Optimization of 2,4-dinitrophenylhydrazine (DNPH) derivatization conditions for the determination of carbonyl compounds in e-vapor products

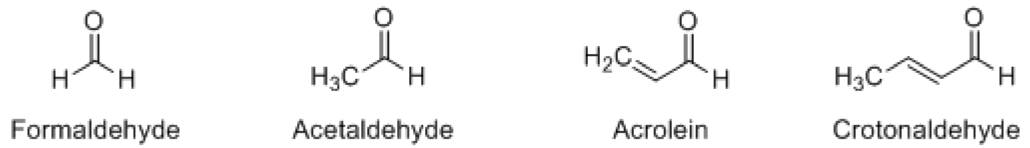
Lena Jeong, John Miller, Niti Shah

Altria

Altria Client Services

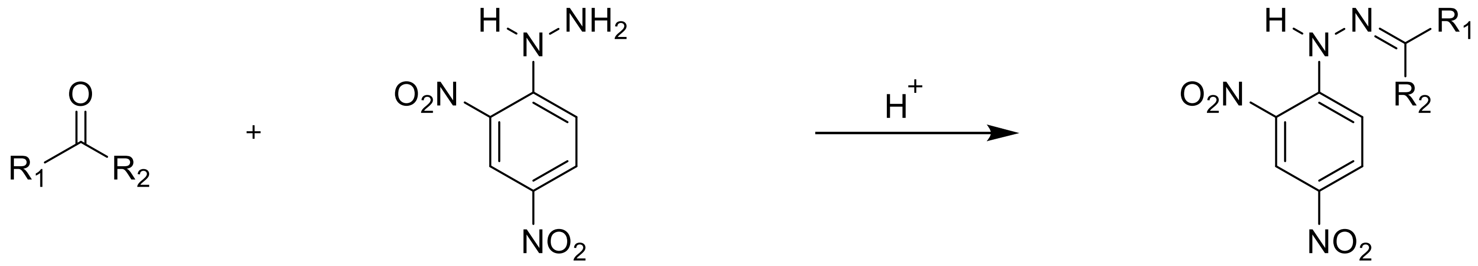
Background

- FDA requires reporting of carbonyls in e-vapor products*:



- ***No CRM available for measuring carbonyls in e-vapor products***
  - CRM 74: mainstream cigarette smoke
  - CRM 86: tobacco and tobacco products

- Carbonyl compounds react with 2,4-dinitrophenylhydrazine (DNPH) the presence of an acidic catalyst to form the respective hydrazones



*FDA Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems Final Guidance for Industry. 2019.
CRM: CORESTA recommended method

Current Methods

	DNPH conc.	Acid	Diluent
CRM 74	11.65 mM	2.05 M phosphoric acid	50/50 ACN/H_2O
Altria (published)	17.5 mM	1.82 M perchloric acid	ACN

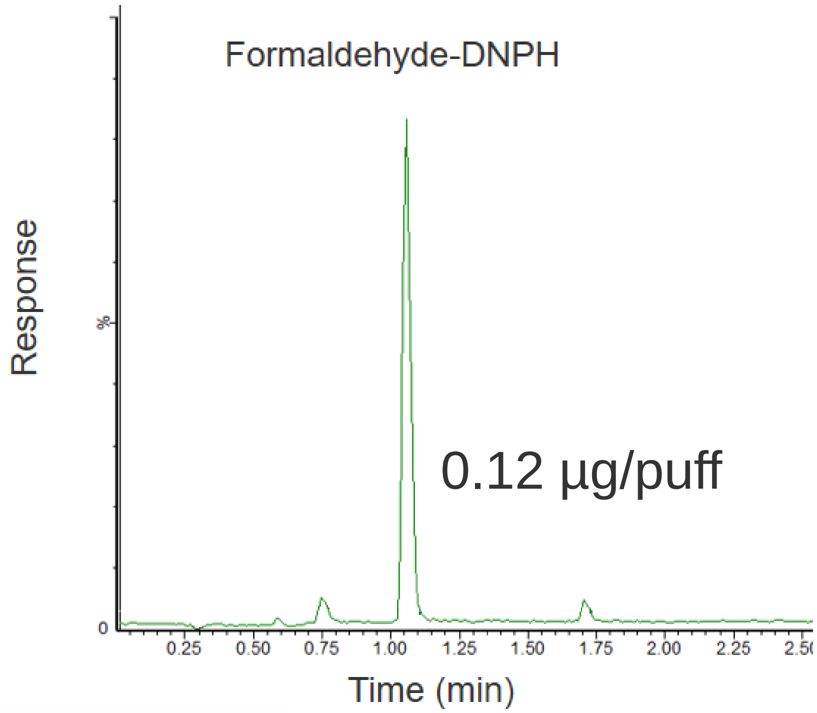
Challenges with current methods:

- ***High background for formaldehyde in current DNPH***
- ***Low and unstable recovery for acrolein***

J.W. Flora et al., Method for the Determination of Carbonyl Compounds in E-Cigarette Aerosols, *Journal of Chromatographic Science*, 55 (2017), 1421-148

