Evaluation of the Formaldehyde Hemiacetals and Acetals Relevant to Electronic Cigarettes

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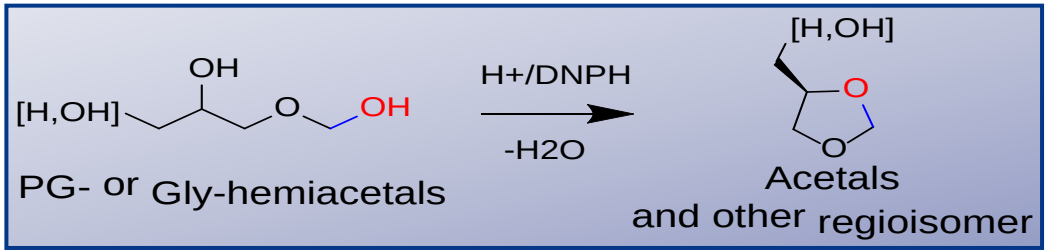
Background

- Formaldehyde is recommended for reporting in the FDA draft guidance for e-vapor products[1]
- Methods for determination of formaldehyde typically use 2,4-Dinitrophenylhydrazine (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]

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

- Reported presence of formaldehyde hemiacetals of propylene glycol (PG) or glycerin (Gly) in e-vapor aerosol[5]
- Suggested conversion of hemiacetal to acetal in acidic DNPH solution[6]

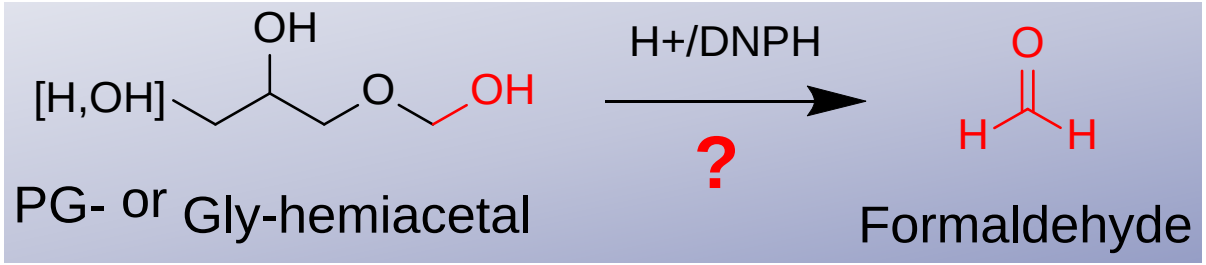


- Claimed that the commonly-used DNPH methods underestimate formaldehyde levels in e-vapor

Objective

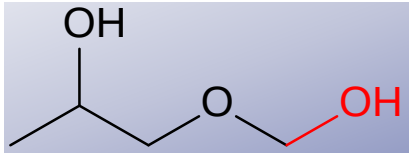
- Evaluate the DNPH method performance for analysis of formaldehyde in e-vapor aerosol:
  1. Do PG- and Gly-hemiacetals hydrolyze to formaldehyde in acidic DNPH solution?
  2. Do PG- and Gly-hemiacetals convert to the corresponding acetals in acidic DNPH solution?
- Evaluate if acetals are formed in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

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

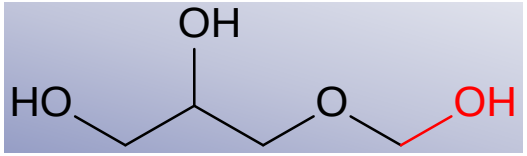


Approach

- 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?

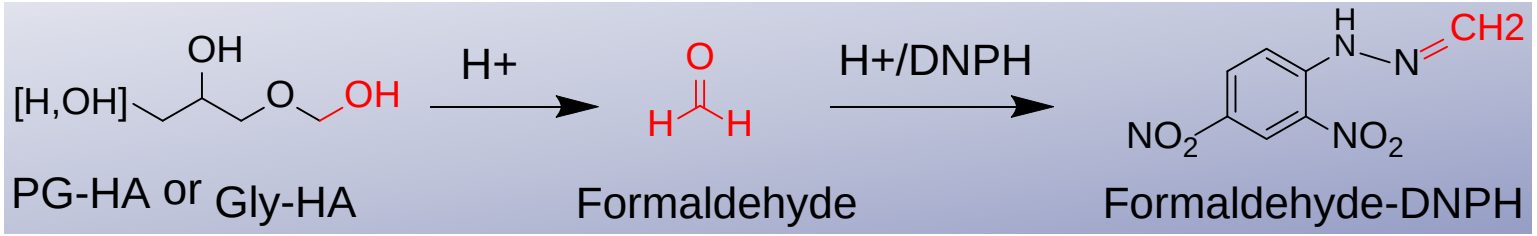
	Formaldehyde theoretical (µg/mL)	Formaldehyde determined (µg/mL)	Recovery (%)
PG-HA (3.19 µg/mL)	0.93	0.96	**103**
Gly-HA (5.86 µg/mL)	1.44	1.50	**104**

