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

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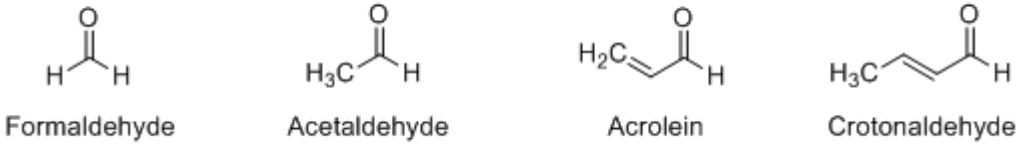
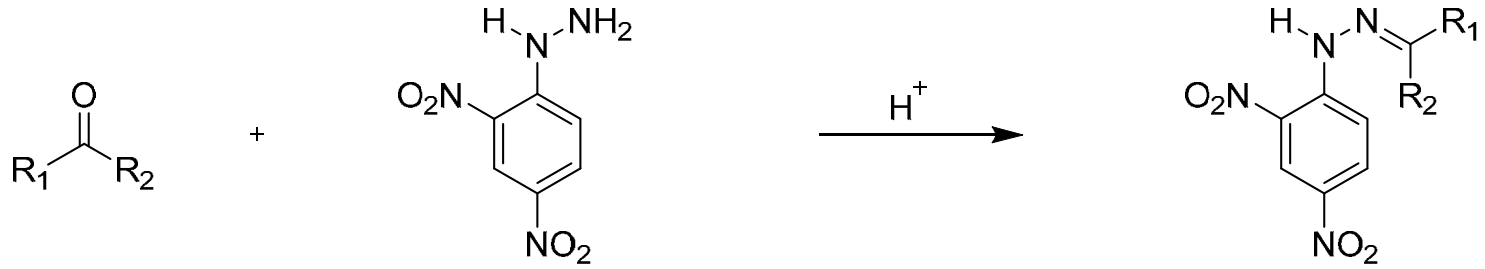
Background

- 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

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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

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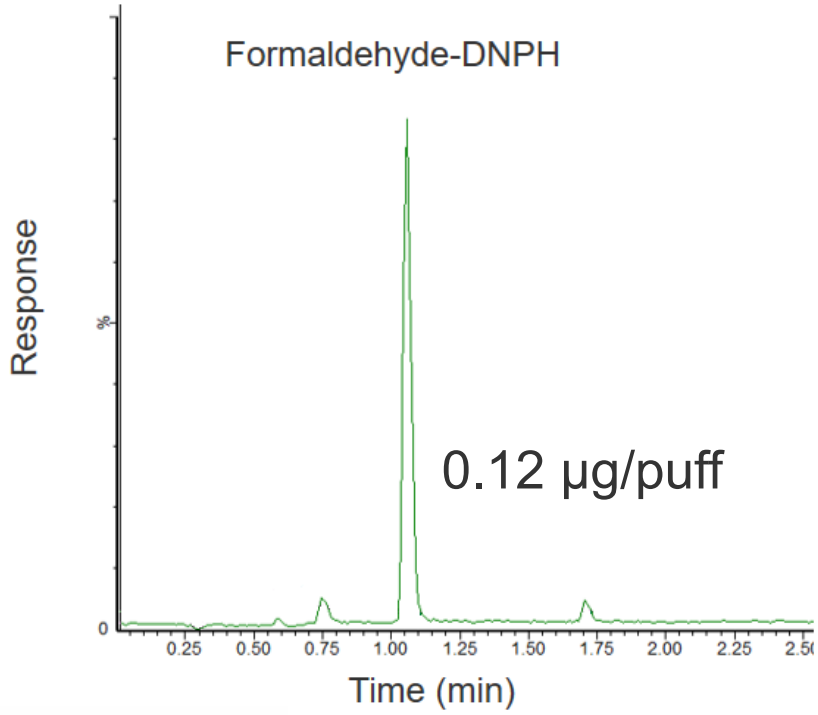
1. Formaldehyde Contamination in DNPH

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

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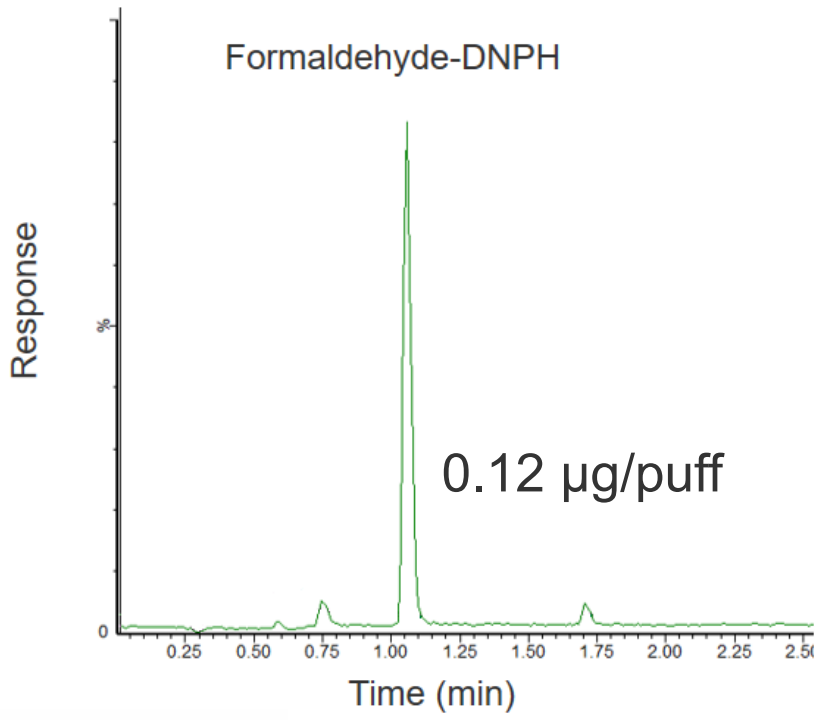
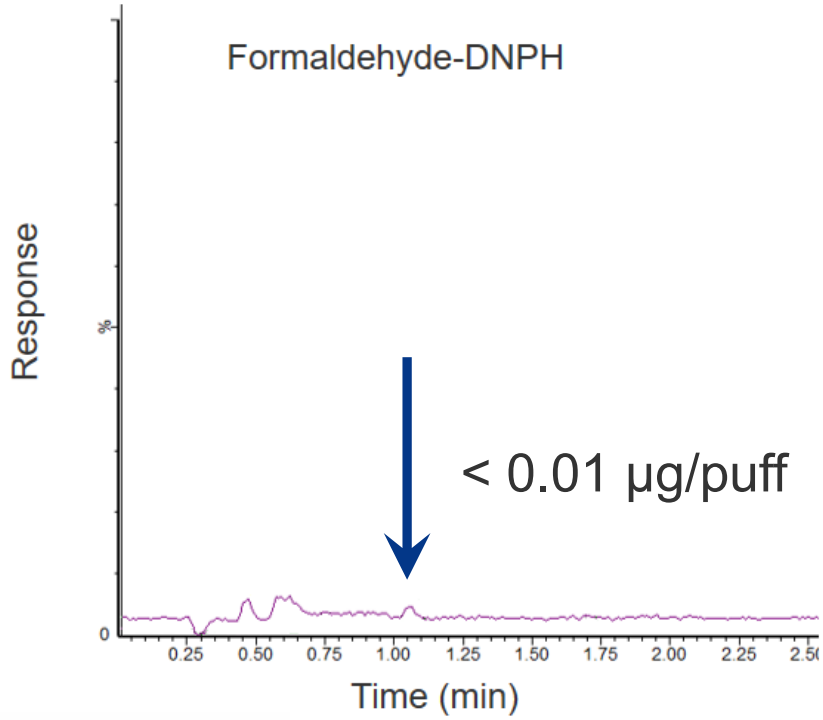
1. Formaldehyde Contamination in DNPH

