Capillary Zone Electrophoresis for Charge Heterogeneity of Antibody Drug Conjugates

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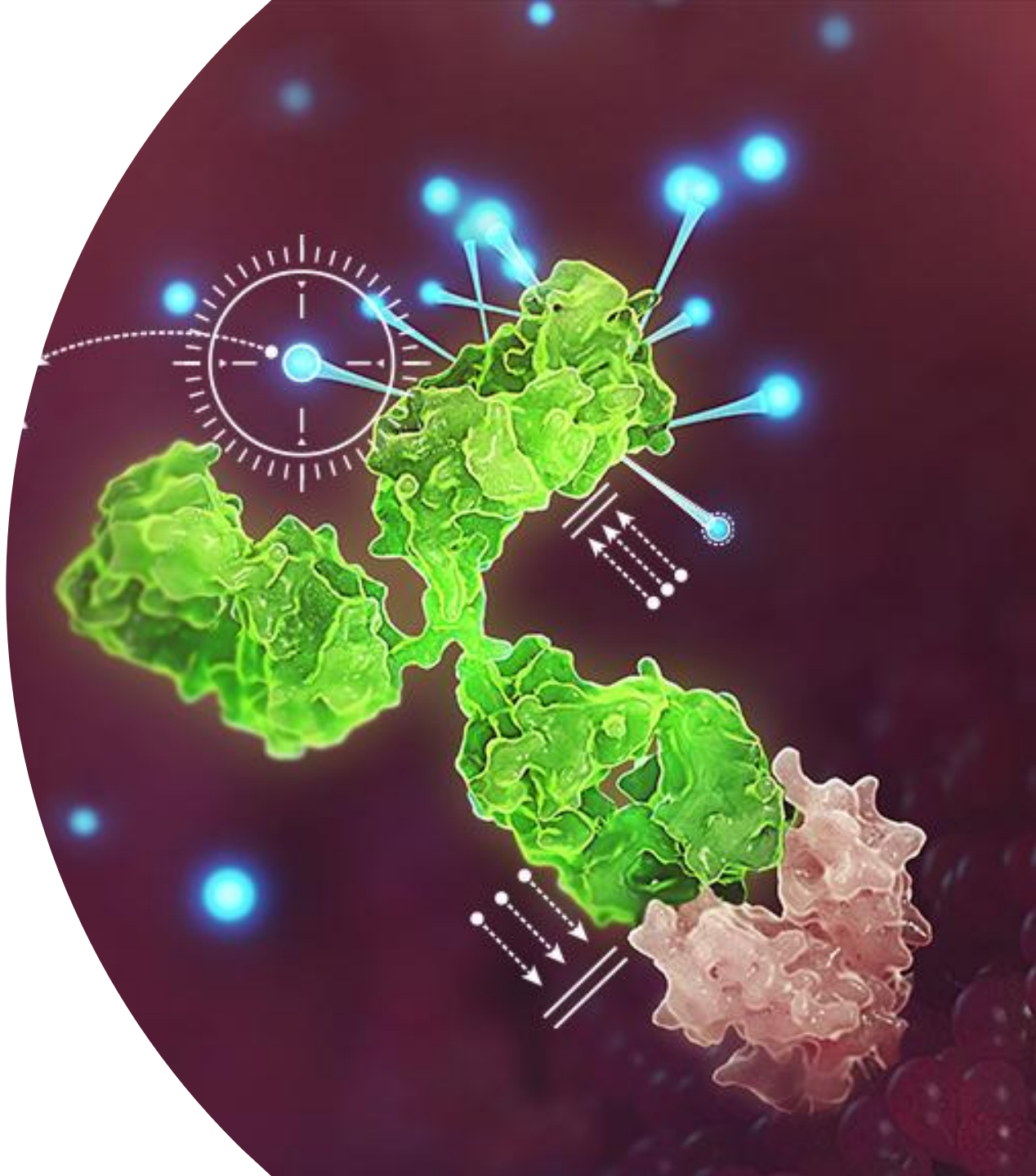
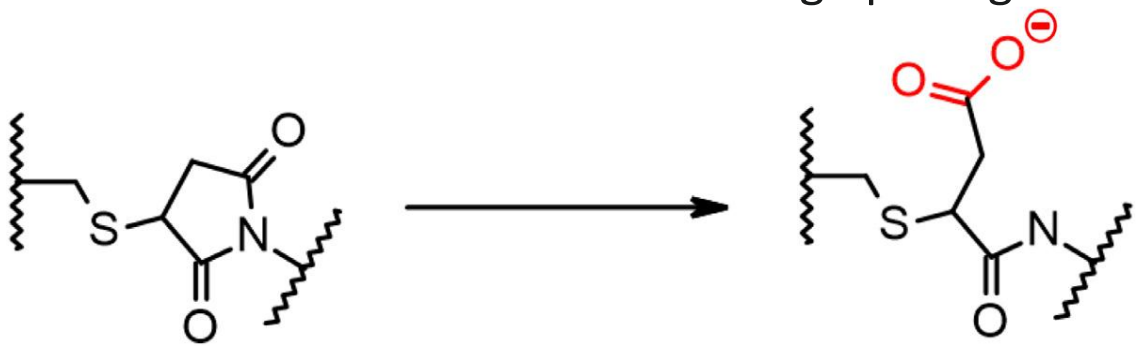
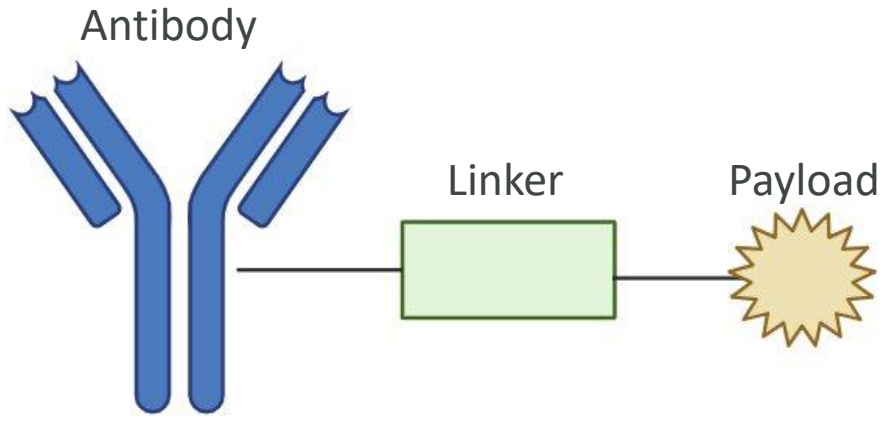


ADCs- Amazingly Difficult Charge (analysis)

- Many antibody drug conjugate (ADC) drugs on the market and in clinical trials are conjugated at a cysteine residue and contain a succinimide-thioether functional group
- Charge heterogeneity profiles are heavily convoluted by contributions from the lactone and succinimide rings in the linker-payload structures
  - Both Ion Exchange Chromatography and Capillary Isoelectric Focusing yield complex profiles
  - Lot-to-lot variability of ring opening events due to in-process hold time variability muddies profile comparisons
  - Ring hydrolysis happens quickly in vivo, so levels at release are not critical quality attributes

