Reporting Formaldehyde in Electronic Cigarette Aerosol and the Relevance of Formaldehyde Hemiacetals and Acetals

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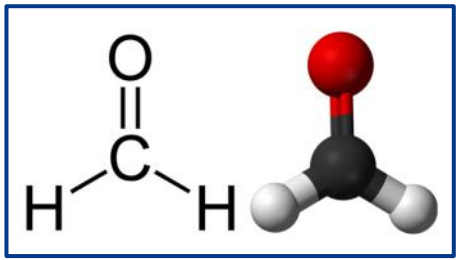
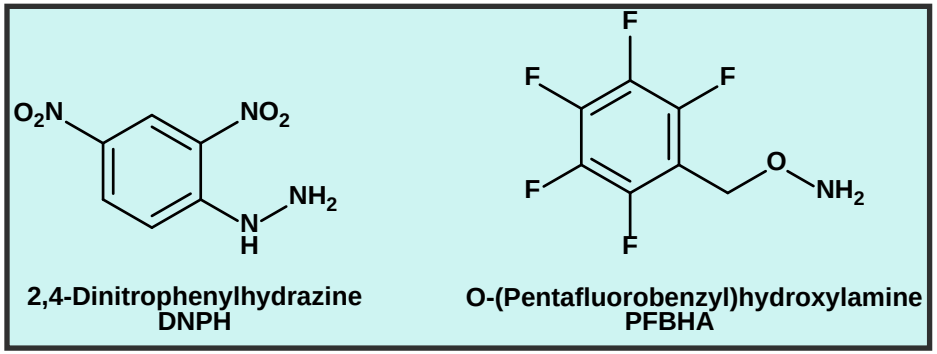
Background - Formaldehyde



- Formaldehyde is recommended for reporting in the FDA draft guidance for e-vapor products[1]
- Group 1 carcinogen in humans by the International Agency for Research on Cancer (IARC)
- Found in varying levels in EVP aerosols depending on device type (cig-a-likes, pods, open tanks, sub-ohm devices, etc)
- Evidence supports formation through thermal degradation of propylene glycol (PG) and glycerin (Gly)

1) FDA Draft Guidance for Industry, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems, 2016

Methods of Analysis



2,4-Dinitrophenylhydrazine
DNPH

O-(Pentafluorobenzyl)hydroxylamine
PFBHA

- Analytical approaches for formaldehyde analysis:
  - Direct analysis by GC-MS
  - Pentafluorobenzylhydroxylamine (PFBHA) derivatization with GC-MS analysis of the oxime
  - 2,4-Dinitrophenylhydrazine (DNPH) derivatization with LC-UV or -MS analysis of the hydrazone

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DNPH Applications

- Methods for determination of formaldehyde typically use DNPH derivatization followed by analysis of the resulting hydrazone:
  - EPA method 8315A for waste water, air samples, etc.[2]
  - CORESTA CRM 74 for cigarette smoke[3]
  - Various methods for e-vapor products[4]
- No standardized methods for formaldehyde analysis in e-vapor products:
  - ISO TC/126
  - CORESTA EVAP subgroup
  - Other national standardization bodies

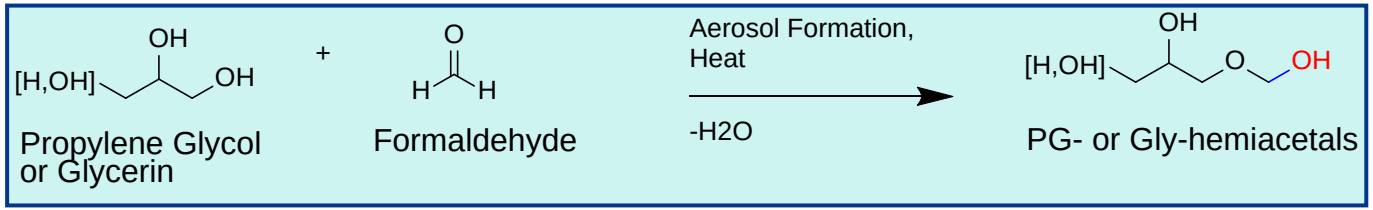
2) EPA method 8315A "Determination of Carbonyl Compounds by High Performance Liquid Chromatography" Rev.1, 1996