DNPH ~30% H_2O



DNPH-HCl



Switching to HCl salt form dramatically reduced background carbonyl levels

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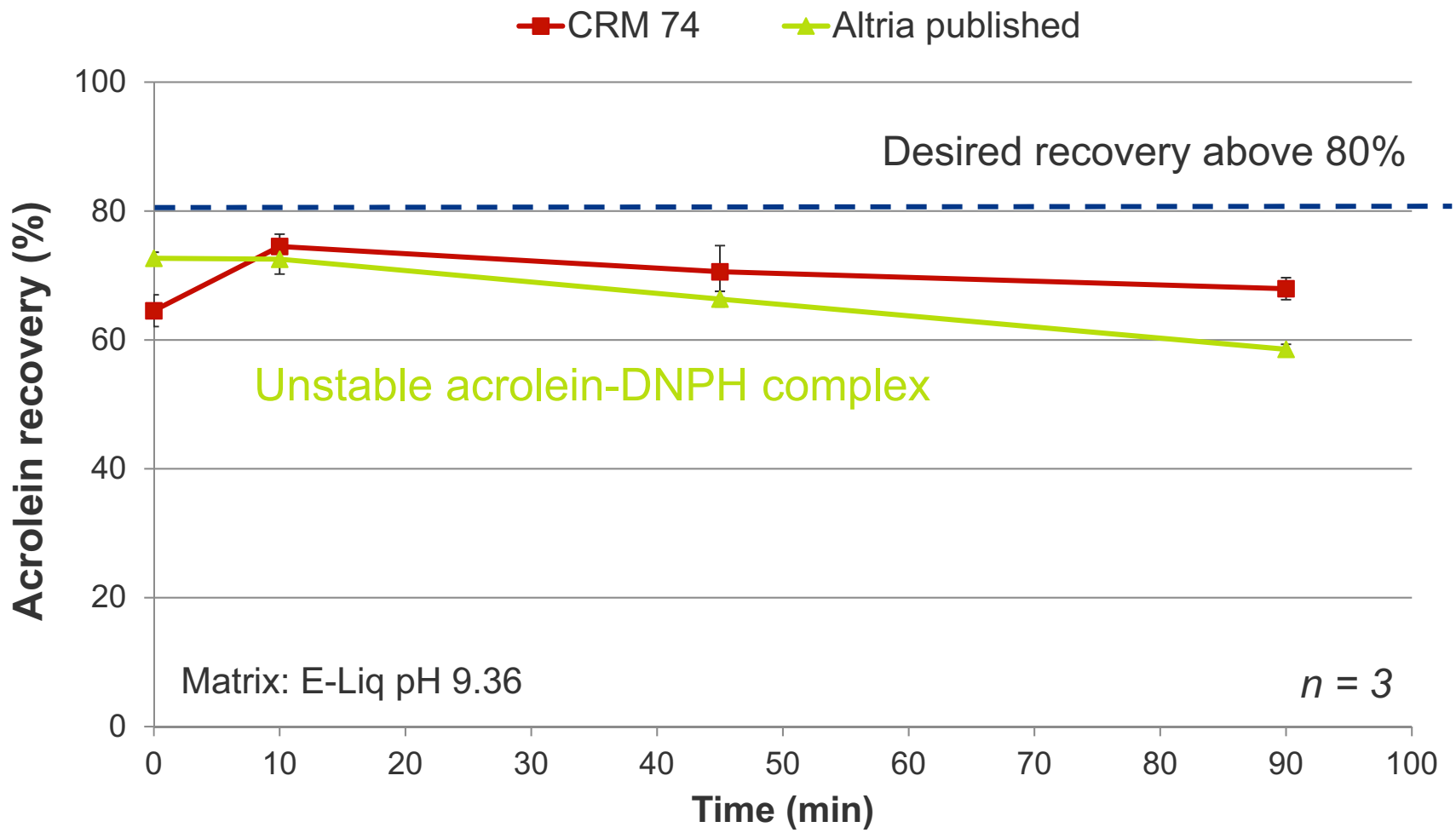
2. Low and Unstable Acrolein Recovery



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

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Investigation into Low Acrolein Recovery