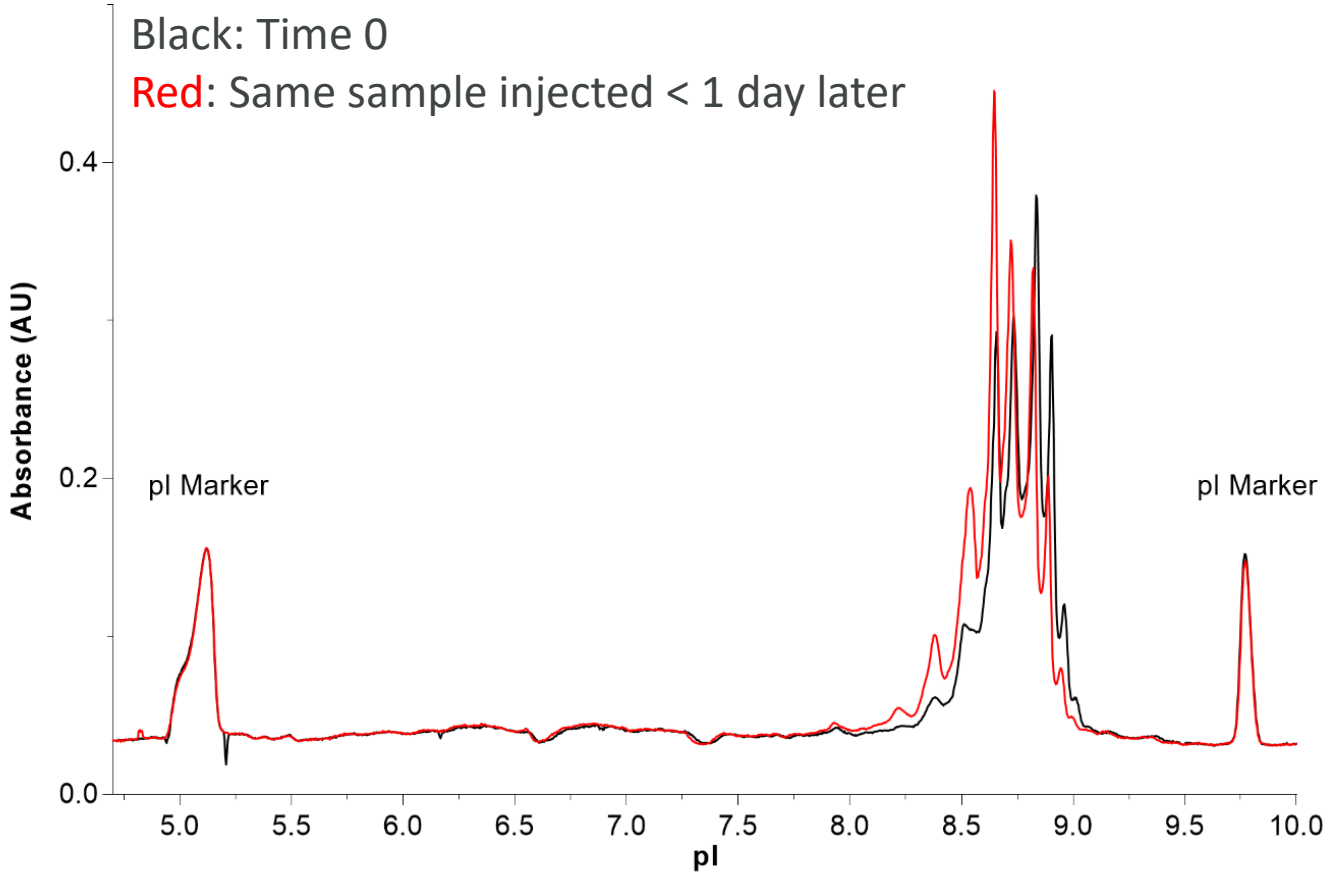
Irreversible succinimide-thioether ring opening





Platform cIEF analysis is not fit for purpose for ADCs

- Profile is inconsistent across multiple preparations
- The pH gradient in pharmalytes results in poor autosampler stability
  - There is a major acidic shift
- Limited peak ID work has indicated the major species are related to reversible and irreversible ring opening species
  - Ring opening species cloud other changes to the protein backbone



Capillary Zone Electrophoresis (CZE) as an Alternate Charge Method

- CZE is well documented as an alternate charge heterogeneity method using electrophoretic mobility
  - Widely used for characterization of mAbs
- Dilute and Shoot- minimal sample manipulation and preparation
  - Can minimize potential interactions with sample matrix
- UV detection at 214 nm enables high sensitivity
- Less sensitive to salt concentration
- Kit based option from Sciex should allow for easy experiments with existing PA800s

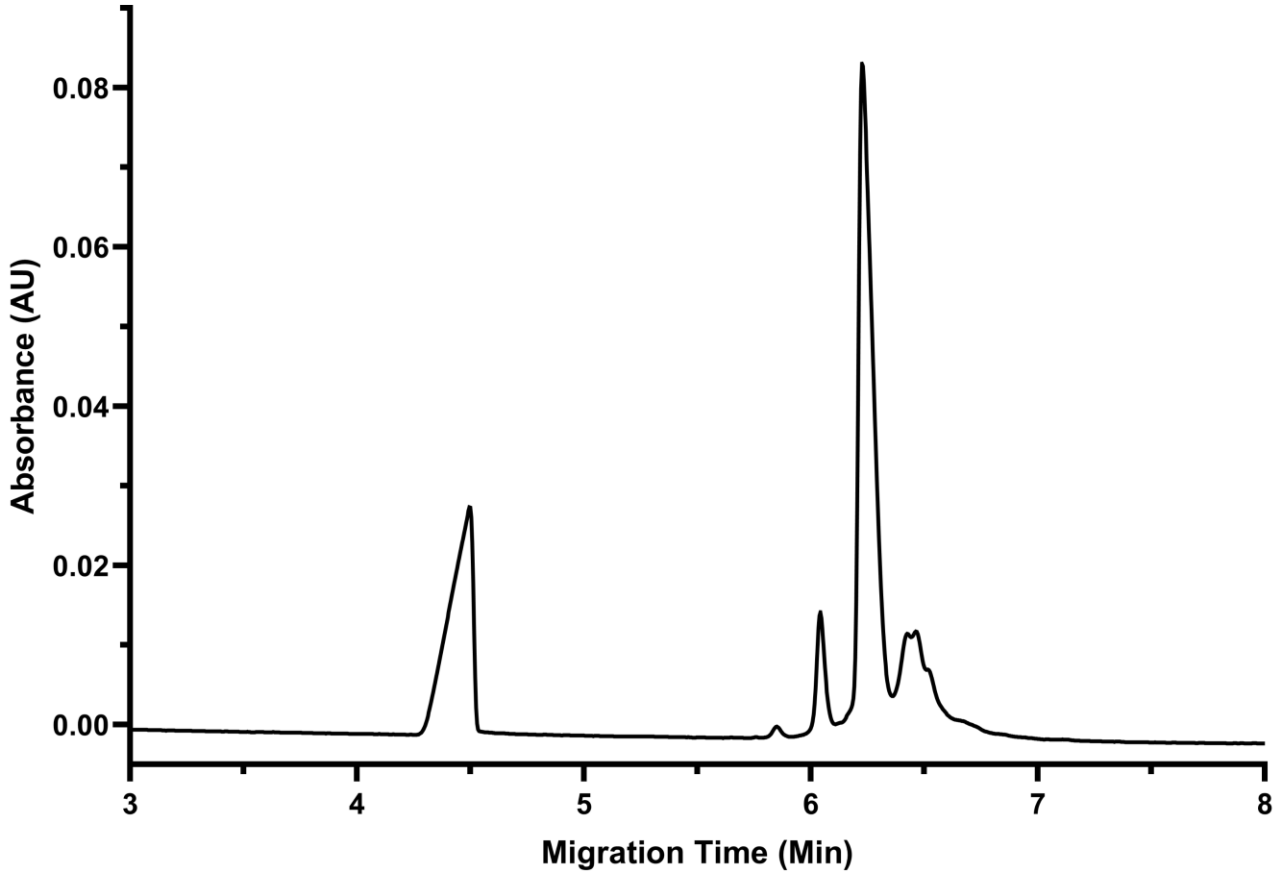
Image generated in BioRender

He et al. / Anal. Chem. 2010, 82, 8, 3222–3230 https://doi.org/10.1021/ac9028856

He et al. / J. Sep. Sci. 2011, 34, 548–555 https://doi.org/10.1002/jssc.201000719

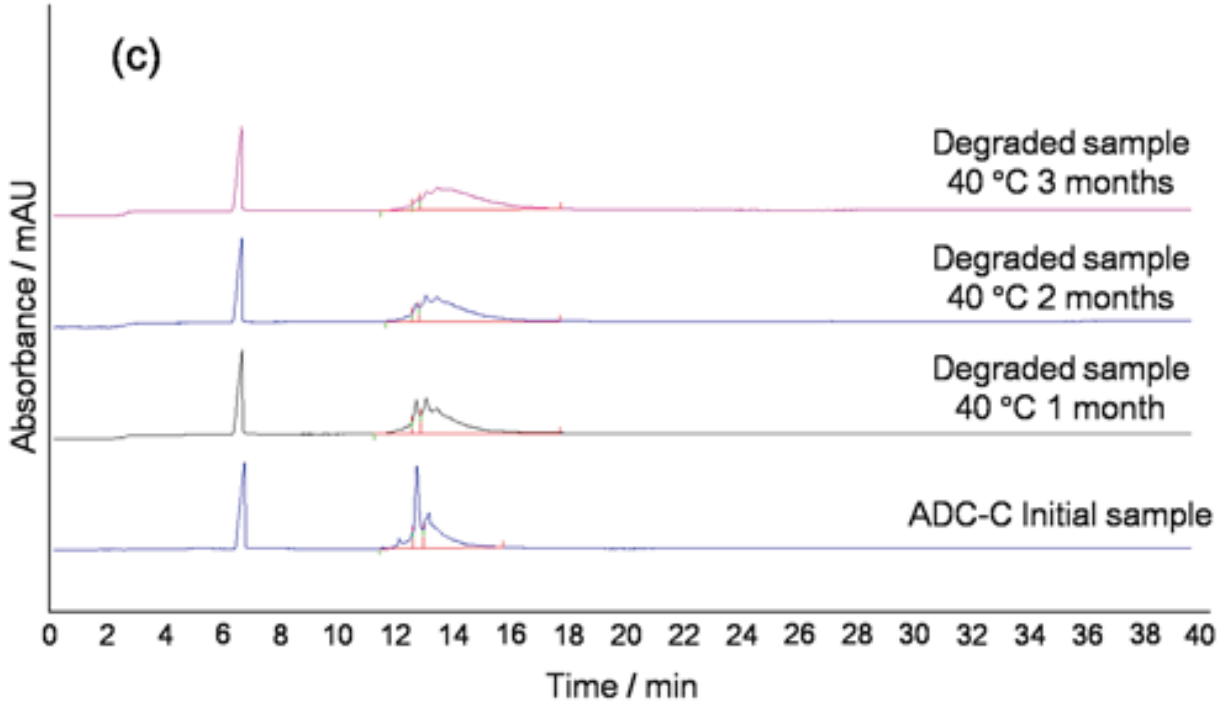
CZE Development on Antibodies- NIST mAb

- Some modifications to Sciex Rapid Charge Variant Kit showed good resolution on in-house antibodies
- This shows the potential viability of separation on troublesome molecules
- **If it works on an antibody why not try it on an ADC?**