3) CORESTA CRM 74 "Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC", Rev.4, 2018

4) Flora, J.W., Wilkinson, C.T.; Wilkson J. W.; Lipowicz P. J.; Skapars J. A.; Anderson, A.; and Miller J. H., Method for determination of Carbonyl Compounds in E-Cigarette Aerosols, J Chromatogr Sci. 2017 Feb; 55(2): 142-148

“Hidden Formaldehyde” (Jensen *et al.*; 2015)

- Reported presence of formaldehyde as the hemiacetals formed from a condensation with PG and Gly in e-vapor aerosol[5]

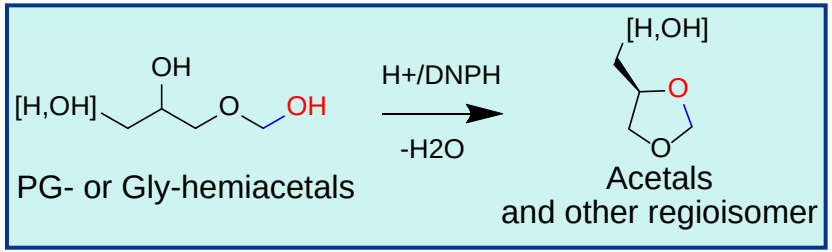


- Quantitated using nuclear magnetic resonance (NMR) after trapping aerosol in cold trap; expensive instrumentation; low sensitivity
- Hemiacetals only detected at high voltage (5.0 V; atomizer resistance not stated)

5) Jensen R.P.; Luo W.; Pankow J. F.; Strongin R. M.; and Peyton D. H., Hidden Formaldehyde in E-Cigarette Aerosols, N. ENGL. J. MED. 372:4, NEJM.ORG, JANUARY 22, 2015

Conversion to Acetals (Salamanca *et al.; 2017)*

- Obtained 67% recovery of formaldehyde from synthetic formaldehyde hemiacetals when treated with acidic DNPH[6]



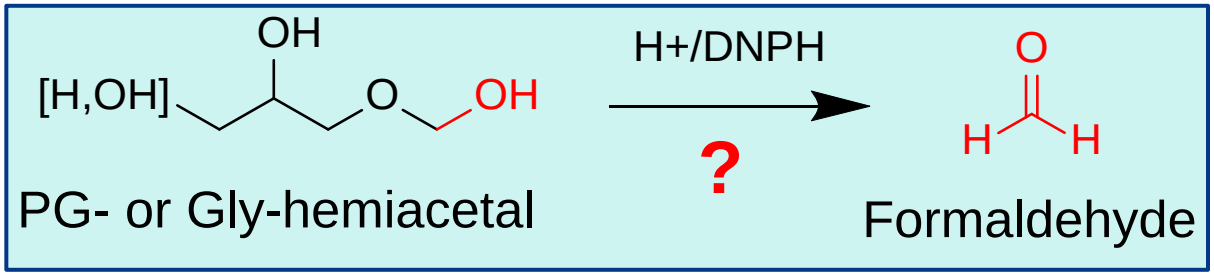
- Suggested conversion of hemiacetal to acetal in acidic DNPH solution
- Claimed that this leads to the underestimation of formaldehyde levels in e-vapor when using the common DNPH methods

6) Salamanca J.C.; Ian, M.; Escobedo J. O.; Jensen R. P.; Shaw A.; Campbell R.; Luo W.; Peyton D. H.; Strongin R. M., Formaldehyde Hemiacetal Sampling, Recovery, and Quantification from Electronic Cigarette Aerosols, Scientific reports, 7:11044, September 8, 2017

Study Objectives and Questions

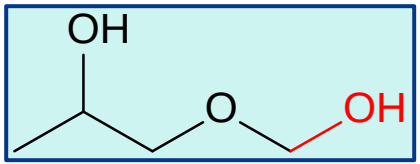
- Evaluate the DNPH method performance for analysis of “Hidden Formaldehyde” in e-vapor aerosol:
  1. Do PG- and Gly-hemiacetals hydrolyze to formaldehyde in acidic DNPH solution?
  2. Do PG- and Gly-hemiacetals convert to the corresponding acetals in acidic DNPH solution?
- Evaluate if acetals are formed in e-vapor aerosol

Do PG and Gly-hemiacetals Hydrolyze to Formaldehyde in Acidic DNPH Solution?