Polyderivatization of Acrolein*

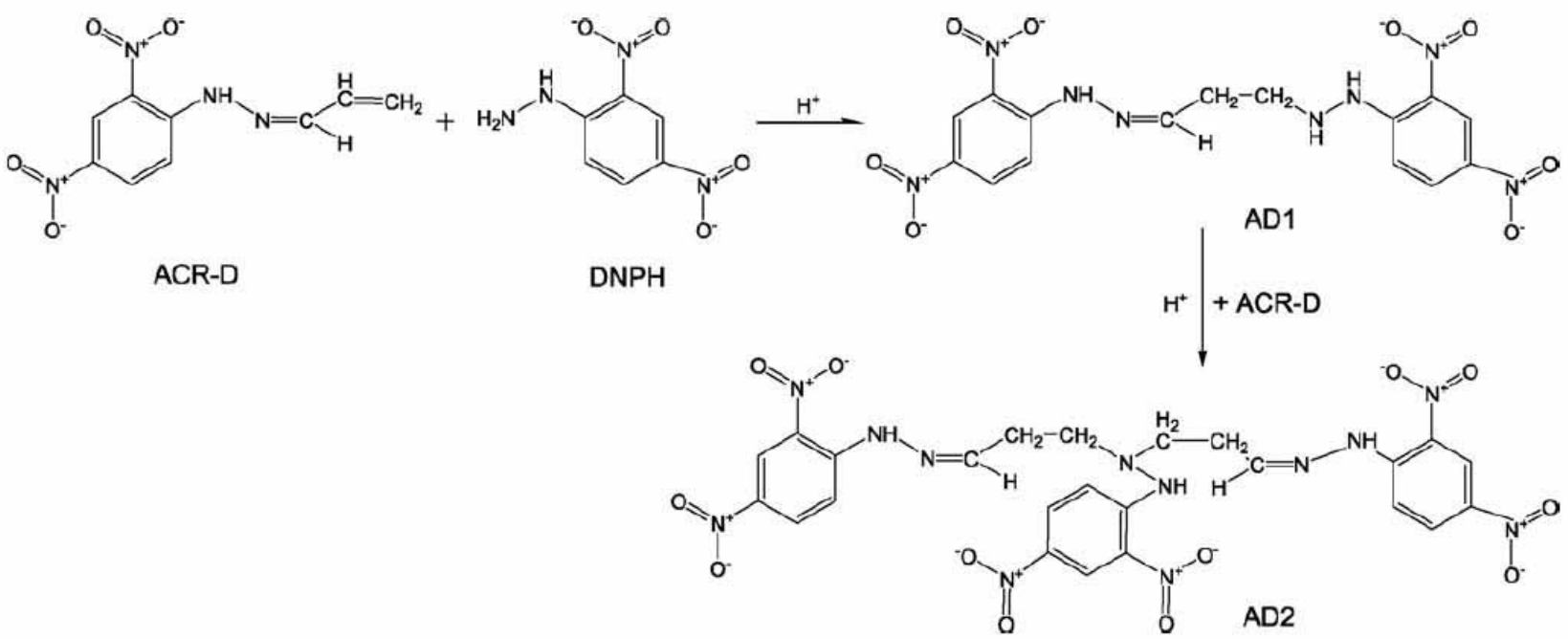

Fig. 2. Decomposition of ACR-D with DNPH.

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

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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

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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

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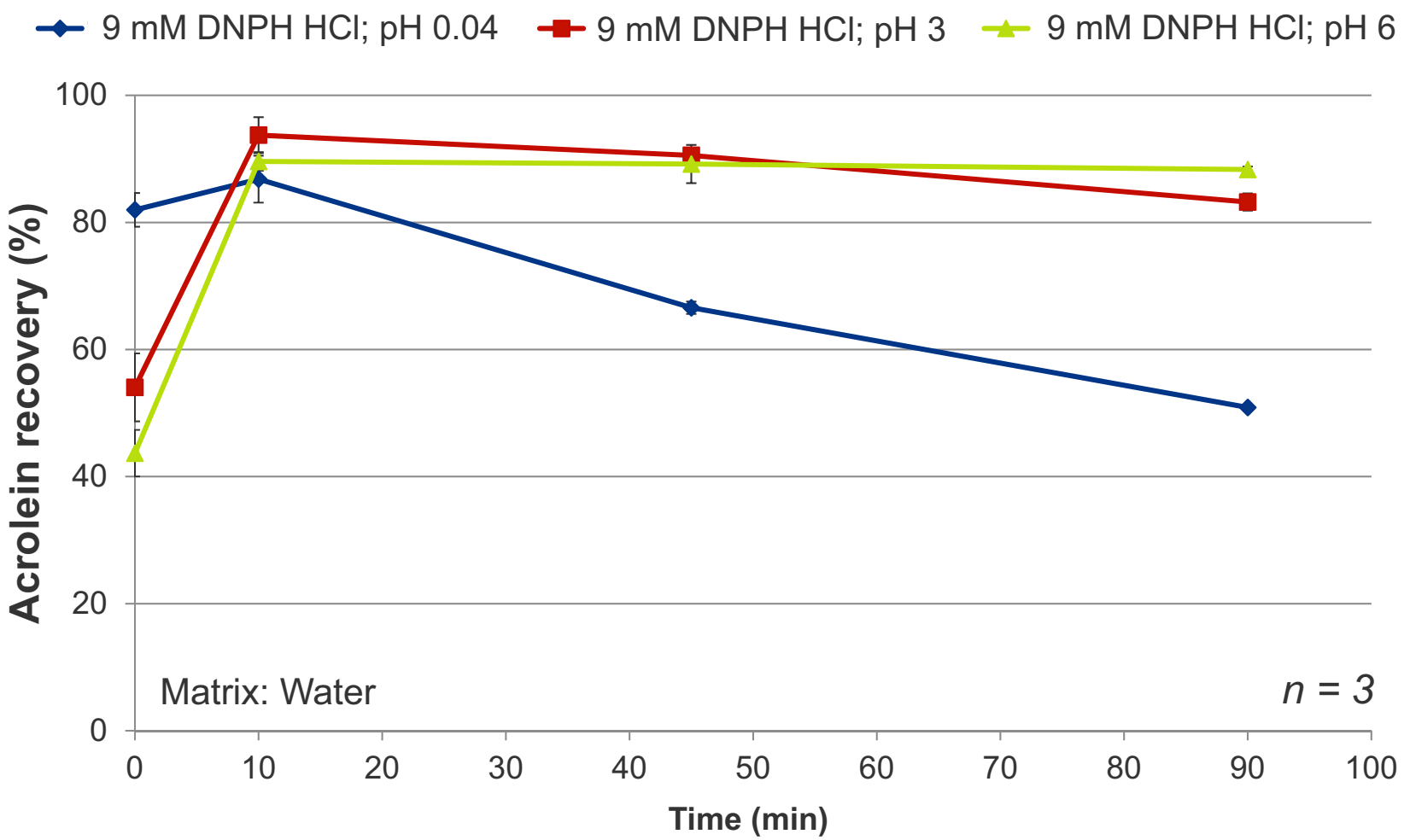
Effect of pH



