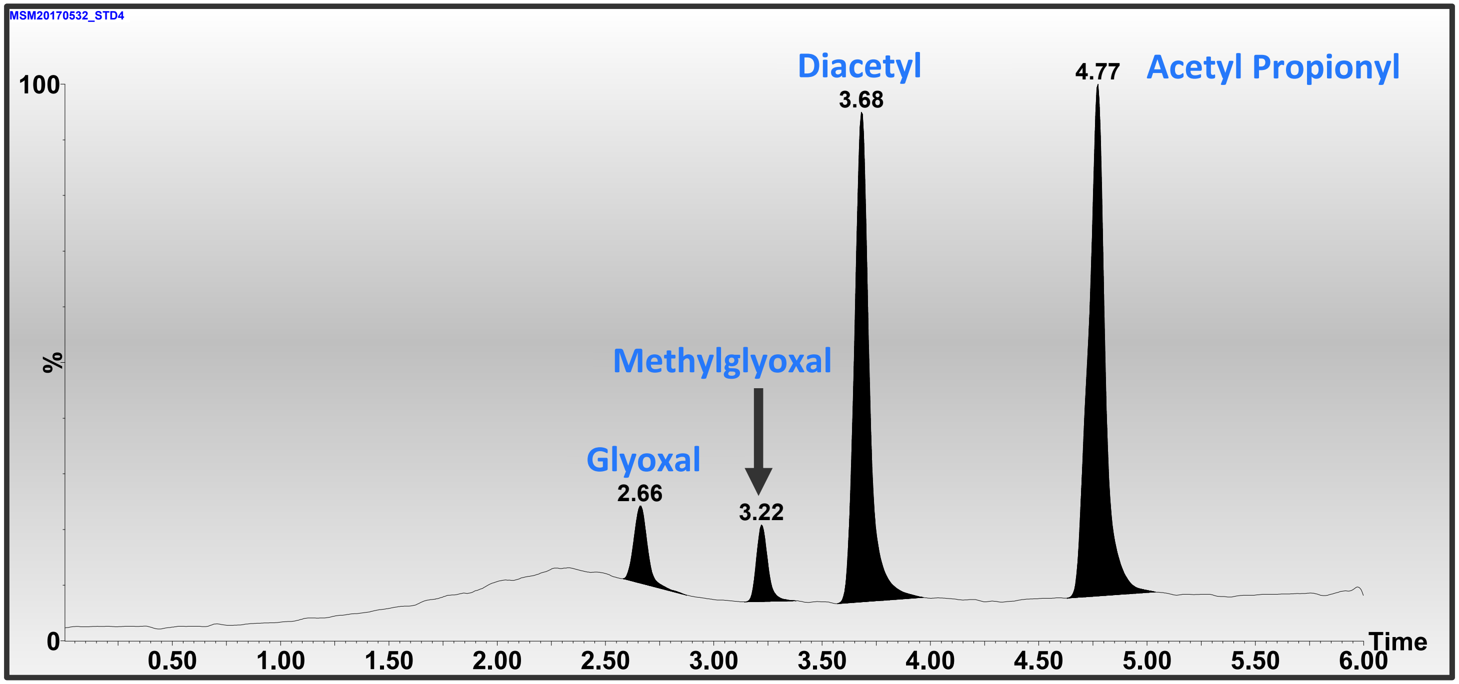
- Commercially available:
  - Quinoxaline (G-Q)
  - Methylquinoxaline (MG-Q)
  - Dimethylquinoxaline (DA-Q)
  - Quinoxaline-d6
- Synthesized from OPD and corresponding α-diketone
  - Methylethylquinoxaline (AP-Q)
  - Dimethylquinoxaline-d6
  - Methylethylquinoxaline-d5
- Initial derivatization reagent consisted of 50 mM OPD in 0.2% (v/v) acetic acid