Daiichi Sankyo Successfully Published a CZE Method for ADCs

- Validated method performance of CZE on an ADC molecule
  - Demonstrated consistent and robust method results
- Large acidic shift in stressed material only described as an “ability to monitor the degradation of samples”
- **CZE Should be possible on a representative ADC**

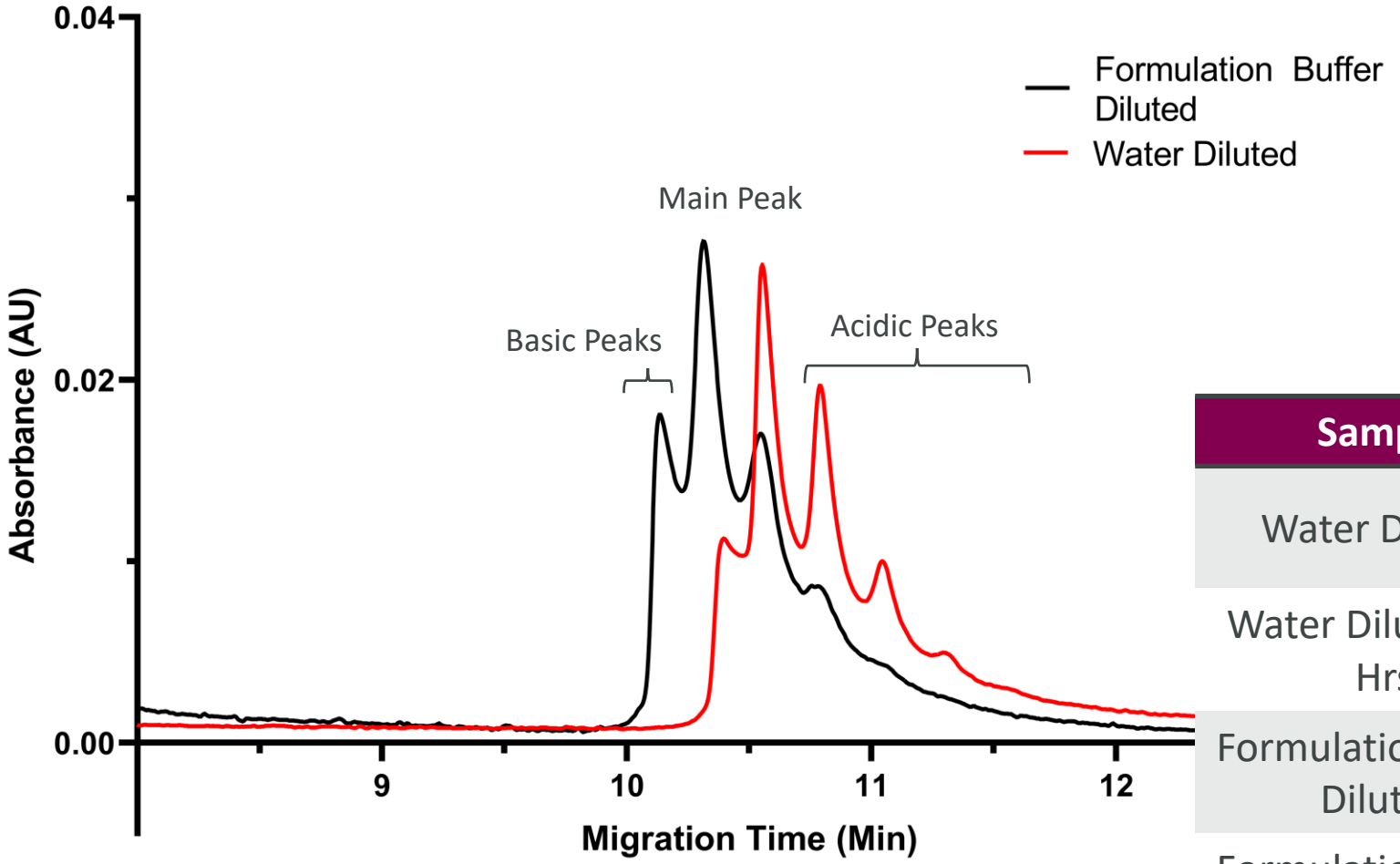


Data and Figures from [*Kubota e. al.; Chromatography* **2016**, *37*, 117-124]

Method Description- Initial Development

- Tested Material:
  - Representative ADC Molecule; Unstressed and 3 month 40℃ stressed
  - Started with modified Sciex Rapid Charge Variant methods:
    - 1mg/mL sample load
    - 20kV separation for 20 mins
    - 30 cm effective length uncoated OR neutral coated capillary
    - 15℃ sample storage
    - Diluted samples in both CE grade water and Formulation Buffer; injected in triplicate, single preparation
    - 24 Hr 15℃ autosampler stability
    - PDA Detection at 214nm

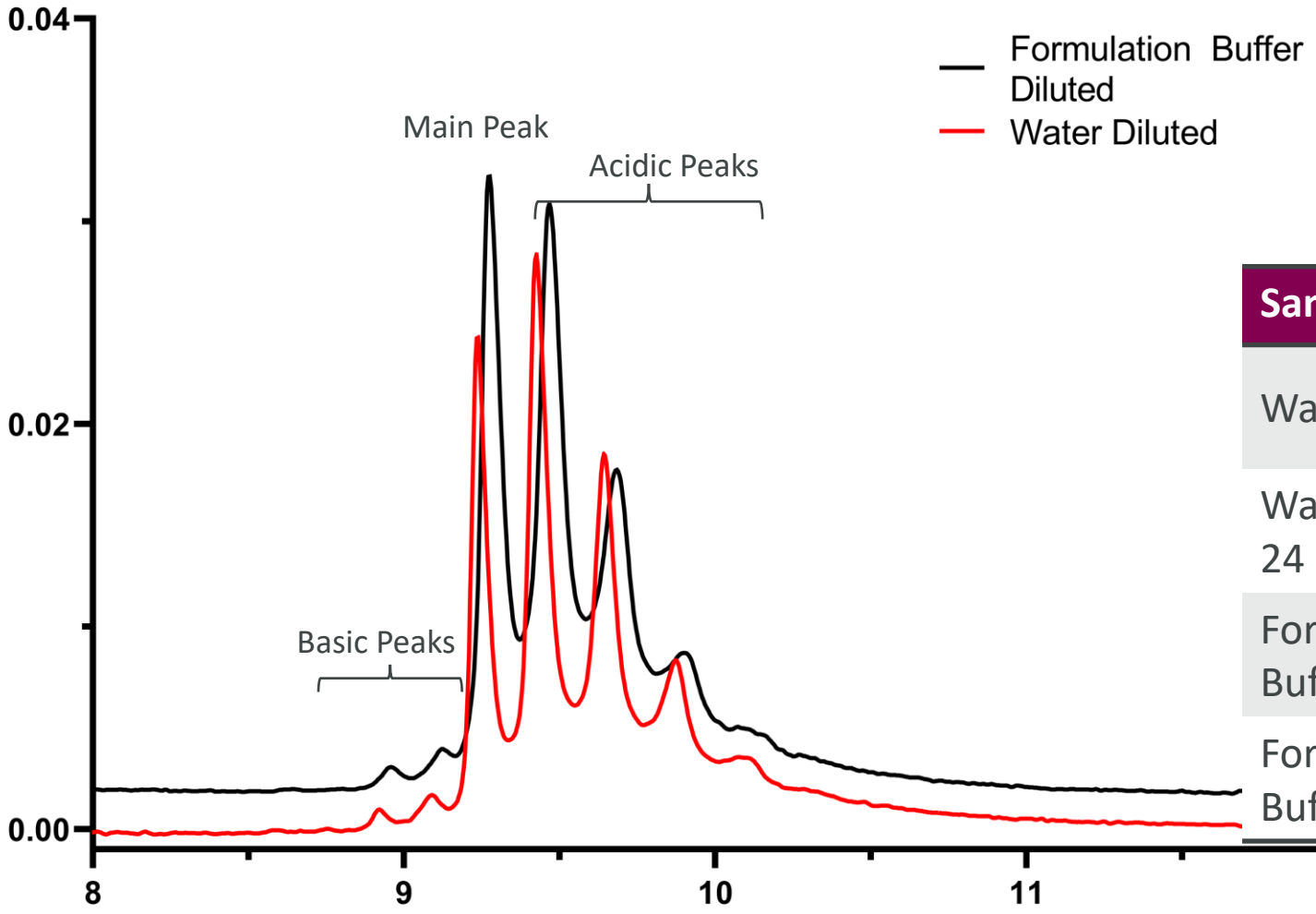
Uncoated Capillary Separation: CZE Shows Promise



- 4 major peaks observed in both conditions
- Similar autosampler stability in water and FB diluted samples
- 3 Month 40°C too degraded for any meaningful data

Sample	% Acidic	% Main	% Basic
Water Diluted	57.3	31.9	10.8
Water Diluted- 24 Hrs	61.8	29.2	9.0
Formulation Buffer Diluted	49.9	33.8	16.2
Formulation Buffer Diluted- 24 Hrs	46.9	34.1	19.0

Changing to a Neutral Coated Capillary Makes Improvements



- Improved peak resolution over uncoated capillary
- Better autosampler stability in FB diluted samples

Sample	% Acidic	% Main	% Basic
Water Diluted	79.2	17.7	3.1
Water Diluted- 24 Hrs	83.1	13.9	3.0
Formulation Buffer	73.1	23.8	3.1
Formulation Buffer- 24 Hrs	73.1	23.1	3.8

What if We Try and Control Reversible Ring Opening Species?