Low pH results in decomposition of acrolein-DNPH complex

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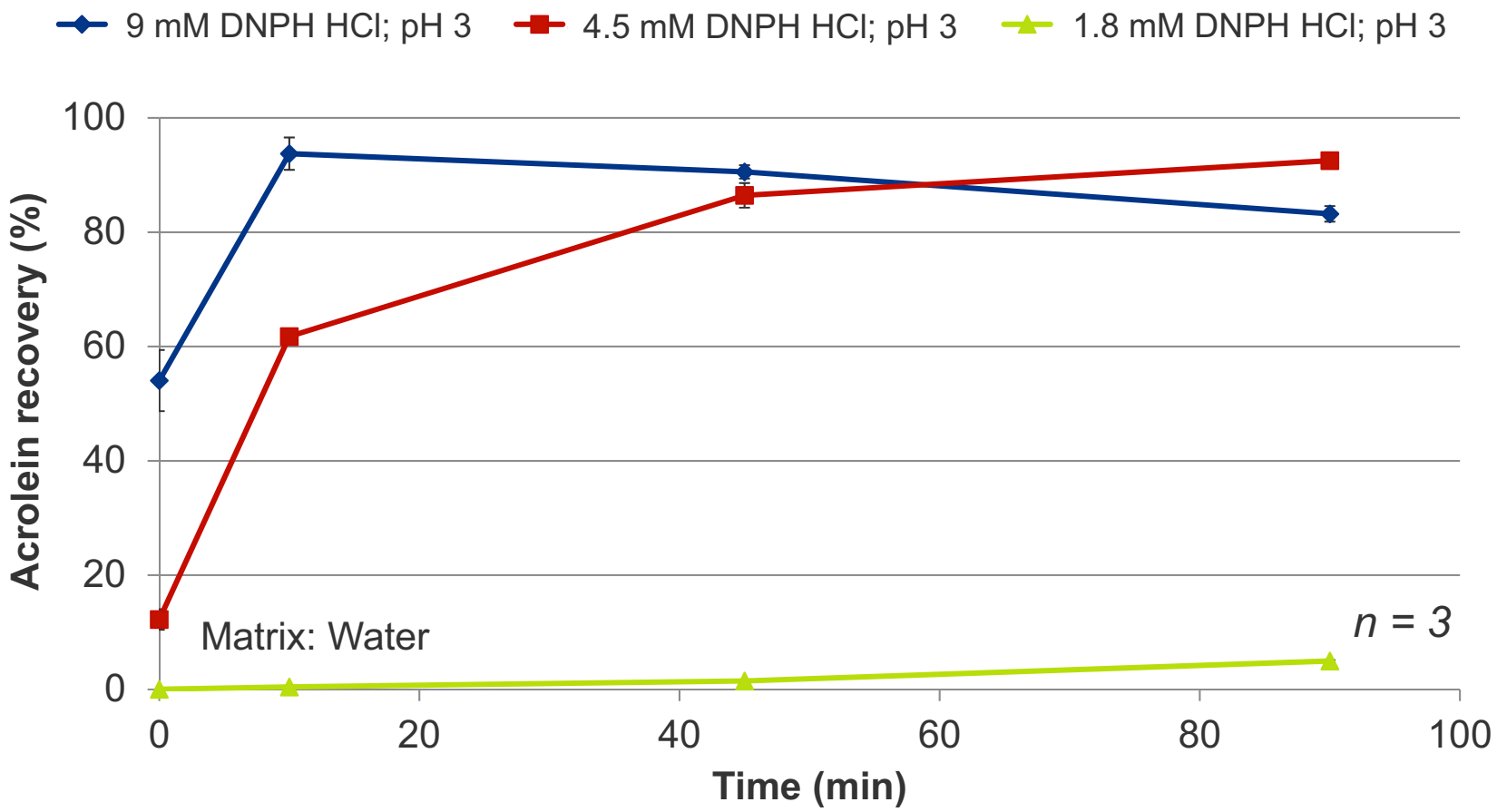
Effect of DNPH Concentration



DNPH concentration is directly related to the derivatization rate

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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