Instrumental Conditions

Parameters	
LC Column	Agilent PoroShell Bonus-RP 2.1 X 100 mm; 2.7 µm
Column Temperature	50°C
Injection Volume	2 µL
Flow Rate	0.6 mL/min
Mobile Phase *gradient elution*	A: Type-1 Water B: Acetonitrile
Run Time	6 min
Ionization Mode	Positive Electrospray
Source Temperature	150°C
Desolvation Temperature	500°C
SIR MS Mode (m/z) *as the corresponding quinoxaline*	Glyoxal = 131; Methylglyoxal = 145; Diacetyl = 159; Acetyl Propionyl = 173

Example Chromatogram: Standard

- Analyzed as the corresponding quinoxaline derivative



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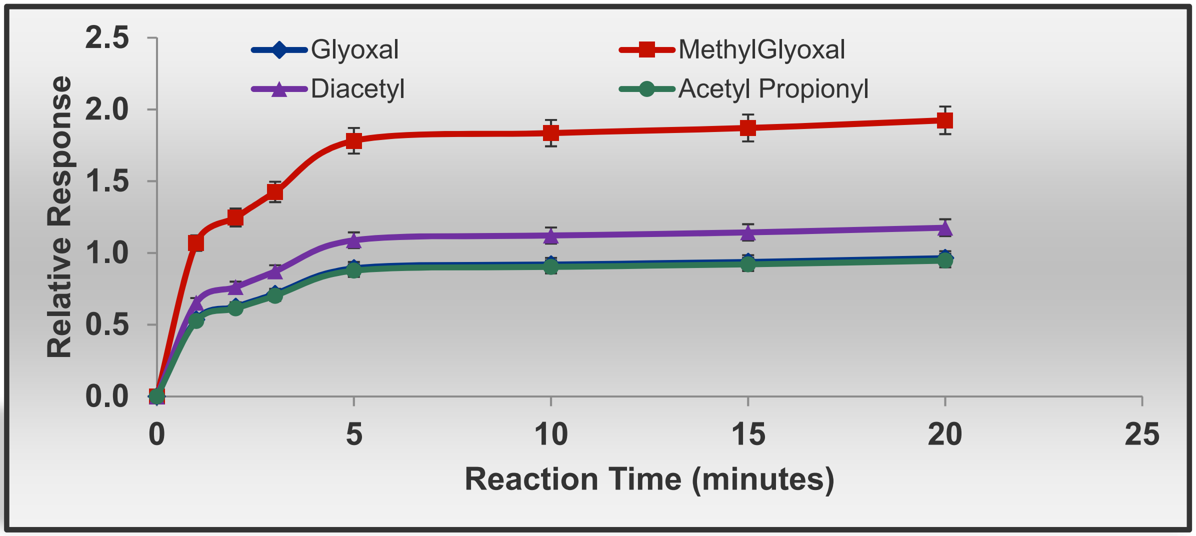
OPD Derivatization Time

- **Individual samples were prepared and extracted after the indicated time**



- Reaction was > 90% complete after 5 minute in 0.2% (v/v) acetic acid
- The method derivatization time was set at 10 minutes

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Optimization for E-Vapor Matrix

- Reference e-liquid = 50:50 propylene glycol:glycerin + 15% Water + 2.5% Nicotine (on a weight basis)
- 50 mM OPD in 0.2% (v/v) acetic acid
- Perform biphasic extraction into hexane
  - Removes propylene glycol, glycerine, and basic components of matrix
  - Quinoxalines (pKa~0.6) transfer to organic phase, top hexane layer
- Matched Internal Standards

E-Liquid Sample Preparation Work Flow

1
- Weigh 500 mg of e-liquid into vial

2
- Add 5 mL of 50 mM OPD in 0.2% (v/v) acetic acid
- Add internal standards solution
- Add 2 mL of hexane

3
- Extract for 10 minutes
- Analyze hexane layer by LC-MS/MS
- 2 µL injection volume