- Ring opening known to be an issue impacting profiles
- Shown to be highly pH dependent by Oguma et al. and other studies
- Other assays utilize a pH 4 acid treatment step; forces reversible lactone ring openings to close
  - **Succinimide-linked ring openings are irreversible and can't be controlled**
- Used acid treatment step before running CZE analysis on neutral coated capillary

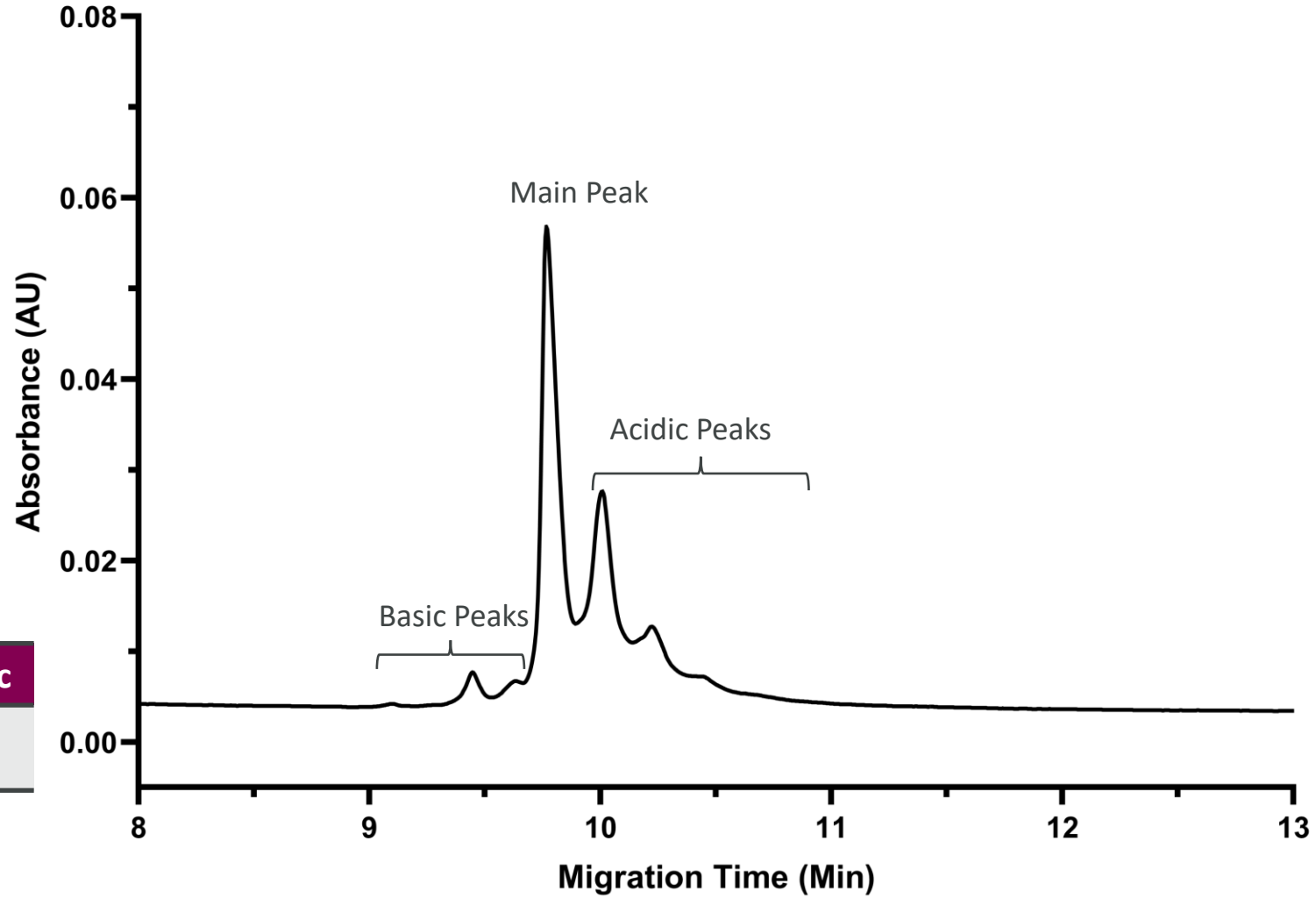
Oguma et al. / Biol Pharm Bull. 2001 Feb;24(2):176-80. doi: 10.1248/bpb.24.176.

K. Zheng et al. / Journal of Pharmaceutical Sciences 108 (2019) 133-141

Acid Pretreatment Simplifies the Profile

- Reversible lactone ring opening species forced closed
- Acidic species simplified while maintaining resolution
- More apparent main peak after acid treatment

Sample	% Acidic	% Main	% Basic
Representative ADC	51.9	42.0	6.1



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Can the Method Pass a Reproducibility Experiment?

- Neutral coated capillary improved resolution over uncoated
- Acid treatment minimized ring opening species- simpler profiles in representative material
- Dilution in formulation buffer indicated better autosampler stability
- Tried changing separation parameters (capillary length, separation voltage, time, sample loading method, etc.)- no beneficial impact observed
- **Prepared multiple samples mimicking method qualification parameters to demonstrate method suitability**

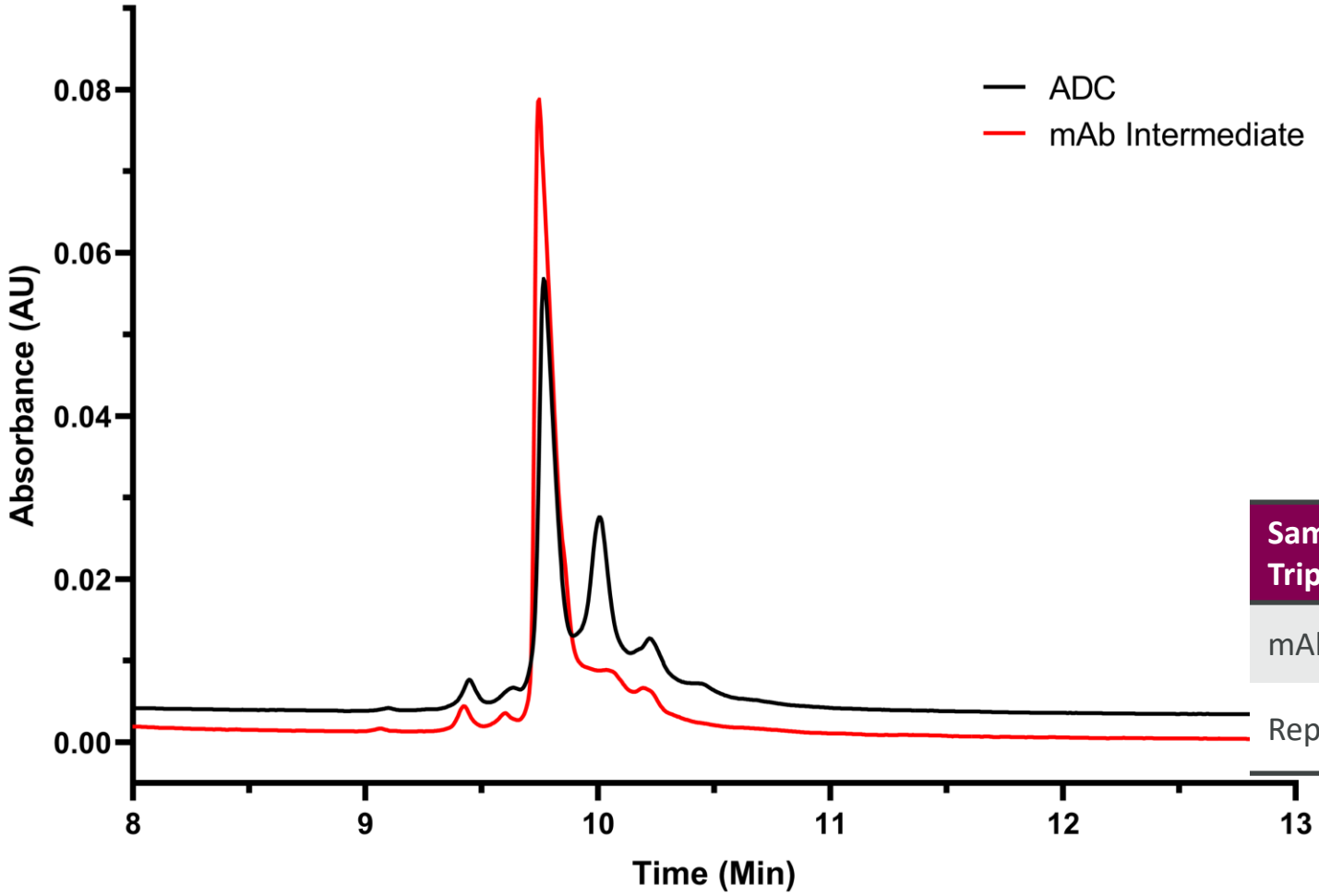
Putting CZE to the test: Reproducibility Gauntlet