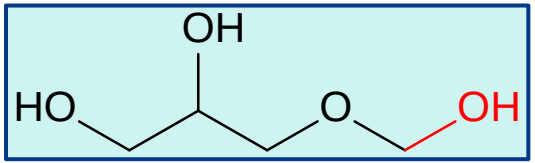


Approach

- Do hemiacetals hydrolyze to formaldehyde?



PG-hemiacetal (PG-HA)*



Gly-hemiacetal (Gly-HA)*

- Add PG-HA or Gly-HA to acidic DNPH solution
- Determine formaldehyde by UPLC/MS

* Custom synthesized by Dr. Sönke Peterson (Worms, Germany)

LC/MS Method for the Analysis of Formaldehyde

- Do hemiacetals hydrolyze to formaldehyde?

Parameters	
Instrumentation	UHPLC-MS
Column	BEH C18 (1.7µm)
Ionization mode	ESI negative, SIM
Trapping solution	DNPH in acetonitrile w/perchloric acid
Internal standard	Formaldehyde-DNPH-d3
Calibration range	0.01 - 4 µg/mL (1.5 - 60 µg/g)
Limit of quantification	1.5 µg/g
Recovery	97 - 105%

Results

- Do hemiacetals hydrolyze to formaldehyde?

	Formaldehyde theoretical (µg/mL)	Formaldehyde determined (µg/mL)	Recovery (%)
PG-HA (3.19 µg/mL)	0.93	0.96	**103**
Gly-HA (5.86 µg/mL)	1.44	1.50	**104**

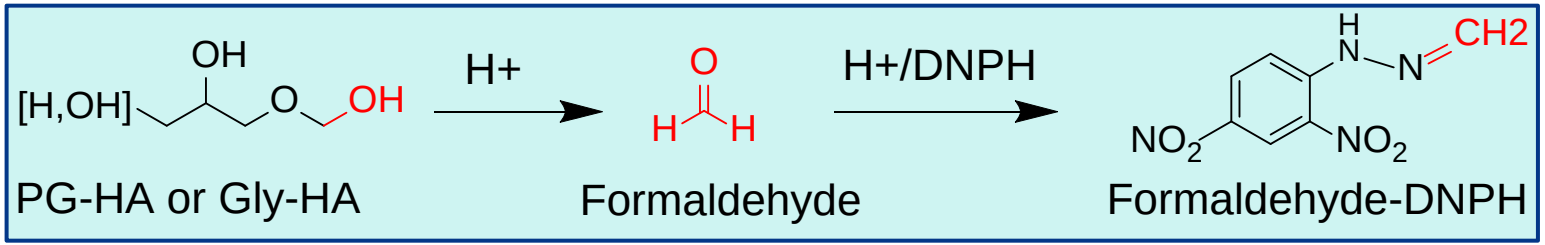
- PG-HA and Gly-HA quantitatively release formaldehyde and form the corresponding hydrazone in acidic DNPH solution
- These results corroborate those reported by Knorr, *et al*.[7]

7) Knorr A.; Gautier L.; Tekeste E.; Buchholz C., Almstertter M.; Arndt D.; Bently M., Formaldehyde-Glycerol Hemiacetal _Absence of "Hidden" Formaldehyde in THS 2.2 Aerosols, https://www.researchgate.net/publication/291833243