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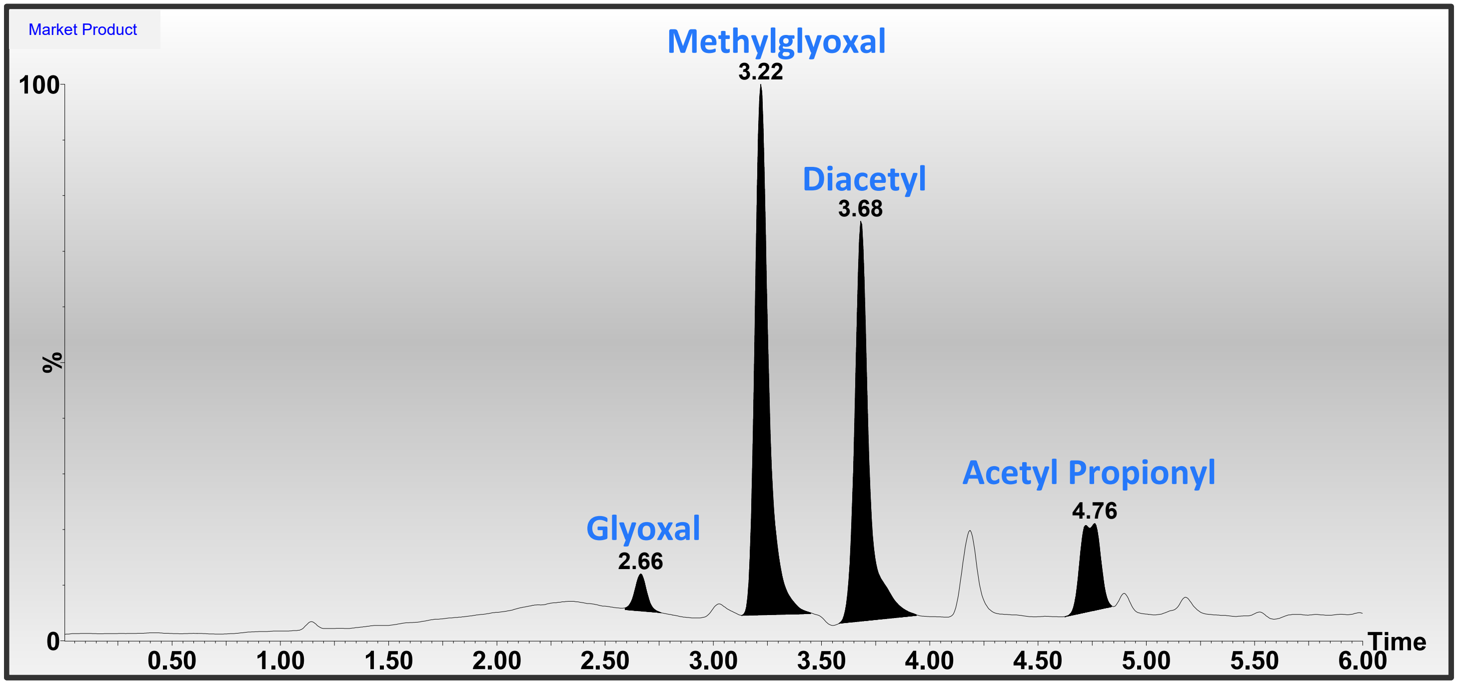
Example Chromatogram: Market Product

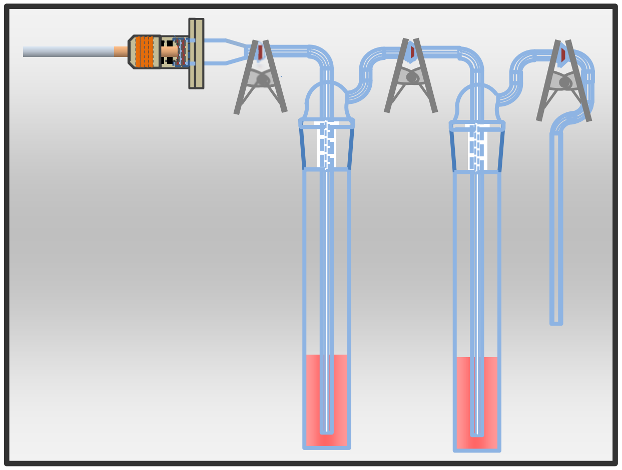
- Analyzed as the corresponding quinoxaline derivative