- Run by 2 analysts:
  - Representative ADC in triplicate preparations, FB diluted and Acid Treated
  - 1 Week 40℃ for representative ADC in triplicate, FB diluted and Acid Treated
  - mAb intermediate in triplicate; diluted in FB Only
  - mAb intermediate as a single acid treated sample
- Additional materials tested as single injections; prepared both as FB diluted and Acid treated
- **Observed averages, standard deviations, and absolute differences between analysts**

Test Method Details

- 2 Sample preparations:
  - **FB**- Dilute to 1mg/mL in Formulation Buffer
  - **Acid Treated**- Dilute to 1.2 mg/mL in FB, add 20uL 100mM Acetate (pH 4.0) buffer to 100uL sample, incubate @ 40℃ for 2 hours (final concentration 1 mg/mL)
- 30 cm effective length neutral coated capillary
- 15℃ sample storage temperature
- 20kV separation for 20 minutes
- PDA detection at 214nm

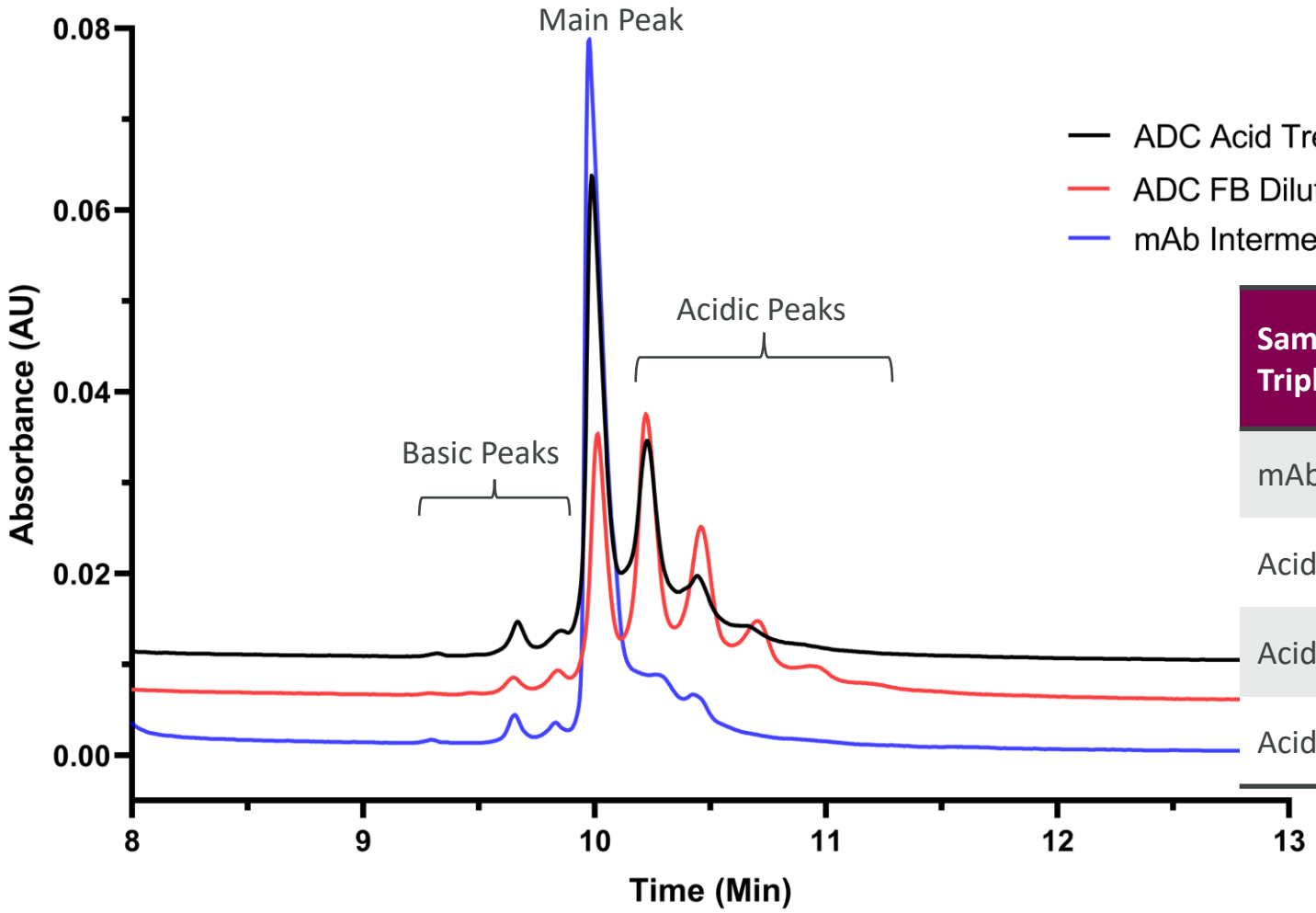
mAb Intermediate vs. ADC



- Main Peak manually aligned for profile comparison
- Significant difference in % acidic peaks
  - Due to linker-payload conjugation
  - Major acidic species do align

Sample- Average of Triplicate	% Acidic	% Main	% Basic	Main Peak MT
mAb Intermediate	11.0	84.6	4.5	9.7
Representative ADC	51.9	42.0	6.1	9.8

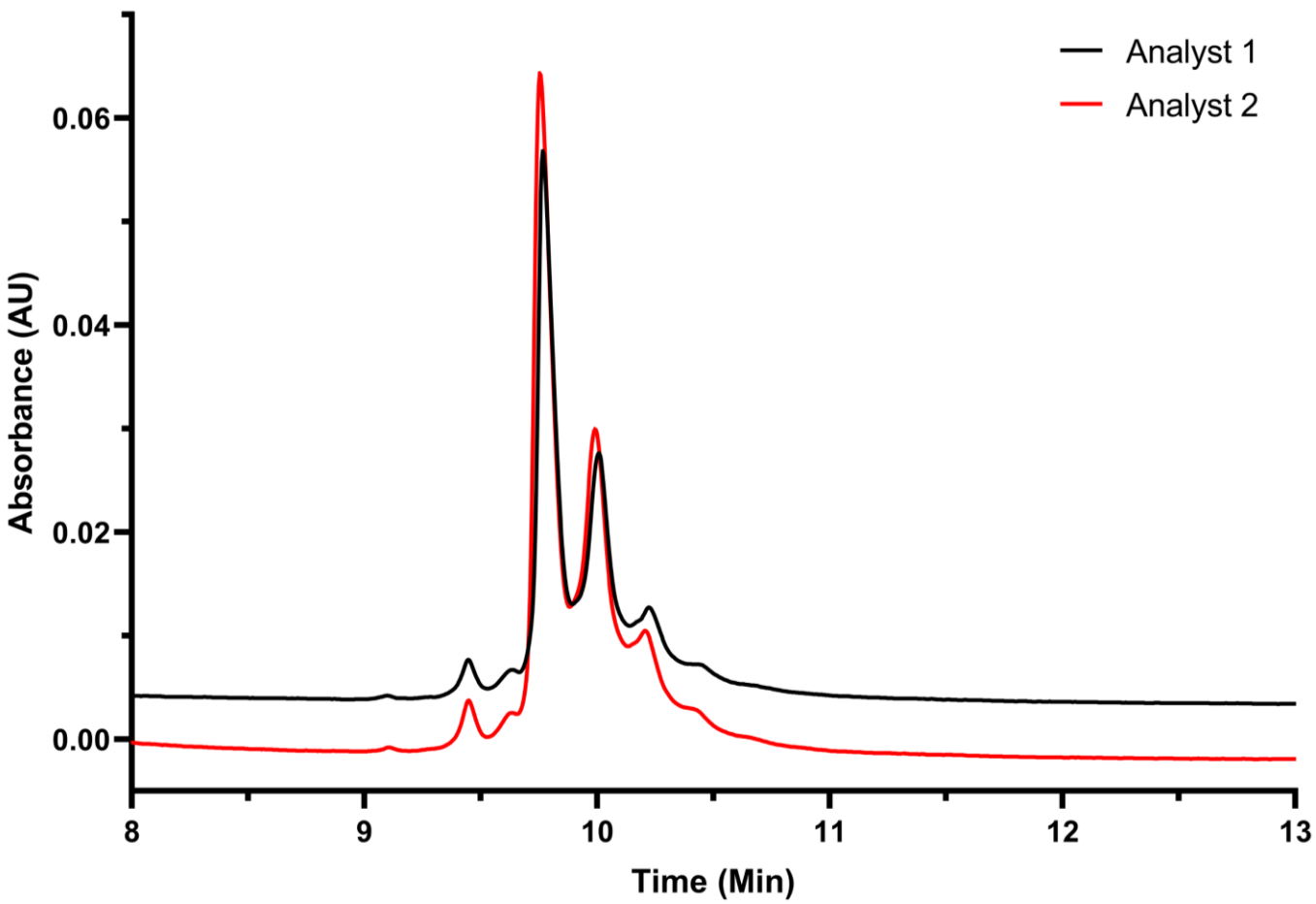
Unstressed ADC Replicates



- Main Peaks Manually aligned for profile comparison
- Unstressed material has reproducible peak distributions