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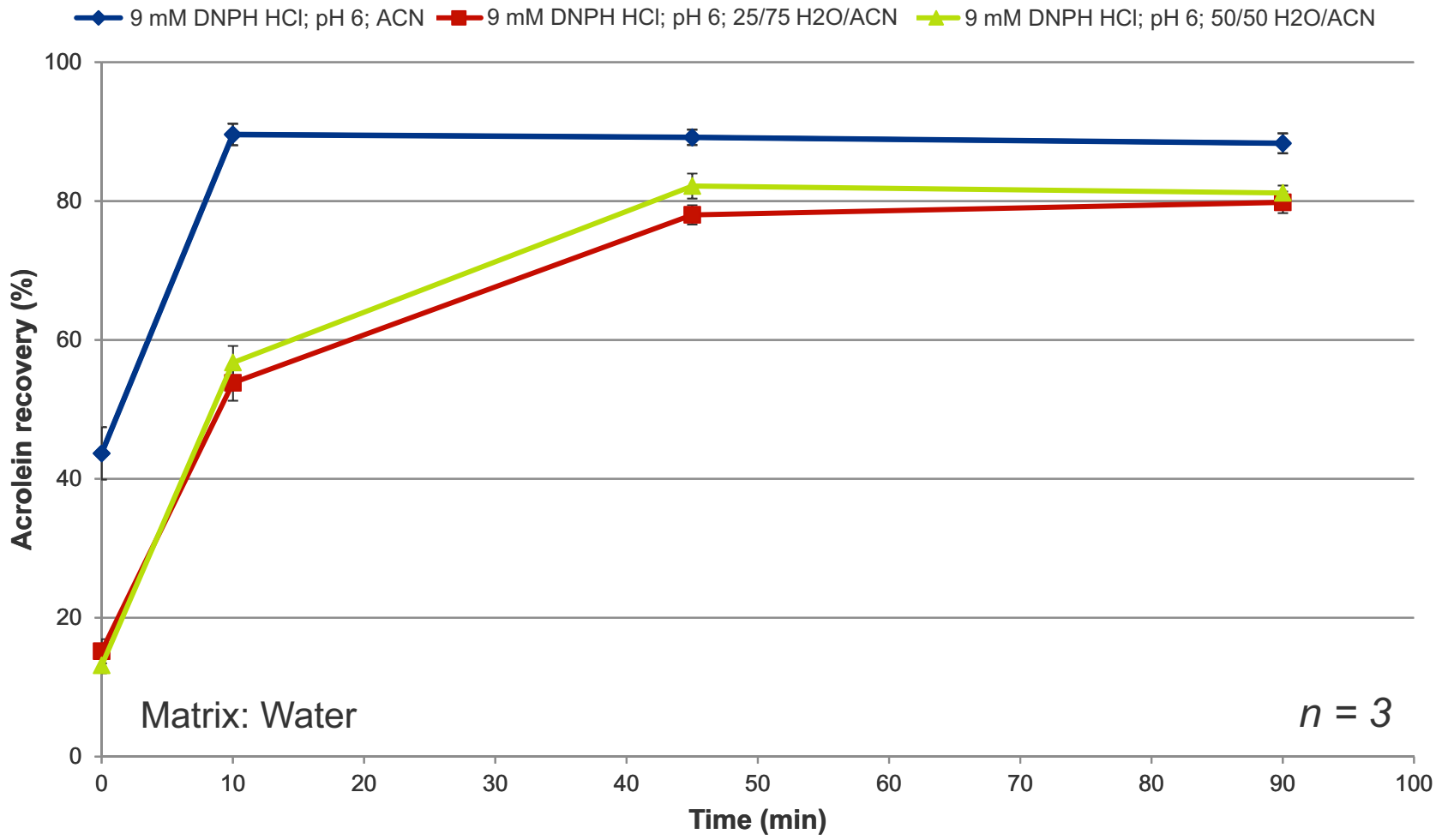
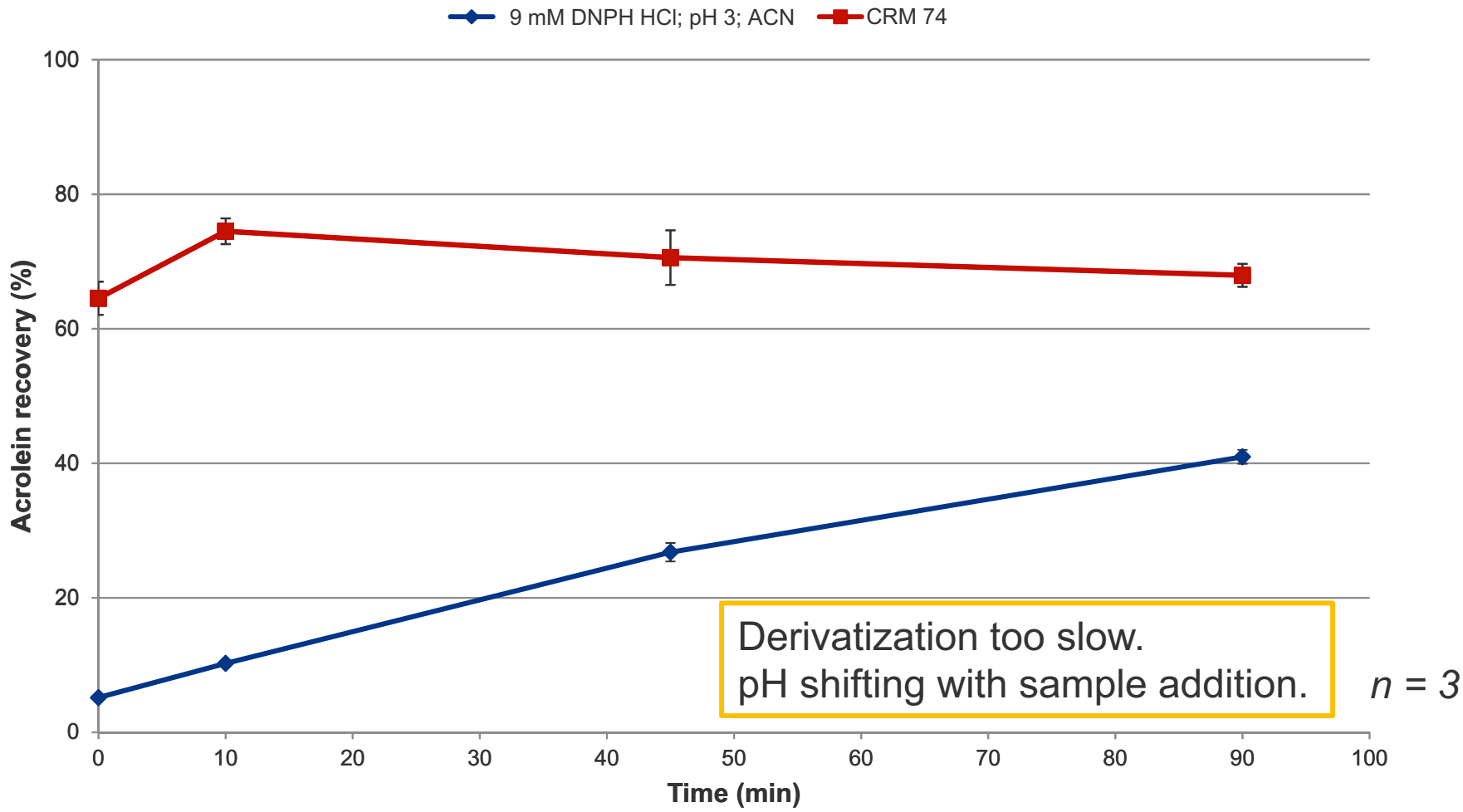
Water Content Comparison