- 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]

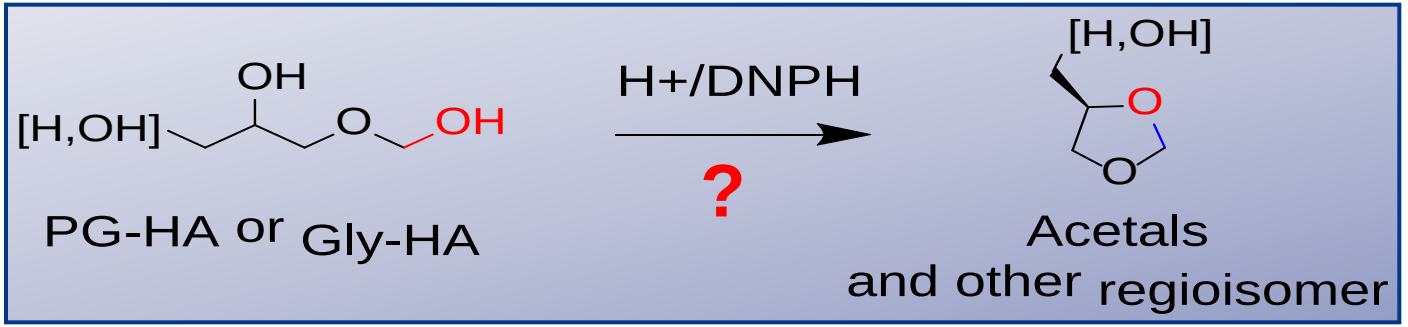
Summary

- 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



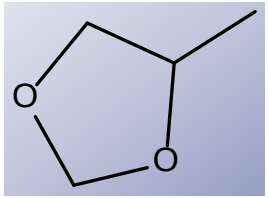
Do PG-HA and Gly-HA Convert to the Corresponding Acetals in Acidic DNPH Solution?



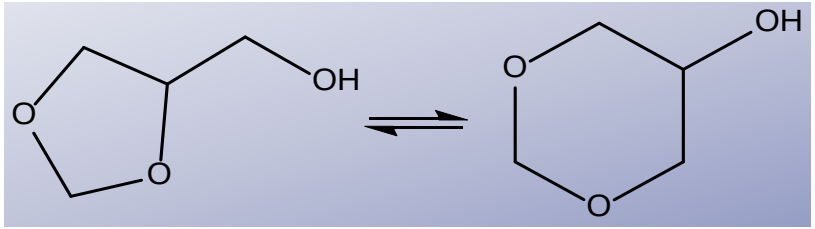
Approach

- 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

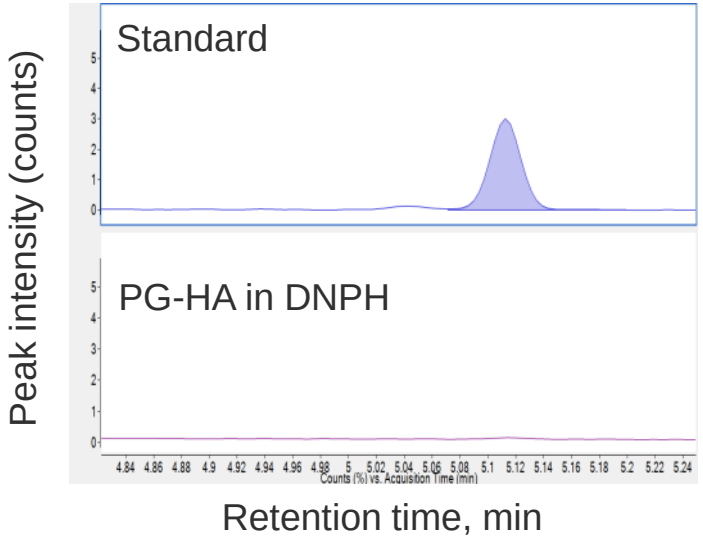
- Do hemiacetals convert to acetals?