Sample- Average of Triplicate	% Acidic	% Main	% Basic	Main Peak Migration Time
mAb Intermediate FB	11.0	84.6	4.5	9.7
Acid Treated ADC	51.9	42.0	6.1	9.8
Acid Treated SD	0.3	0.4	0.1	0.0
Acid Treated % CV	0.6	1.0	1.9	0.0

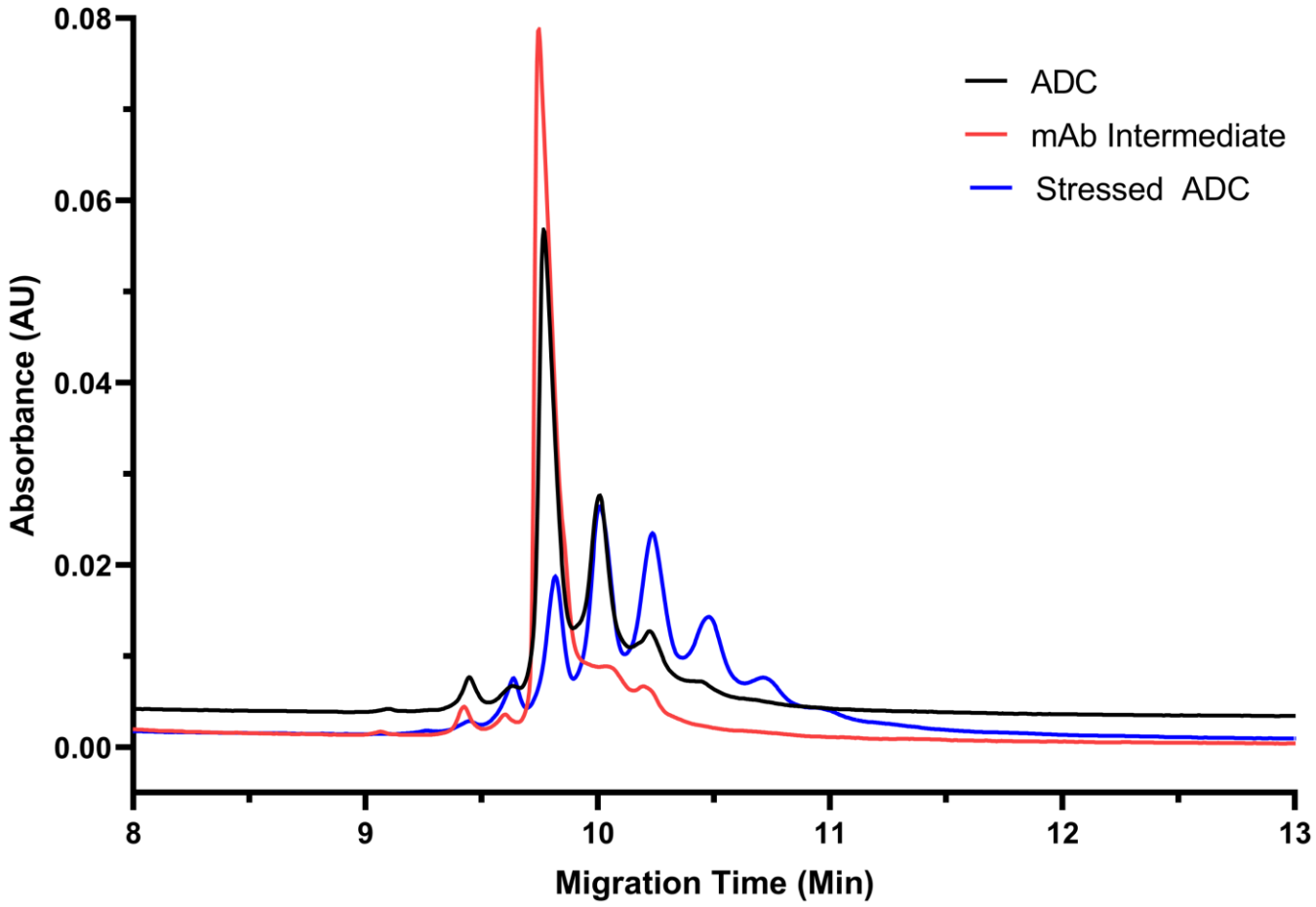
Analyst to Analyst- Unstressed ADC



Sample- Average of Triplicate	% Acidic	% Main	% Basic	Main Peak Migration Time
Analyst 1 Average	51.9	42.0	6.1	9.8
Analyst 2 Average	50.3	44.0	5.7	9.6
Difference	1.5	2.0	0.4	0.2
Standard Deviation	0.9	1.1	0.2	N/A
% CV	1.7	2.6	3.9	N/A

- Main peak aligned for peak comparison
- Comparable quantitation across analysts
- Difference in migration time is a known capillary variability

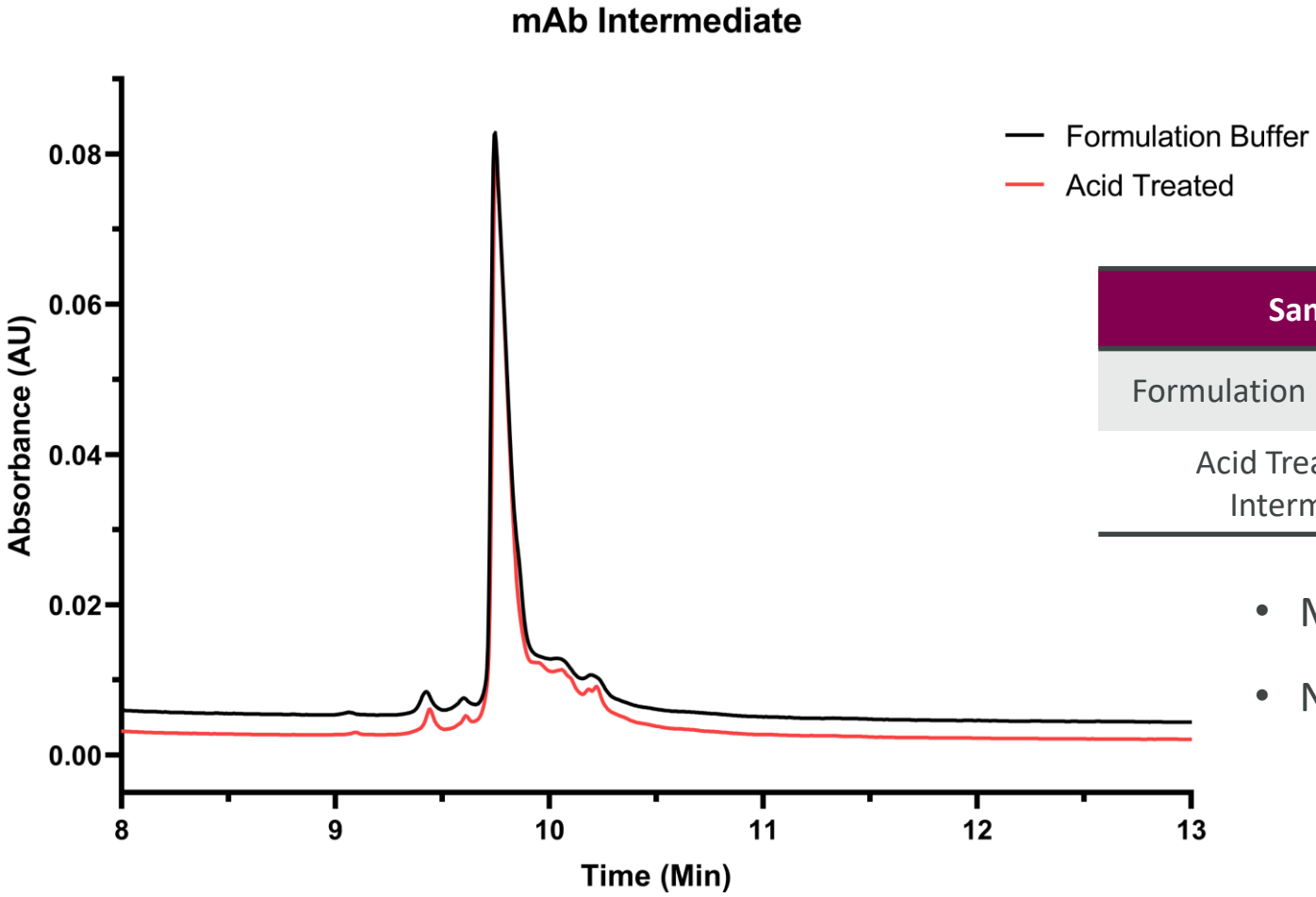
Stresed Material- 1 Week 40C



- Main Peaks Manually aligned for profile comparison
- **Irreversible ring opening impacts profile, quickly produces an acidic shift**
- Any observable deamidation convoluted by ring opening