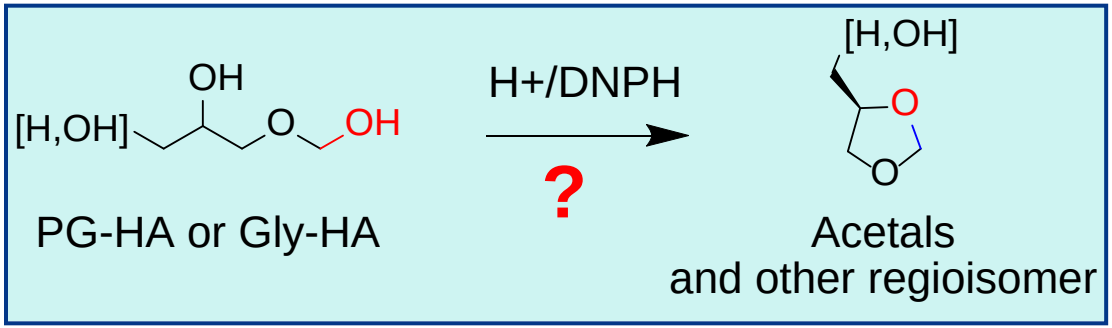
Summary

- Do hemiacetals hydrolyze to formaldehyde?

- PG-HA and Gly-HA quantitatively hydrolyze to formaldehyde in acidic DNPH solution
  - These hemiacetals rapidly hydrolyze in acidic DNPH solution to release formaldehyde
  - The secondary reaction with DNPH to form the hydrazone drives the equilibrium to release more formaldehyde



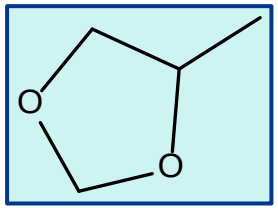
Do PG-HA and Gly-HA Convert to the Corresponding Acetals in Acidic DNPH Solution?



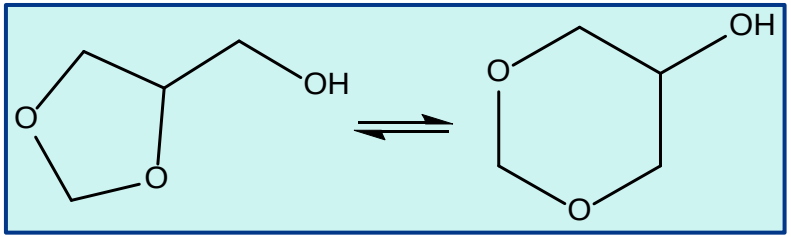
Approach

- Do hemiacetals convert to acetals?

- PG-HA and Gly-HA added to acidic DNPH solution (~5µg/mL)
- Determine PG- and Gly-acetals by GC/MS



PG-acetal
(4-methyl-1,3-dioxolane)



Gly-acetals
(4-hydroxymethyl-1,3-dioxolane and
5-hydroxy-1,3-dioxane)

GC/MS Method for the Analysis of Acetal

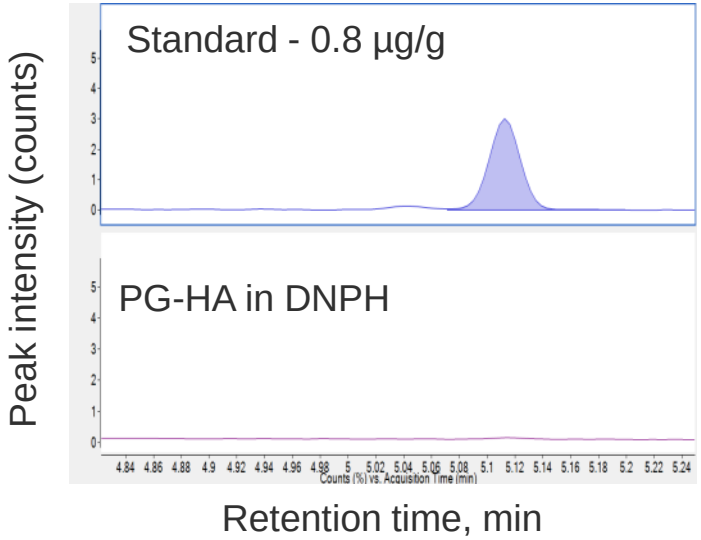
- Do hemiacetals convert to acetals?