Addition of protic solvent slows derivatization reaction

Optimized Method vs. CRM 74



Derivatization too slow.
pH shifting with sample addition.

n = 3

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

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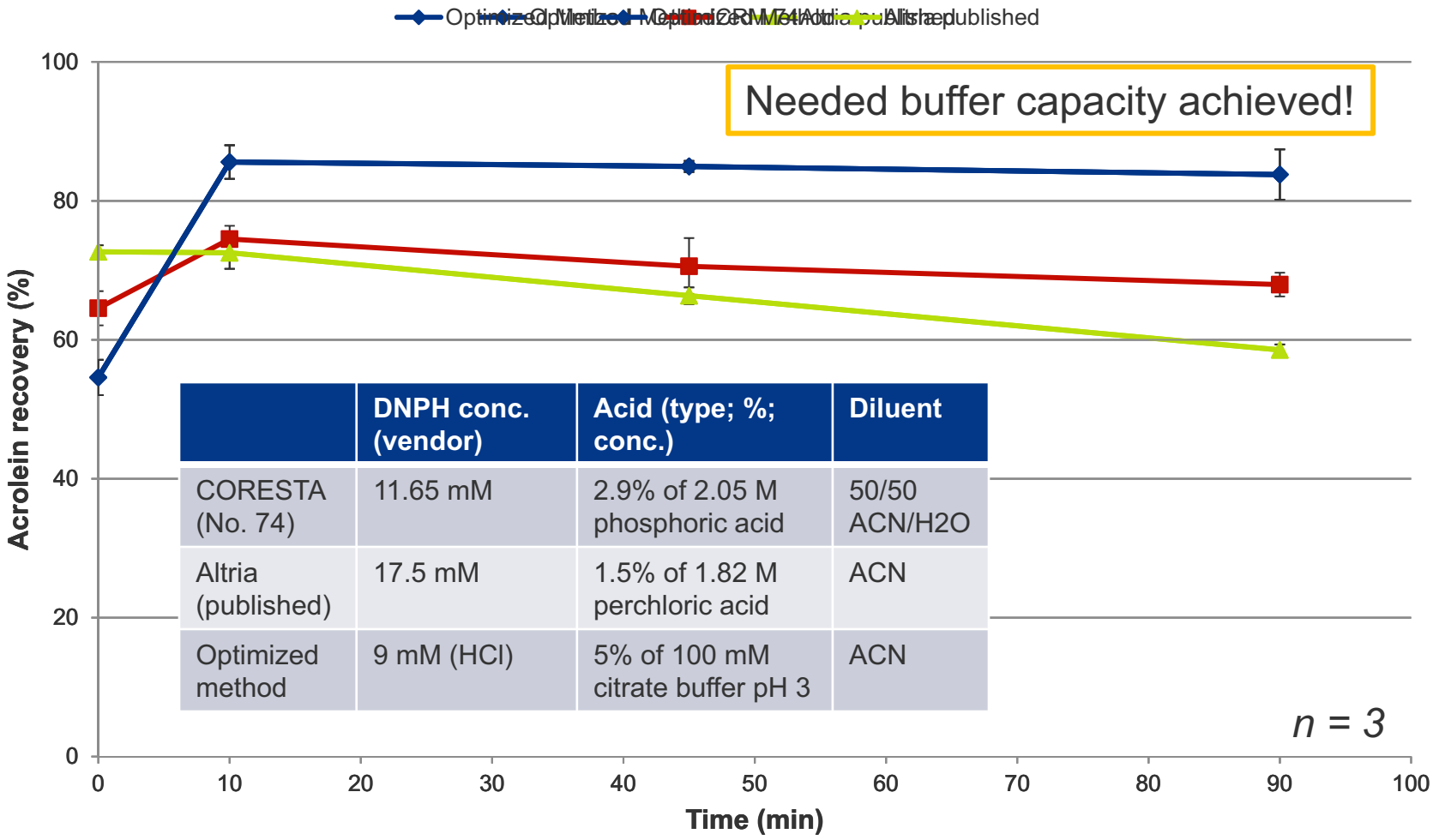
Comparison of Methods: Acrolein Recovery



	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)