Aerosol Trapping Efficiency

(55 mL Puff Volume, 5 sec Puff Duration, 30 sec Puff Interval, Square Wave)

- Cambridge filter pad followed by 2 impingers containing 20 mL of 50 mM OPD in 0.2% (v/v) acetic acid
- 140 puffs yielding approximately 450 mg of aerosol



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Aerosol Trapping Efficiency

(55 mL Puff Volume, 5 sec Puff Duration, 30 sec Puff Interval, Square Wave)

N=5	Glyoxal	MethylGlyoxal	Diacetyl	Acetyl Propionyl
Cambridge Filter Pad	98%	98%	10%	3%
Impinger #1	2%	2%	88%	93%
Impinger #2	0%	0%	2%	3%

- Glyoxal and methylglyoxal primarily trapped on pad
- Diacetyl and acetyl propionyl trapped in first impinger
- < 5% of target analytes in second impinger

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Aerosol Collection Work Flow

1
- Collect aerosol using a trapping system consisting of a CFP and 1 impinger containing 20 mL 50 mM OPD in 0.2% (v/v) acetic acid

2
- Combine CFP and Impinger solution in a 40 mL vial
- Add internal standards solution
- Add 2 mL of hexane

3
- Extract for 10 minutes
- Analyze hexane layer by LC-MS/MS

Method Validation Matrices

Sample ID	PG/Gly Ratio (%)	Water (%)	Nicotine (%)	Analyte Fortification
e-liquid[1]	50/50	15	2.5	NA
e-liquid Low	50/50	15	2.5	Low
e-liquid Mid	50/50	15	2.5	Mid
e-liquid High	50/50	15	2.5	High

Fortification Level	Glyoxal (µg/mL)	Methylglyoxal (µg/mL)	Diacetyl (µg/mL)	Acetyl Propionyl (µg/mL)
Low	0.149	0.075	0.027	0.029
Mid	0.746	0.375	0.135	0.144
High	1.493	0.750	0.270	0.287

1) Added to empty MarkTen® XL cartridges for aerosol collections

Method Validation Summary

Parameter		Glyoxal	MethylGlyoxal	Diacetyl	Acetyl Propionyl
Linearity	R^2	> 0.995	> 0.995	> 0.995	> 0.995
	Range (ng/mL)	50 - 5000	30 - 3000	6 - 600	9 - 900
Recovery	e-liquid (%)	82 - 114	76 - 94	84 - 111	87 - 116
	Aerosol[1] (%)	80 - 110	88 - 98	95 - 103	88 - 108
Precision	e-liquid[2] (n=5; %RSD)	< 5	< 5	< 7	< 7
	Aerosol[3] (n=15; %RSD)	< 16	< 13	< 8	< 11
LOQ	e-liquid (ng/g)	104	54	12	18
	Aerosol[4] (ng/g)	104	54	12	18

1) Transfer efficiency not evaluated
2) Recovery samples used
3) Commercial product used
4) Assuming 0.5 g of generated aerosol