Parameters	
Instrument	GC-MS
GC column	Restek Rtx 624
Ionization mode	EI
Acquisition mode	SIM
Extraction / trapping	Dichloromethane (DCM)
Internal standard	2,3-Hexadione
Calibration range	0.01 - 1 µg/mL (0.8 - 80 µg/g)
Limits of quantification	0.8 µg/g

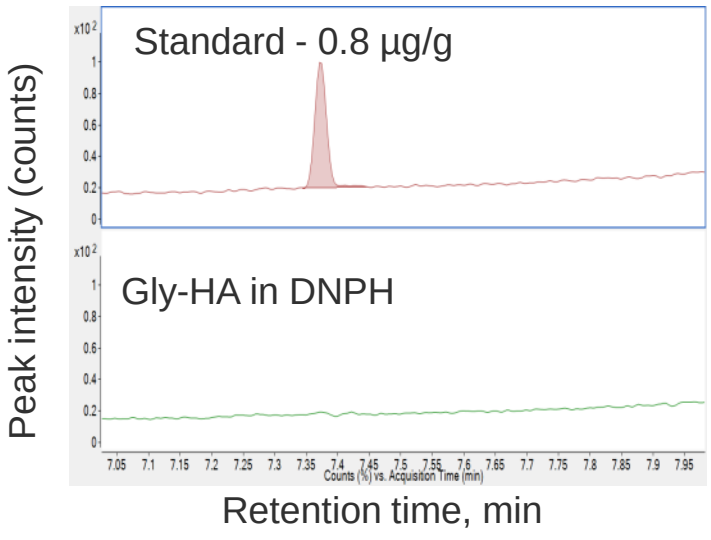
Results

- Do hemiacetals convert to acetals?