1. Formaldehyde Contamination in DNPH

DNPH ~30% H_2O



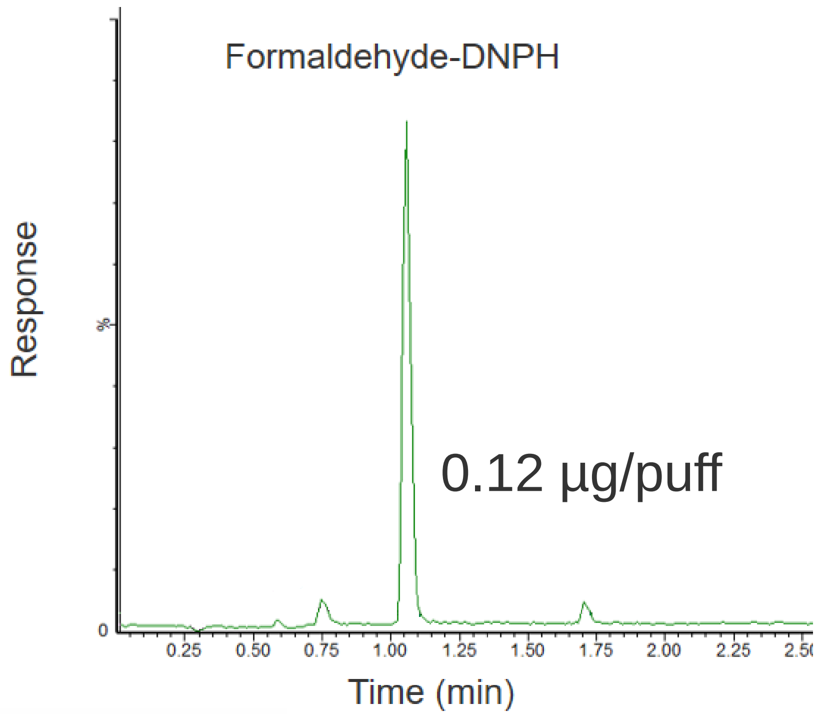
High background signals for Formaldehdye

- Problematic for low level quantitation
- Often requires recrystallization
- Lot-to-lot variation in background levels
- Limited availability (issue for high volume testing)