Sample- Average of Triplicate	% Acidic	% Main	% Basic	Main Peak MT
Acid Treated RS	51.9	42.0	6.1	9.8
Stressed FB	87.1	9.1	3.8	10.3
Stressed Acid Treated	81.6	12.6	5.8	10.1
Acid Treated SD	0.1	0.1	0.1	0.0
Acid Treated % CV	0.1	0.5	2.0	0.0

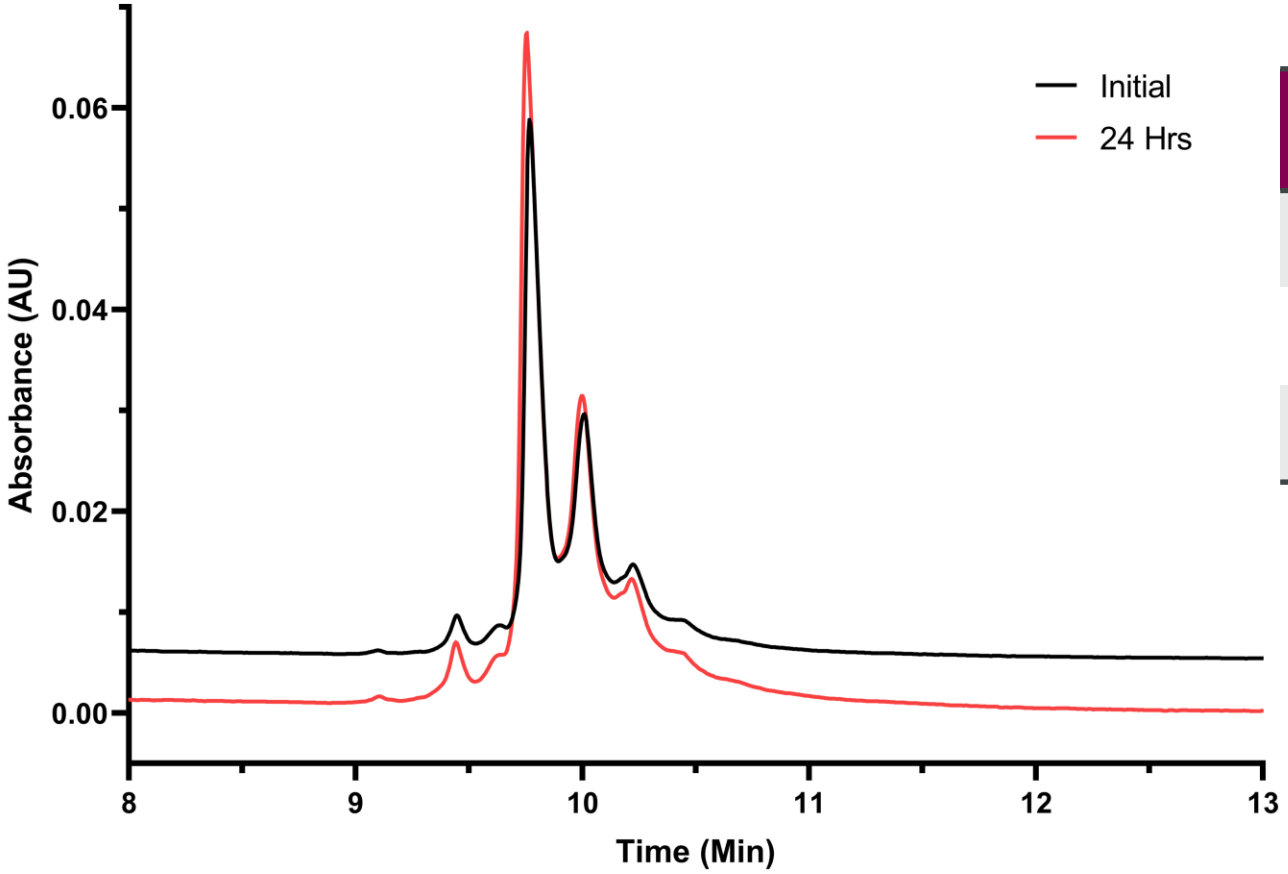
Acid Treated mAb Intermediate



Sample	% Acidic	% Main	% Basic	Main Peak MT
Formulation Buffer Diluted	11.0	84.6	4.5	9.7
Acid Treated mAb Intermediate	12.3	83.6	4	9.6

- Main Peaks manually aligned for profile comparison
- No major differences between profiles

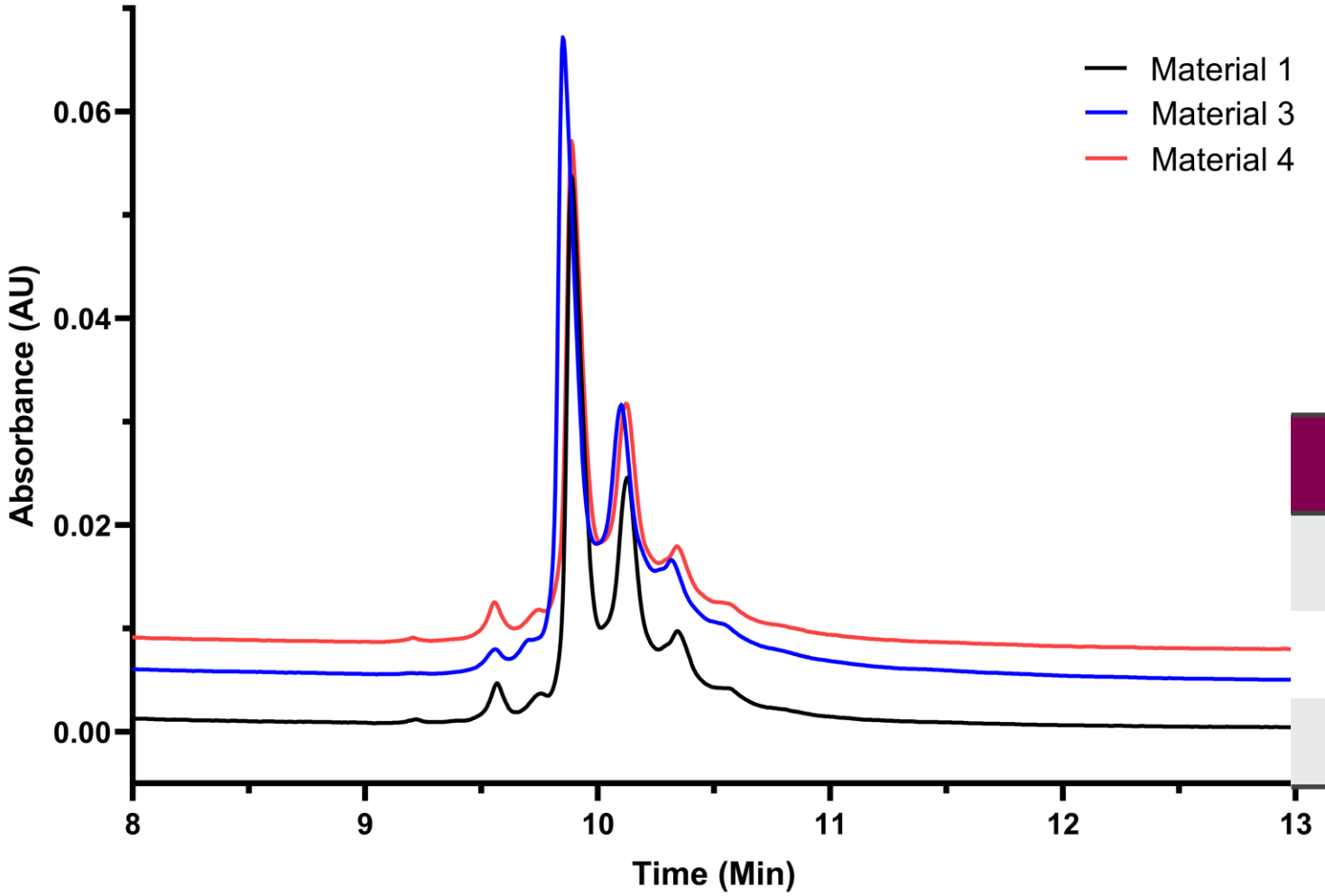
24 Hour Autosampler Stability



Sample- Average of Triplicate	Δ % Acidic	Δ % Main	Δ % Basic	Δ Main Peak MT
Representative ADC	0.2	0.6	0.7	0.0
Stressed ADC	0.3	0.1	0.3	0.1
mAb Intermediate	1.4	1.4	0.0	0.1