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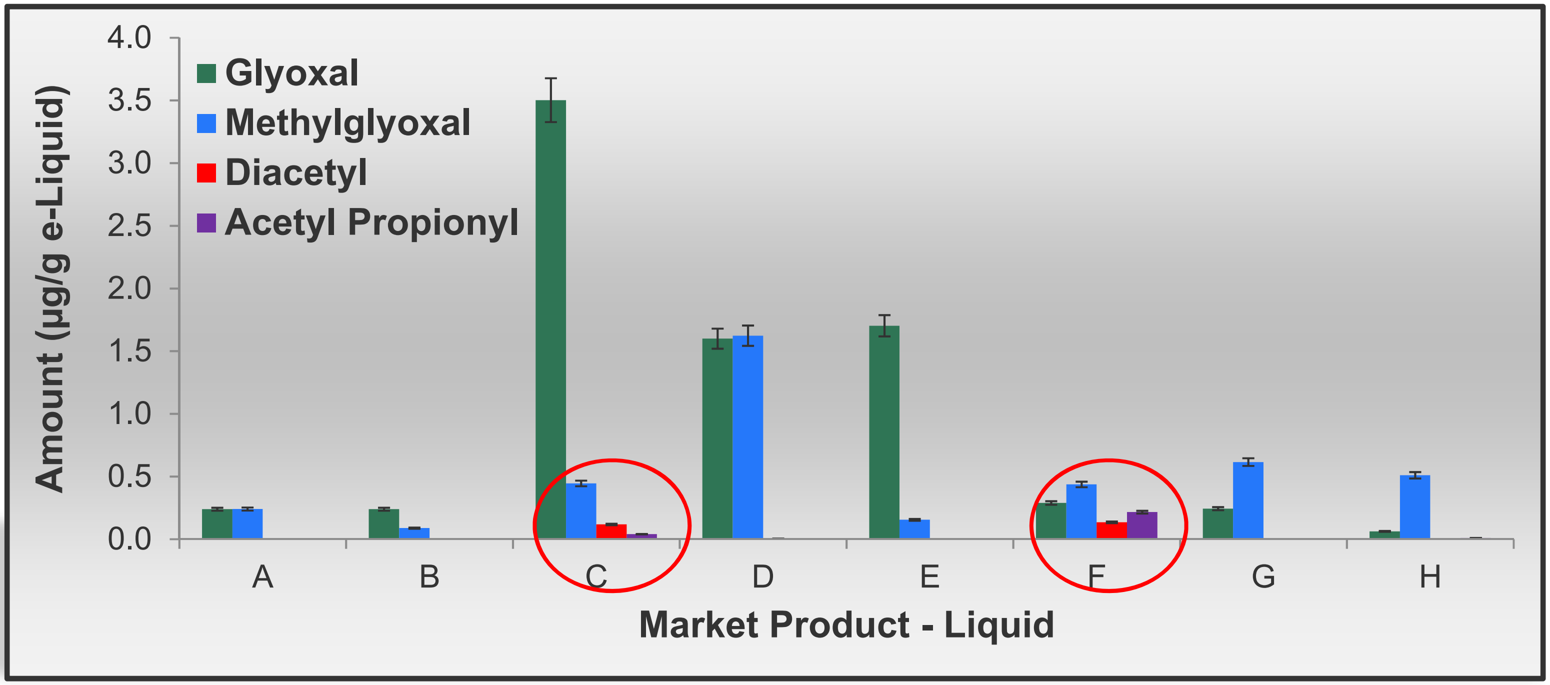
Commercial E-Cigarette: Liquid Analysis



- Glyoxal and Methylglyoxal present in all products
- Diacetyl and acetyl propionyl present in two products