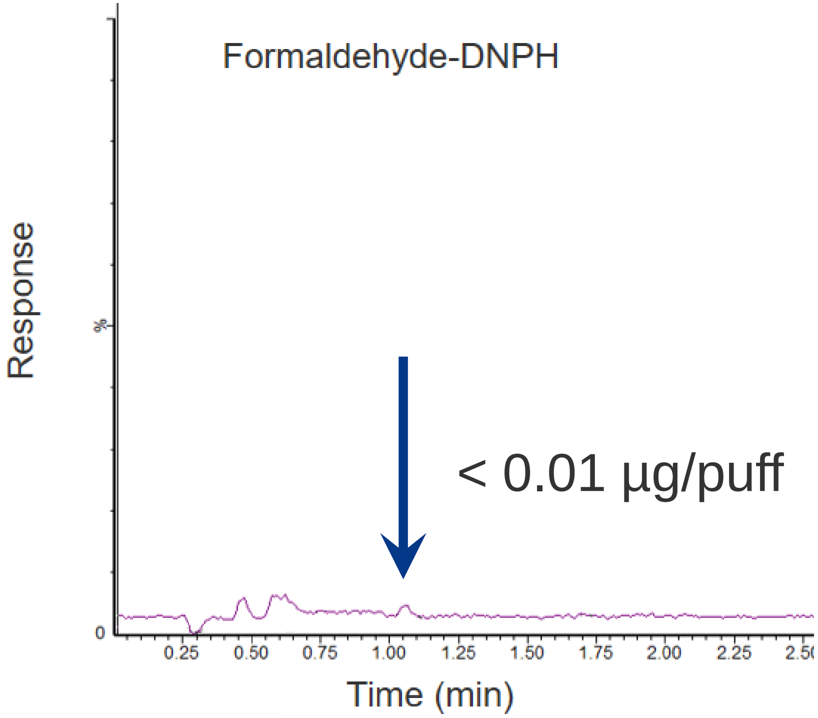
Alternate DNPH type desired

1. Formaldehyde Contamination in DNPH

DNPH ~30% H_2O



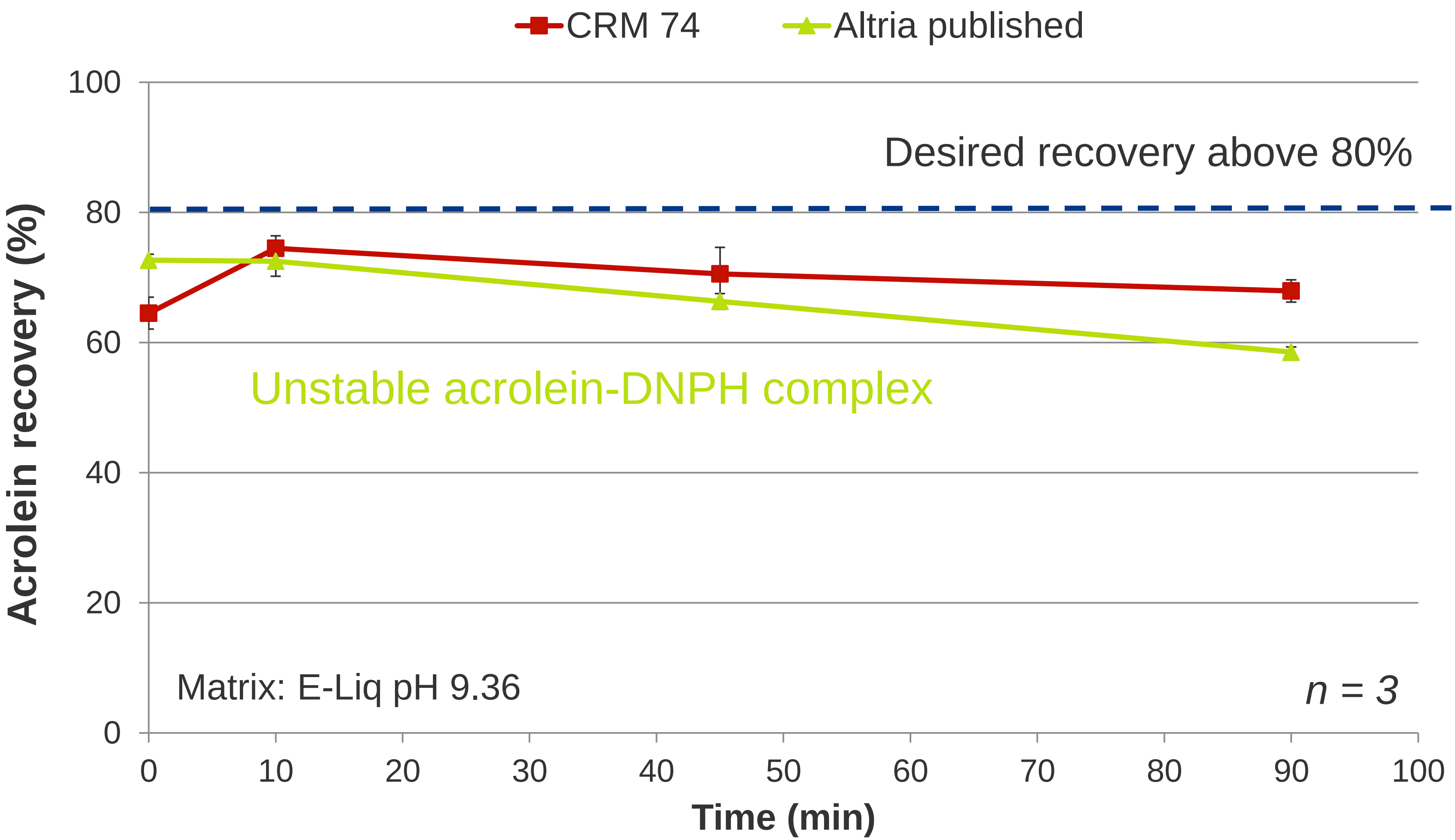
DNPH-HCl



Switching to HCl salt form dramatically reduced background carbonyl levels

Altria
Altria Client Services

2. Low and Unstable Acrolein Recovery



J.W. Flora et al., Method for the Determination of Carbonyl Compounds in E-Cigarette Aerosols, *Journal of Chromatographic Science*, 55 (2017), 1421-148

Investigation into Low Acrolein Recovery

Polyderivatization of Acrolein*

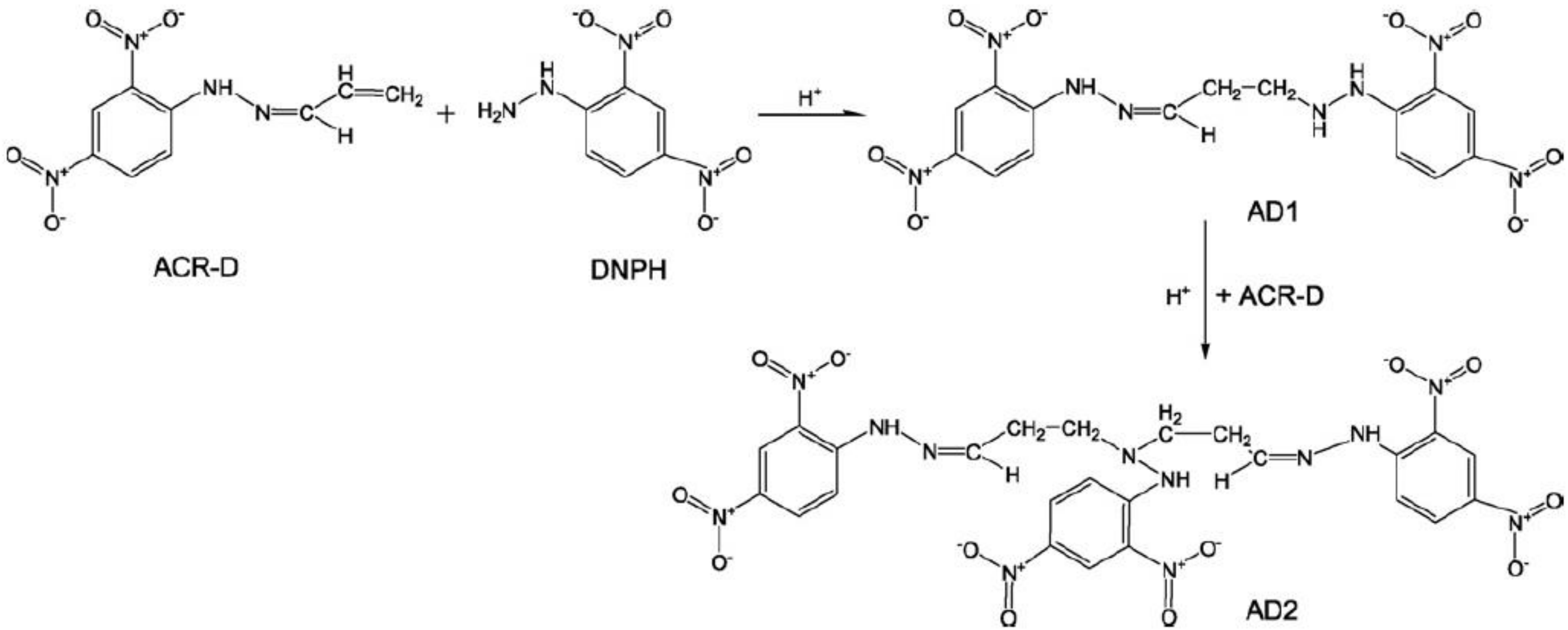


Fig. 2. Decomposition of ACR-D with DNPH.