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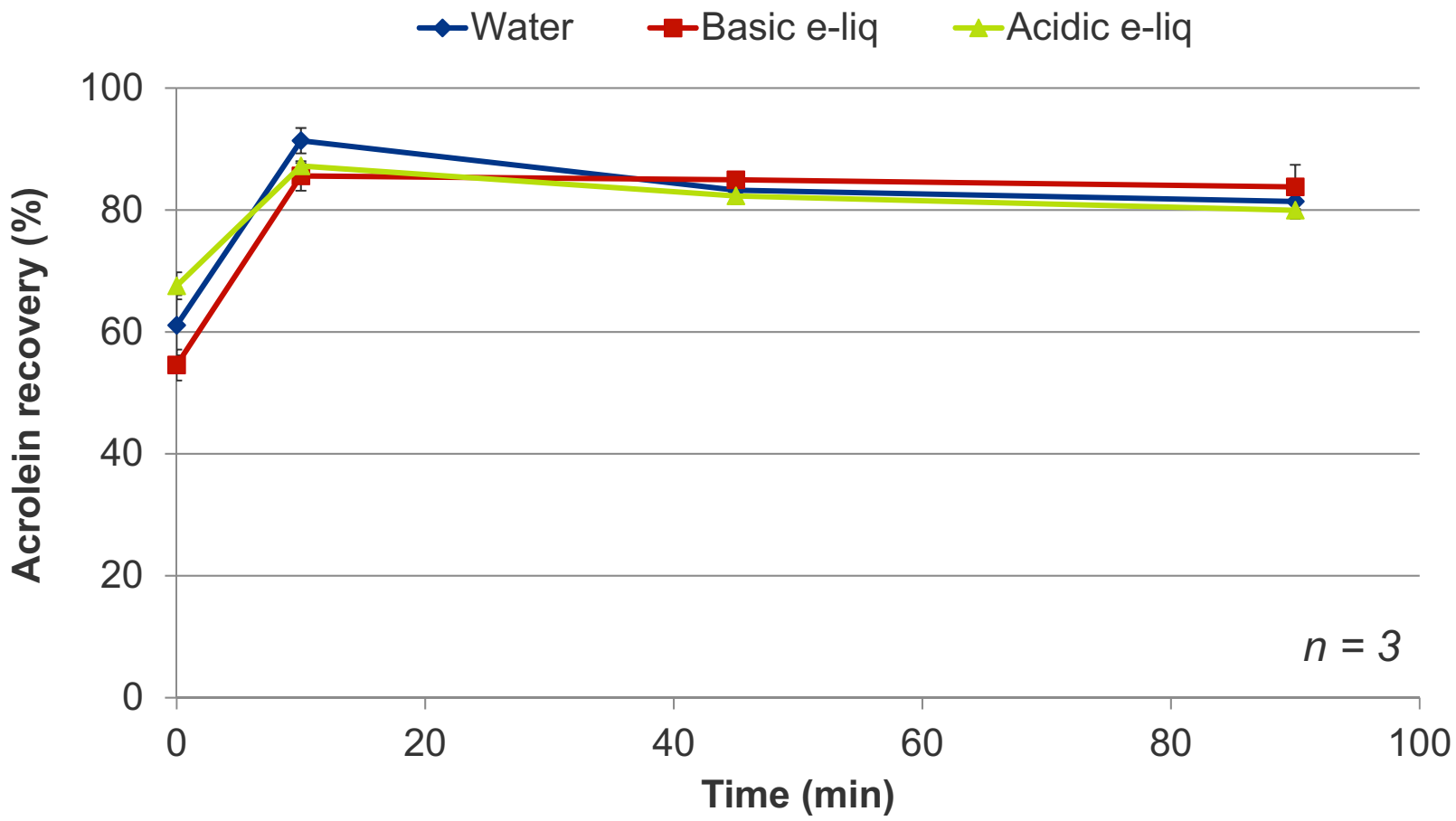
New Method Performance with Varying Sample pH



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)

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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)

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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

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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

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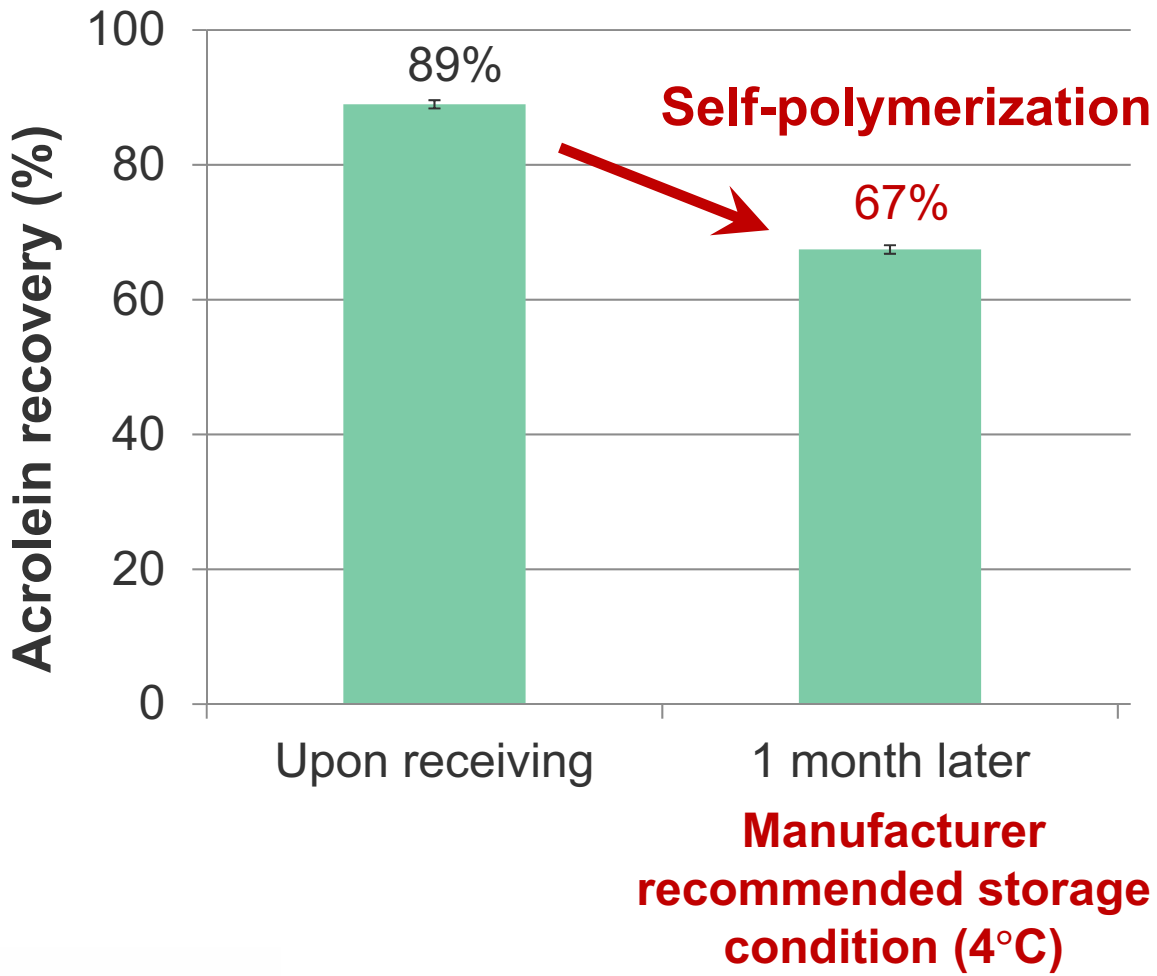
Secondary Issue with Acrolein Recovery