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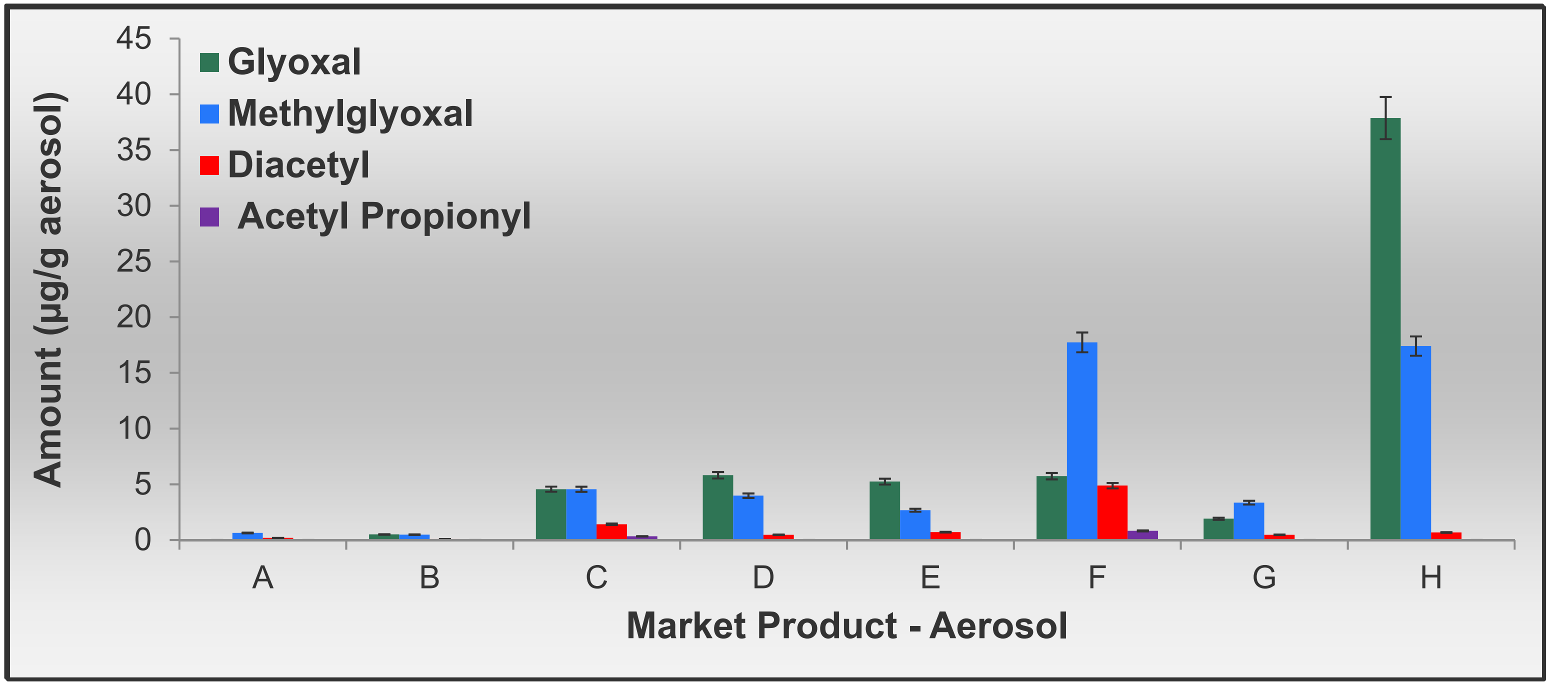
Commercial E-Cigarette: Aerosol Analysis



- Glyoxal and methylglyoxal present in all product aerosols
- Diacetyl and acetyl propionyl appear to be created by some products