Polyderivatization increases under strong acidic conditions

*S. Uchiyama, Y. Inaba, N. Kunugita, J. Chromatogr. A, 1217 (2010) 4383-4388. doi: 10.1016/j.chroma.2010.04.056

Method Optimization

- Need to optimize method to:

  - Reduce formaldehyde background using new DNPH-HCl
  - Obtain higher and more stable acrolein recovery

- Evaluate preparation of DNPH solution:

  - Acid type/concentration
  - DNPH concentration
  - Solvent ratio

Derivatization Optimization

Evaluation of pH and DNPH concentration

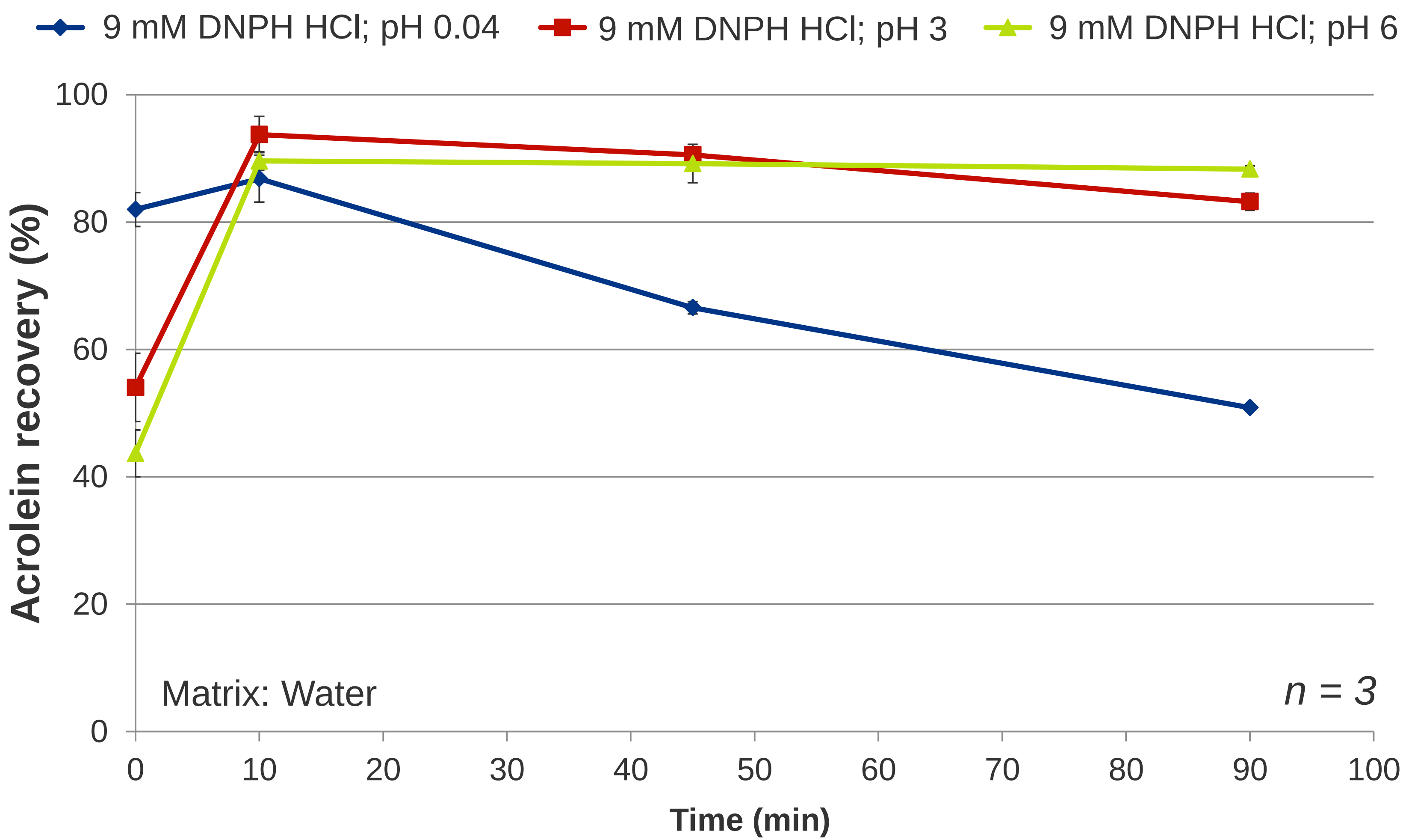
- Concentration of DNPH (9 mM; 4.5 mM; 1.8 mM) in ACN solution prepared with 1.5 % (v/v) of

  - 1.82 M perchloric acid pH 0.04
  - 0.1 M sodium citrate buffer pH 3
  - 0.1 M sodium citrate buffer pH 6

Literature reports that acidity of derivatization solution has a significant impact on reaction rate and stability