Chromatograms of PG-acetal



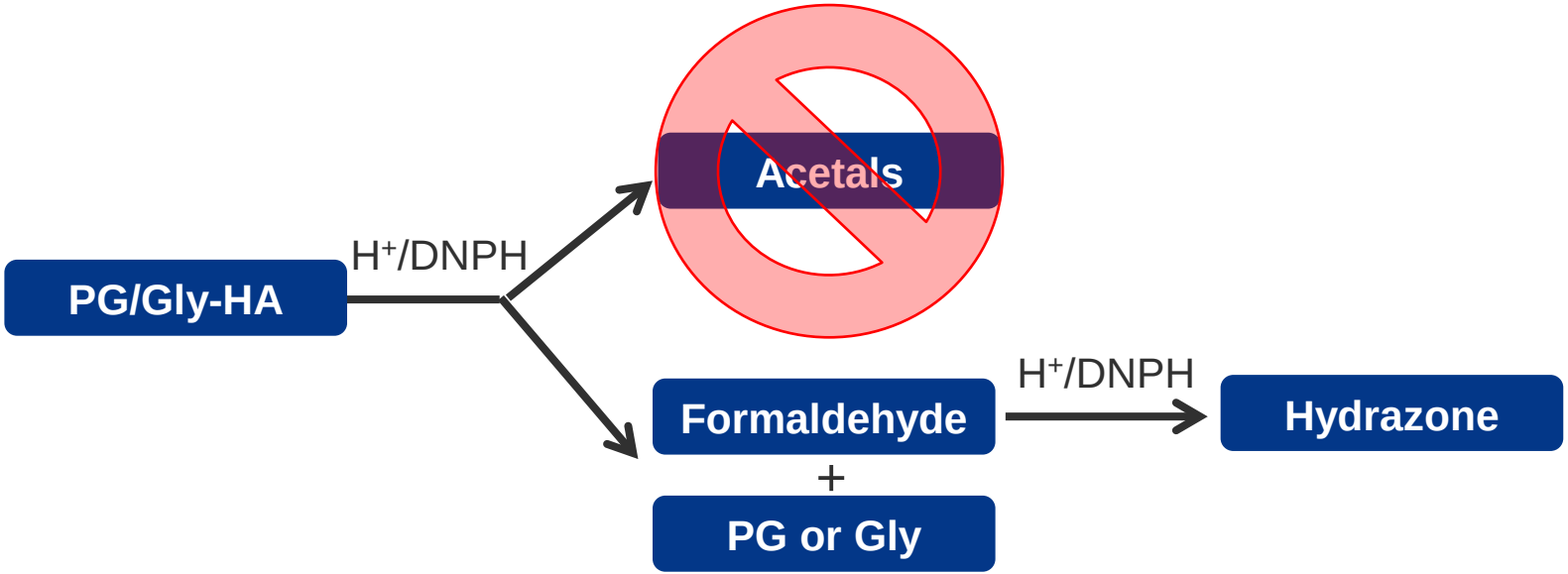
Chromatograms of Gly-acetal



- PG- and Gly-acetals were not formed

Summary

- Do hemiacetals convert to acetals?



- PG-HA and Gly-HA do not convert to the corresponding acetals in acidic DNPH solution

Are PG-Acetal or Gly-Acetals Formed in Aerosol?

Approach

- Do acetals form in e-vapor aerosol?

- Investigate acetal formation during aerosol generation by analyzing the e-liquid and the corresponding e-vapor

Experimental

- Do acetals form in e-vapor aerosol?

1.
   - 0.25 g e-liquid + 20 mL DCM , or
   - 50 puff block collection (55 cc/30 s/5 s)
   - CFP + chilled impinger (20 mL DCM)
2.
   - 100 µL ISTD (2,3-Hexadione)
   - 2 mL water
3.
   - GC/MS analysis (DCM layer)

Results and Conclusion

- Do acetals form in e-vapor aerosol?

Sample	e-Liquid Composition	PG-Acetal (µg/g)		Gly-Acetal (µg/g)	
		e-liquid	aerosol	e-liquid	aerosol
Control	PG:Gly (50:50) (2.5% Nic, 15% H20)	ND*	ND*	ND*	ND*
e-Cig A	Menthol (60:40 PG:Gly; 3.5% Nic)	ND*	ND*	<0.8	<0.8
e-Cig B	Non Menthol (30:70 PG:Gly; 3.5% Nic)	ND*	ND*	1.2±0.1	1.0±0.1

ND*: Not detected or <LOD (PG-acetal: 0.2ng/g, Gly-acetal: 0.3ng/g)

- PG- and Gly-acetals do not form during aerosolization process for the cig-a-like devices tested

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Results and Conclusion

- Do acetals form in e-vapor aerosol?

Sample	e-Liquid Composition	PG-Acetal (µg/g)		Gly-Acetal (µg/g)	
		e-liquid	aerosol	e-liquid	aerosol
Control	PG:Gly (50:50) (2.5% Nic, 15% H20)	ND*	ND*		
e-Cig A	Menthol (60:40 PG:Gly; 3.5% Nic)	ND*	ND*		
e-Cig B	Non Menthol (30:70 PG:Gly; 3.5% Nic)	ND*	ND*	1.2±0.1	1.0±0.1

Indicates transfer not formation

ND*: Not detected or <LOD (PG-acetal: 0.2ng/g, Gly-acetal: 0.3ng/g)

- PG- and Gly-acetals do not form during aerosolization process for the cig-a-like devices tested

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Summary Conclusions

- PG- and Gly-hemiacetals can be determined and reported as formaldehyde when using acidic DNPH
  - Quantitatively hydrolyze to release formaldehyde which forms the hydrazone in acidic DNPH solution
  - Do not convert to the respective acetals in acidic DNPH solution
- PG- and Gly-acetals are not formed during the aerosolization process for cig-a-like type products

➢ Our results demonstrate that commonly-used DNPH methods are fit for the analysis of formaldehyde in e-vapor products

➢ Our results **do not support** the reported claim that “Hidden Formaldehyde” in e-vapor products causes the underreporting of formaldehyde