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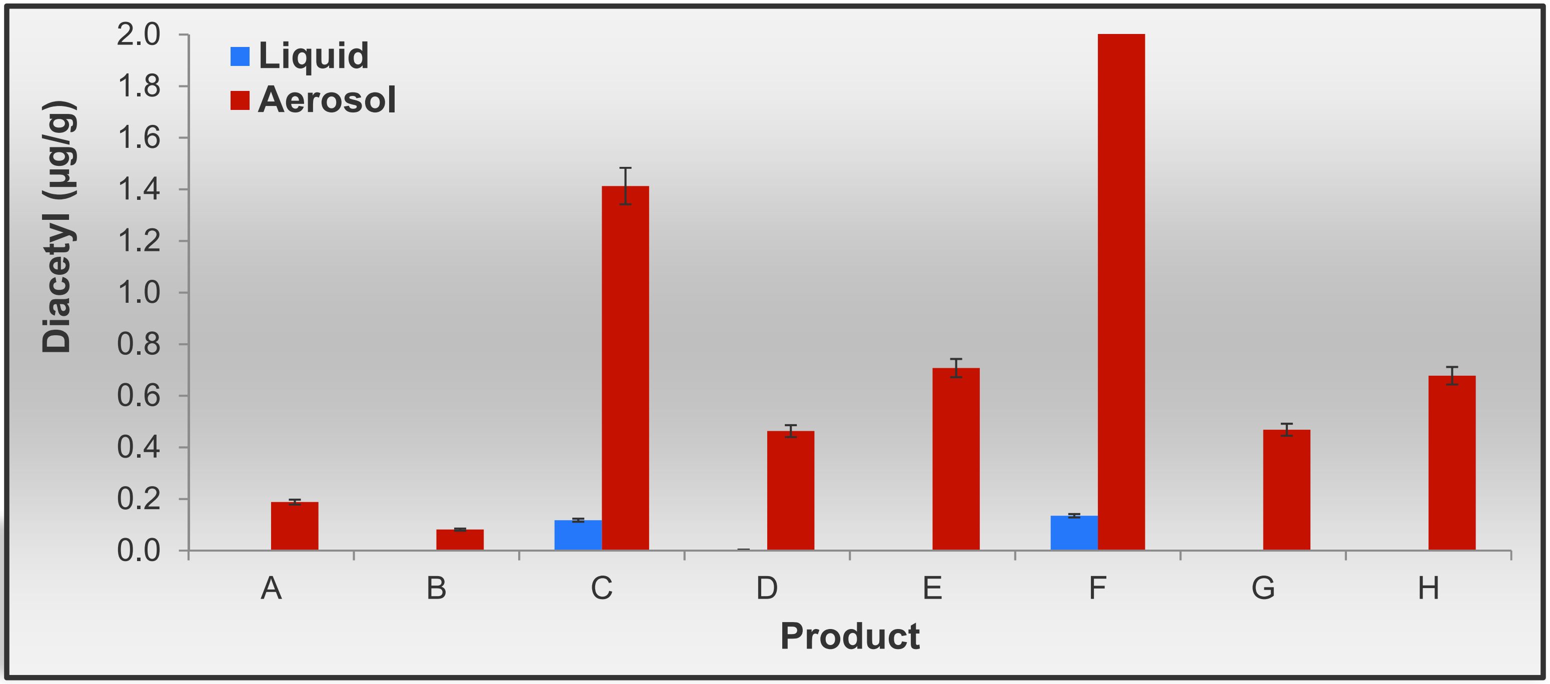
Diacetyl Comparison



- Diacetyl levels elevated in aerosol relative to e-liquid suggesting diacetyl formation during aerosol generation

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Summary

- o-Phenylenediamine is an efficient derivatization agent suitable for the analysis of α-dicarbonyl compounds in electronic cigarette liquids and aerosols
- Method utilizes simple biphasic extraction technique where derivatized the analytes are transferred into hexane
  - Eliminates the need for solid-phase extraction
- Unconventional, water immiscible solvents are compatible with modern UPLC systems
- Many commercial products appear to form α-diketones during the generation of aerosol

Acknowledgments

- George Karles, Yezdi Pithwalla, and Pete Lipowicz
- Thutrang Nguyen, Kathy Jerome, and Ha Nguyen

References

1. Draft Guidance for Industry, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems, May 2016
   http://www.fda.gov/downloads/TobaccoProducts/Labeling/RulesRegulationsGuidance/UCM499352.pdf

2. Coe, J. W., et al. (2005). "Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation." J Med Chem 48(10): 3474-3477.

3. Gensberger, S., et al. (2013). "Analysis of sugar degradation products with alpha-dicarbonyl structure in carbonated soft drinks by UHPLC-DAD-MS/MS." J Agric Food Chem 61(43): 10238-10245.

4. Moree-Testa, P. and Y. Saint-Jalm (1981). "Determination of α-dicarbonyl compounds in cigarette smoke." Journal of Chromatography A 217(C): 197-208.

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