Altria
Altria Client Services

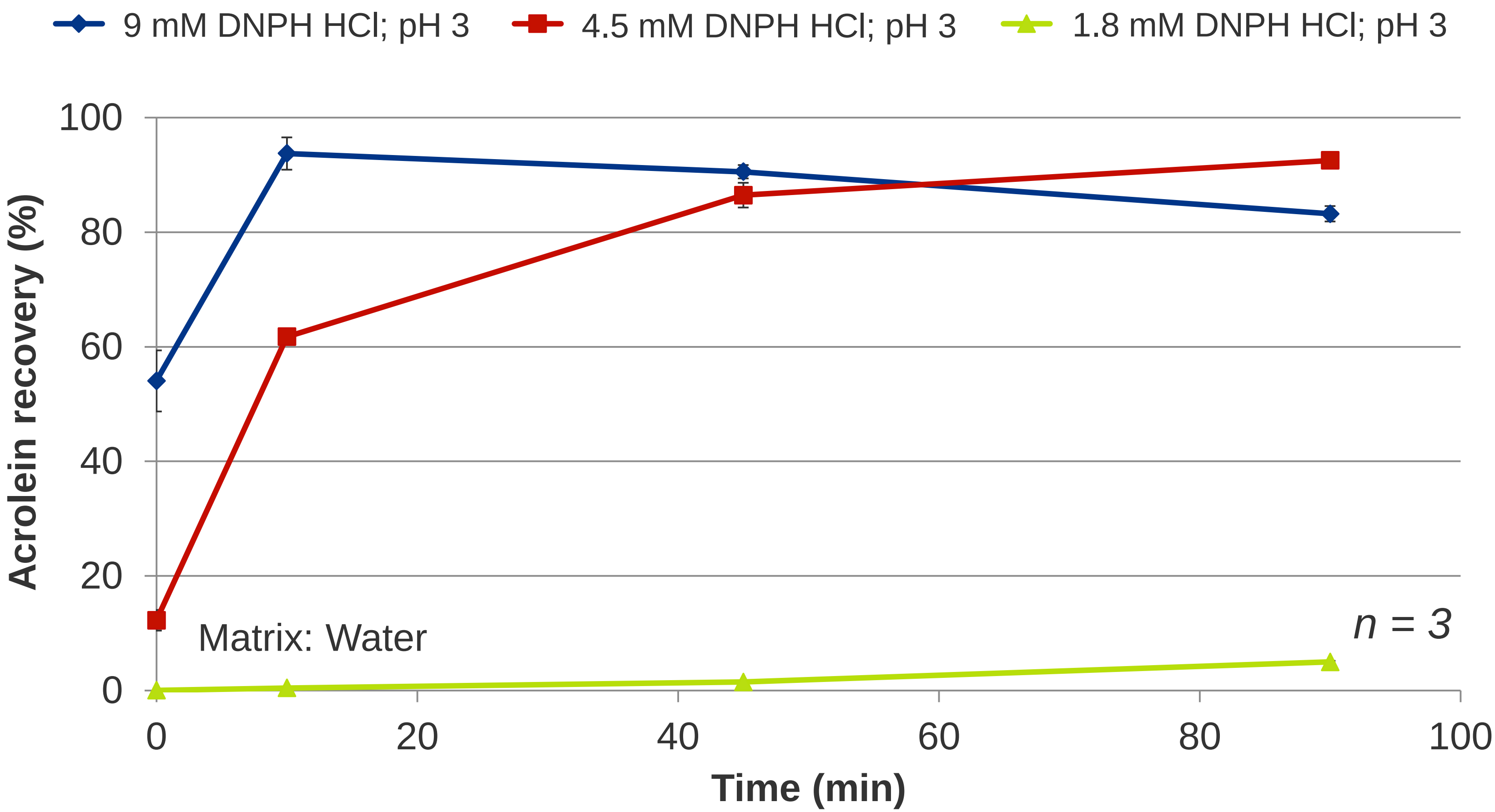
Effect of pH



Low pH results in decomposition of acrolein-DNPH complex

Altria
Altria Client Services

Effect of DNPH Concentration



DNPH concentration is directly related to the derivatization rate

Altria
Altria Client Services

Derivatization Optimization

Effect of Water Content

	DNPH conc.	Acid	Diluent
CRM 74	11.65 mM	2.05 M phosphoric acid	50/50 ACN/H_2O
Altria (published)	17.5 mM	1.82 M perchloric acid	ACN