- New standard mix ordered
- Same lot number
- Expected recoveries to be similar

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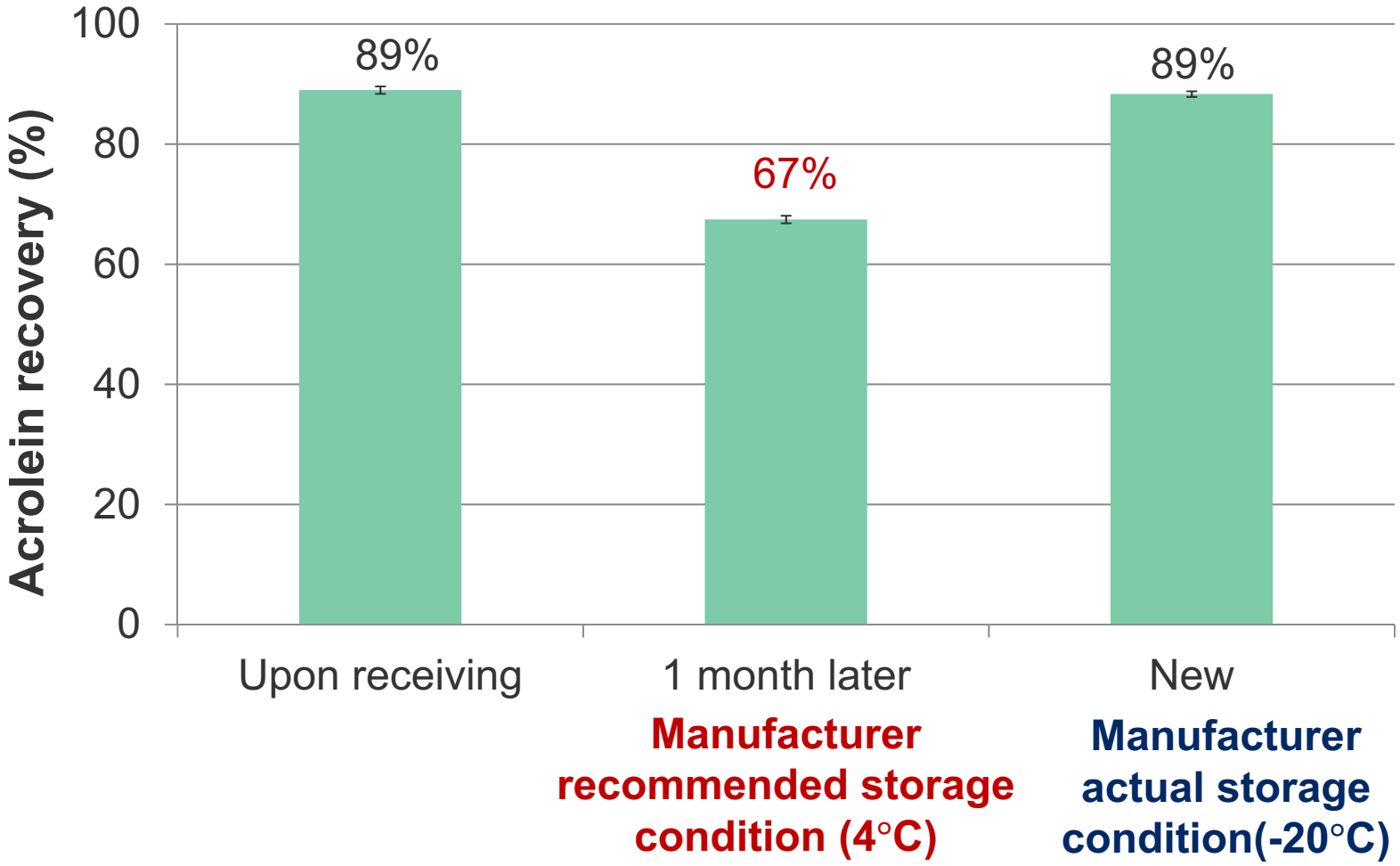
Self-polymerization of Acrolein



Storing vials at -20°C minimizes self polymerization

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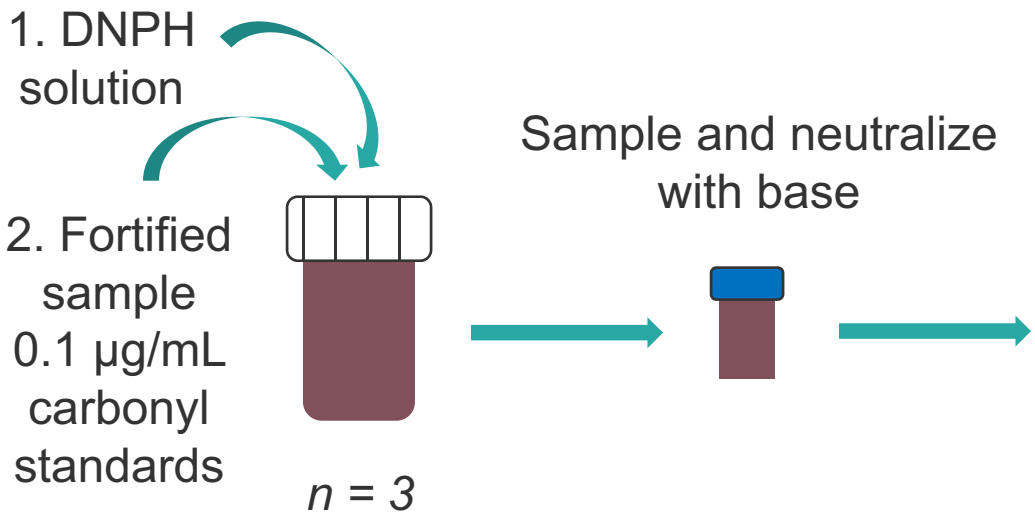
Background (cont.)



ACQUITY UPLC with MS (Waters Quattro Premier)

Formaldehyde, acetaldehyde and crotonaldehyde recoveries were within acceptable range (100%, 80%, 100%, respectively) with varying method conditions.

Data presented here focuses on acrolein recovery.

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