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

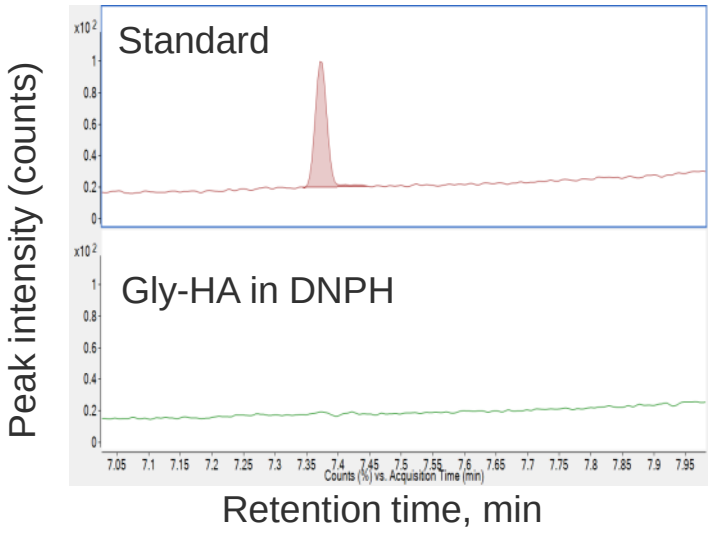
Results

- Do hemiacetals convert to acetals?

Chromatograms of PG-acetal



Chromatograms of Gly-acetal

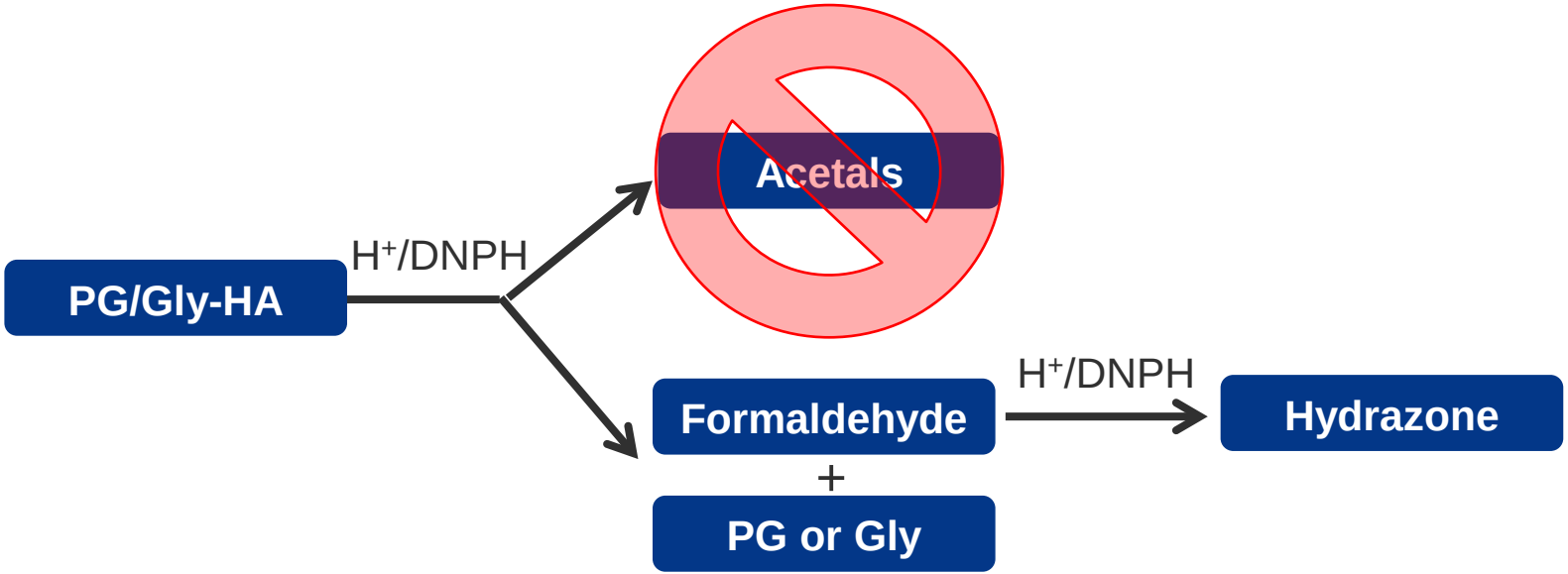


- PG- and Gly-acetals were not formed

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

- 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 (3.5% Nic)	ND*	ND*	<0.8	<0.8
e-Cig B	Non Menthol (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

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

- ➢ Our results demonstrate that commonly-used DNPH methods are fit for the analysis of formaldehyde in e-vapor products
- ➢ Our results **contradict** the reported claim that “Hidden Formaldehyde” in e-vapor products causes the underreporting of formaldehyde

References

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- **Further data and details:**
  www.altria.com/ALCS-Science