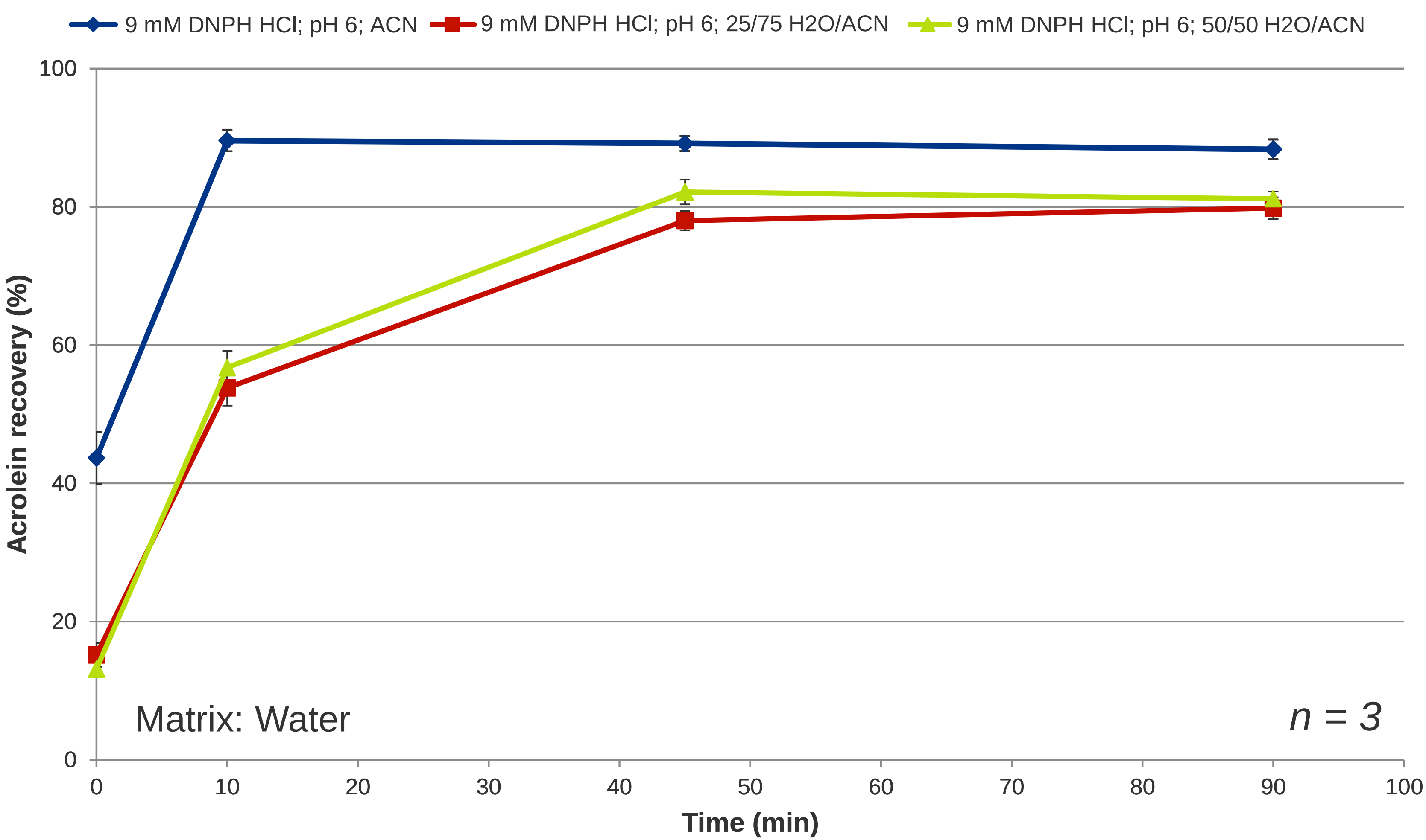
- How is derivatization rate affected in presence of added water?

Varying diluent ratios tested:

- 0/100 H_2O/ACN
- 25/75 H_2O/ACN
- 50/50 H_2O/ACN

Altria
Altria Client Services

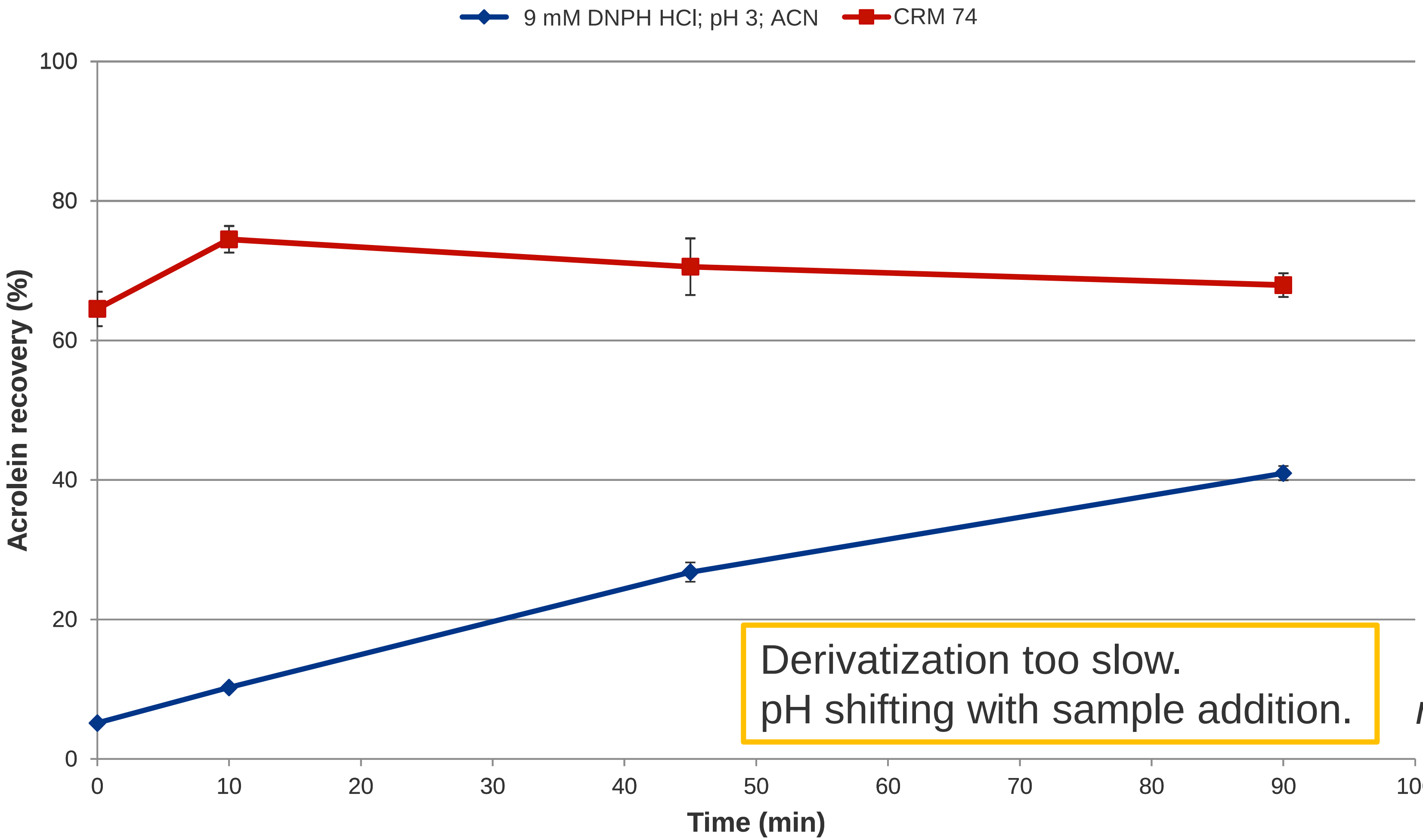
Water Content Comparison



Addition of protic solvent slows derivatization reaction

Altria
Altria Client Services

Optimized Method vs. CRM 74

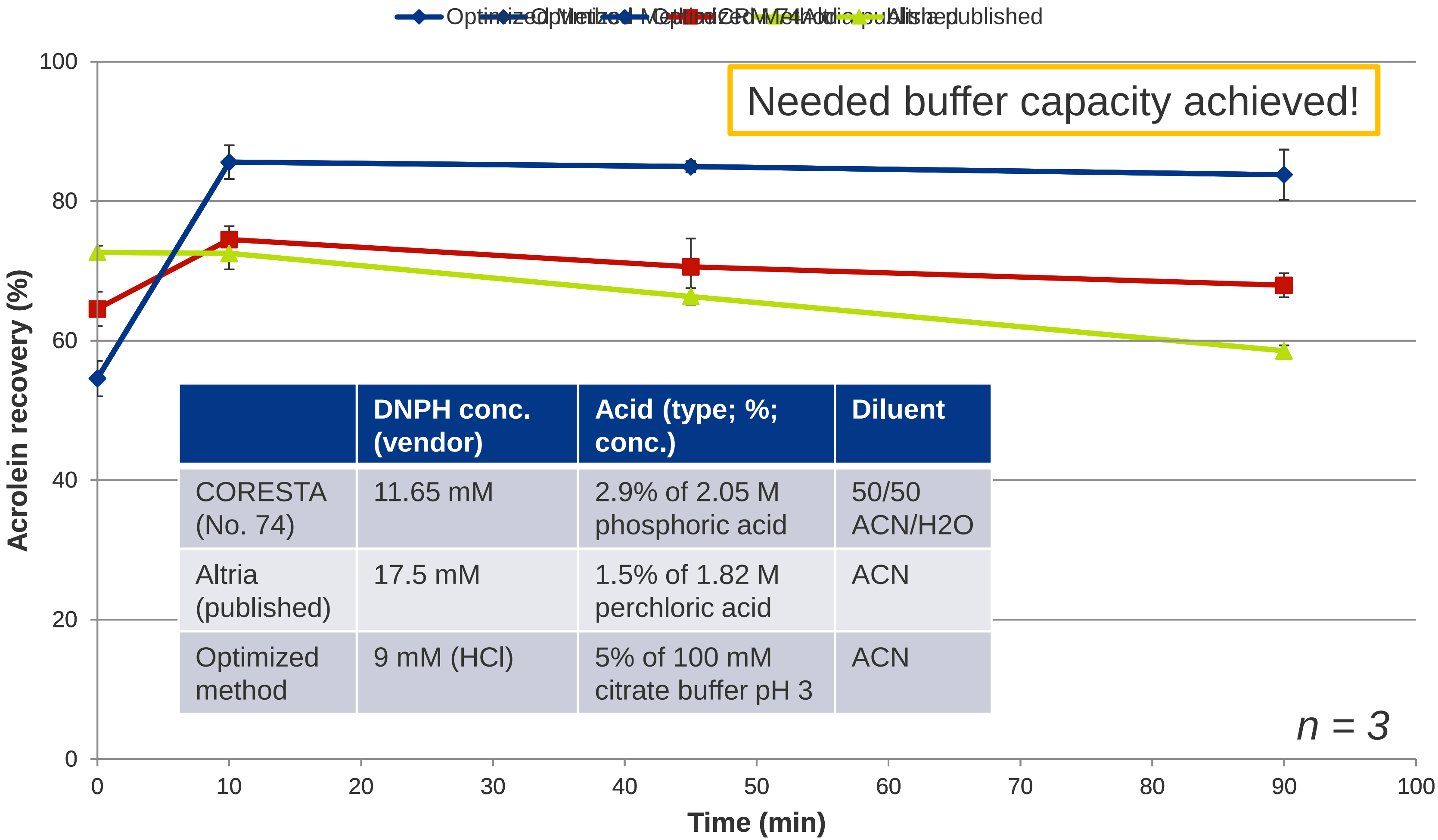


n = 3

Sample matrix: 50/50 PG/GLY with 2.5% nicotine (**pH 9.36**)

Altria
Altria Client Services

Comparison of Methods: Acrolein Recovery

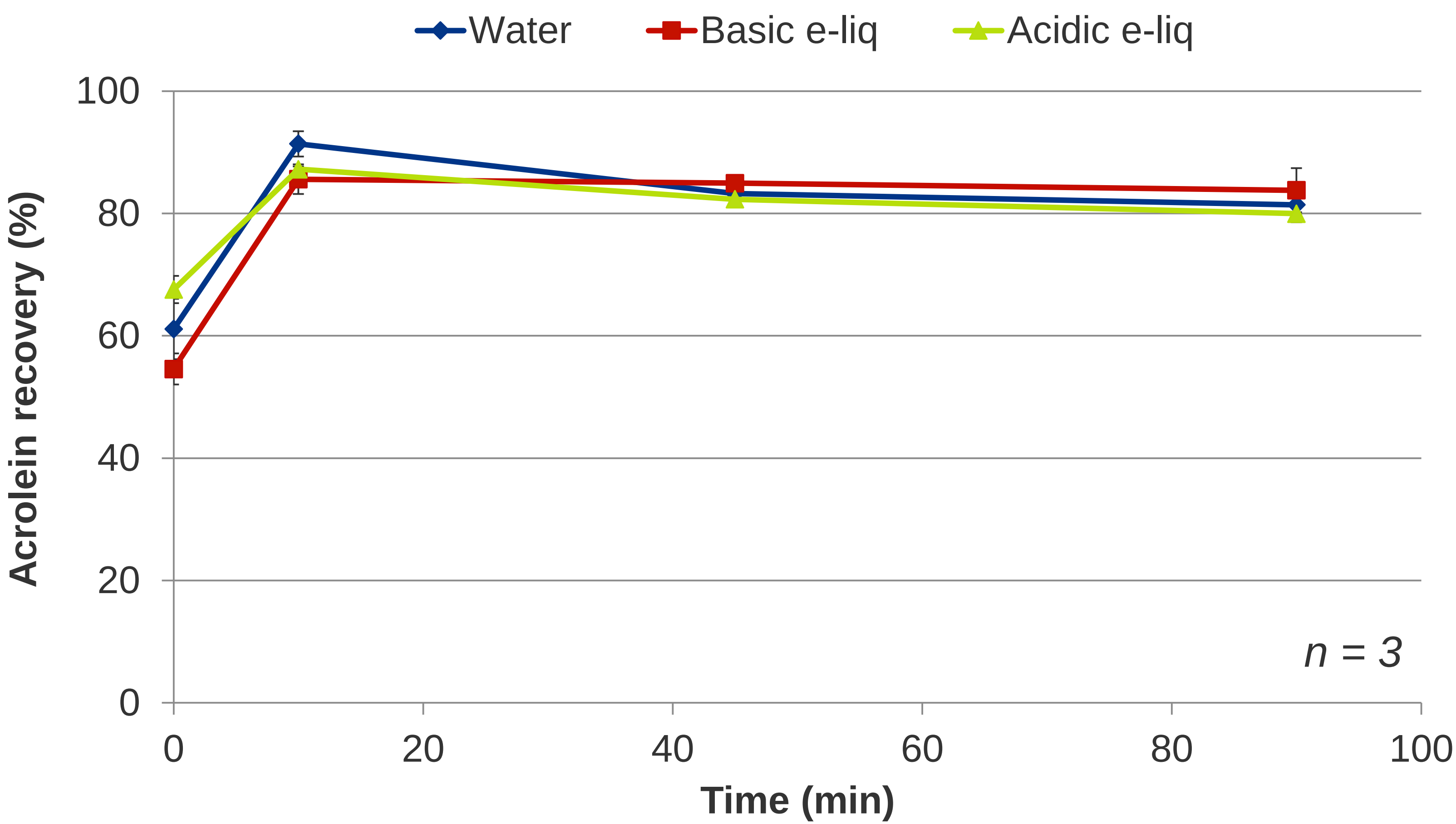


	DNPH conc. (vendor)	Acid (type; %; conc.)	Diluent
CORESTA (No. 74)	11.65 mM	2.9% of 2.05 M phosphoric acid	50/50 ACN/H2O
Altria (published)	17.5 mM	1.5% of 1.82 M perchloric acid	ACN
Optimized method	9 mM (HCl)	5% of 100 mM citrate buffer pH 3	ACN

Basic e-liquid: 50/50 PG/GLY with 2.5% nicotine (pH 9.36)

Altria
Altria Client Services

New Method Performance with Varying Sample pH



Basic e-liquid: 50/50 PG/GLY with 2.5% nicotine (pH 9.36)
Acidic e-liquid: 50/50 PG/GLY with benzoic acid (pH 3.72)

Altria
Altria Client Services

Comparison of Methods: Percent Recovery

Derivatization time: 10 min
n = 3

	Formaldehyde	Acetaldehyde	Acrolein	Crotonaldehyde
CRM 74	100% ± 2.8%	78.7% ± 0.91%	74.5% ± 1.9%	100% ± 2.4%
Altria published	87.2% ± 1.3%	77.7% ± 2.6%	72.5% ± 2.3%	103 ± 2.0%
Optimized method	102% ± 0.84%	80.7% ± 2.9%	85.6% ± 2.4%	103% ± 3.3%

Sample matrix: 50/50 PG/GLY with 2.5% nicotine (pH 9.36)

Altria
Altria Client Services

Summary - Learnings

- Switching to DNPH HCl form dramatically reduced background levels of formaldehyde
- Highly acidic DNPH solution results in polyderivatization of acrolein-DNPH (formation of AD1)
- Use of buffer to control the pH improves and stablizes acrolein recovery for over 90 min derivatization time
- Addition of protic solvent (H_2O) as diluent slows down the derivatization reaction

Altria
Altria Client Services

Conclusions

- DNPH-HCl form reduces background levels of formaldehyde and improves quantitation of carbonyls in e-vapor aerosol
- The DNPH derivatization method was optimized to give acceptable recovery levels for all aldehydes including acrolein
- New conditions allow for better stability of acrolein to extend aerosol collections

Altria
Altria Client Services