- No major change in peak distribution on ADC material

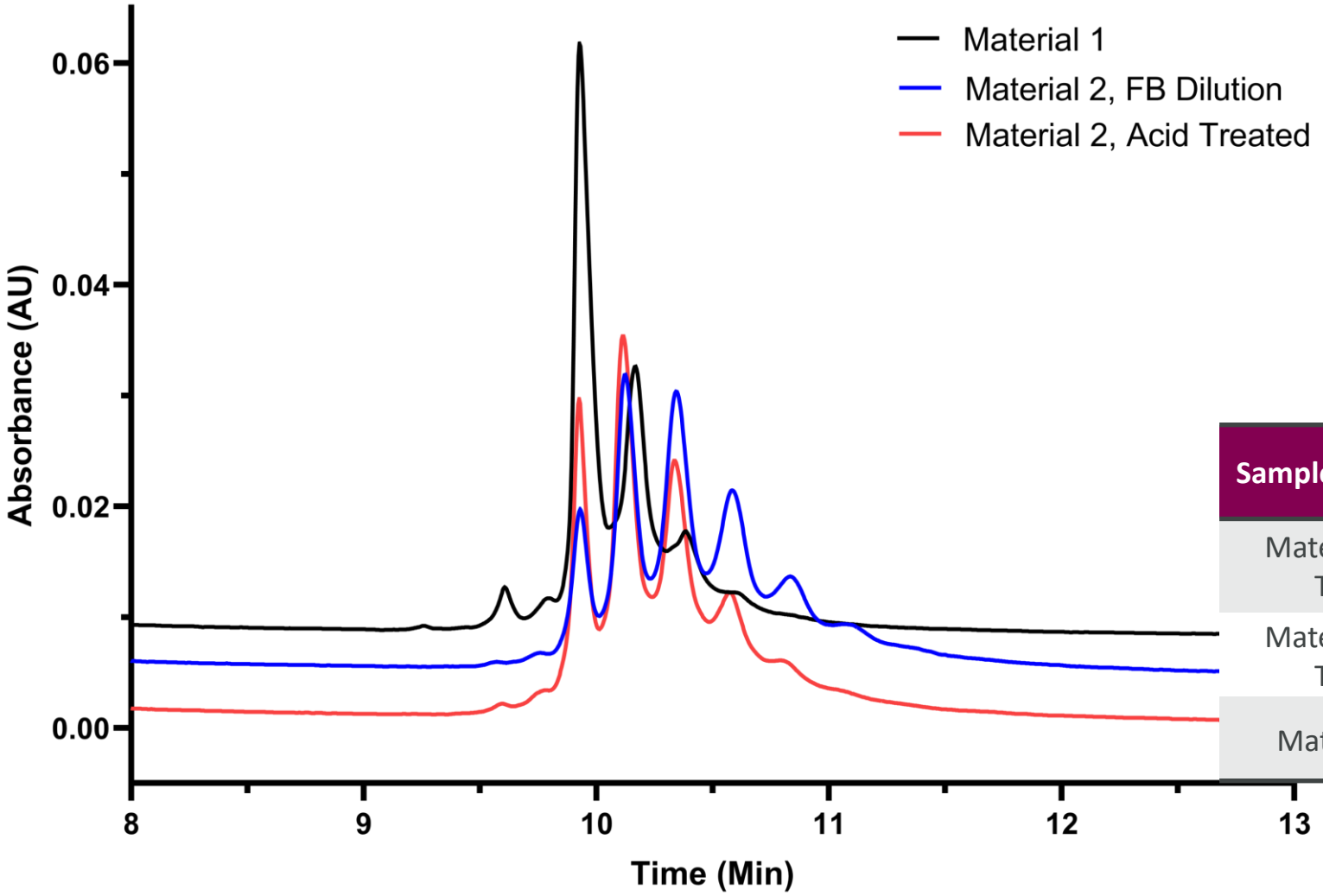
Lots of Representative ADC…



- Materials 3 and Material 4 are comparable to representative ADC material
- Differences to be expected with lot variability
- Comparable hold times during conjugation steps

Sample	% Acidic	% Main	% Basic	Main Peak MT
Material 1 Acid Treated	51.9	42.0	6.1	9.8
Material 3 Acid Treated	52.4	44.1	3.5	9.9
Material 4 Acid Treated	54.4	38.7	6.9	9.9

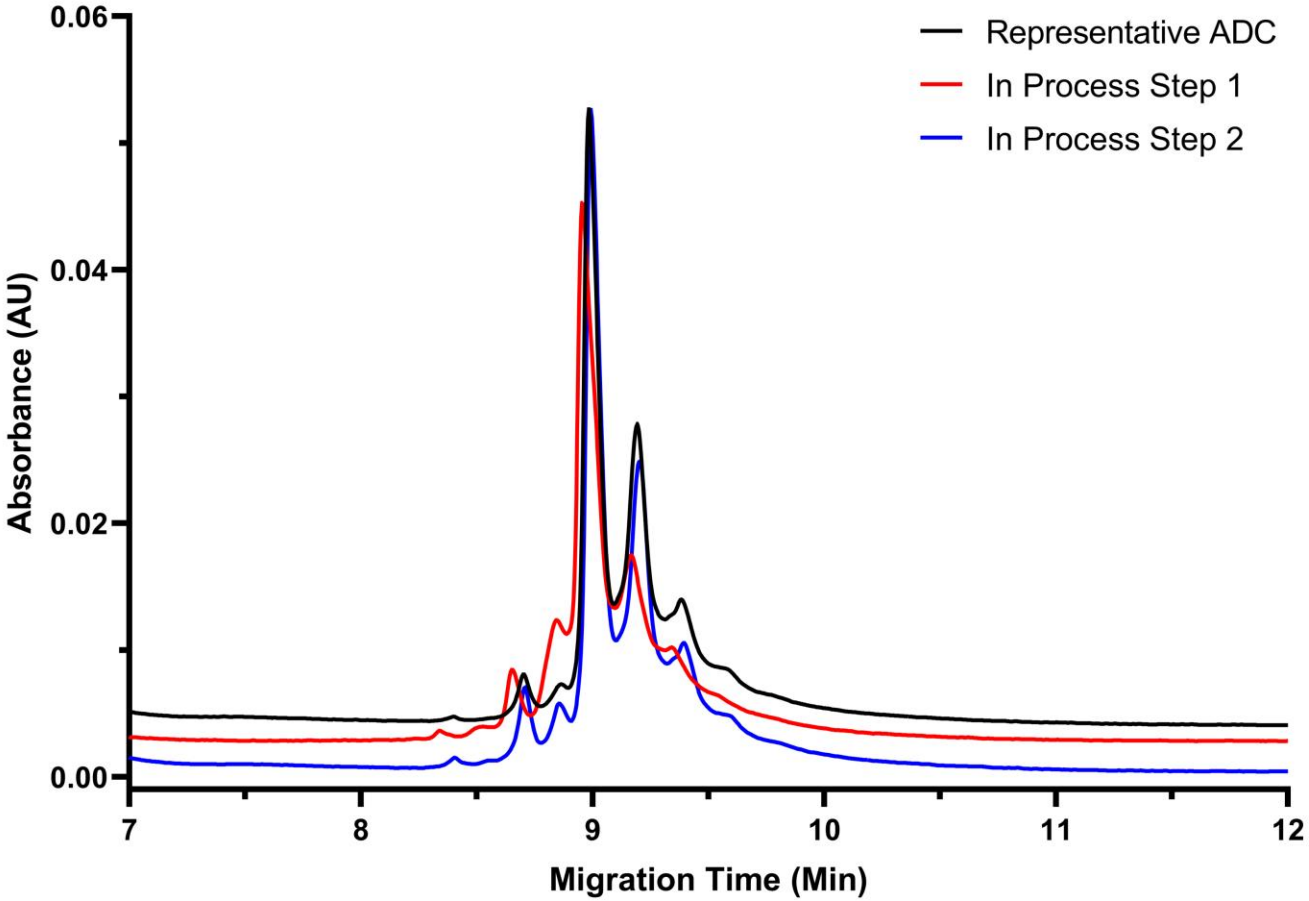
…But Variability is Still Seen



- Material 2 shows inconsistent profile after acid treatment
- **Inconsistencies related to irreversible succinimide ring openings, not any observed PTMs**
- Variability related to in process hold time; longer hold time used in Material 2

Sample	% Acidic	% Main	% Basic	Main Peak MT
Material 1 Acid Treated	50.1	36.1	13.8	11.7
Material 2 Acid Treated	41	43.3	15.7	11.7
Material 2 FB	57.7	31.1	11.2	11.8

Representative In Processs Hold

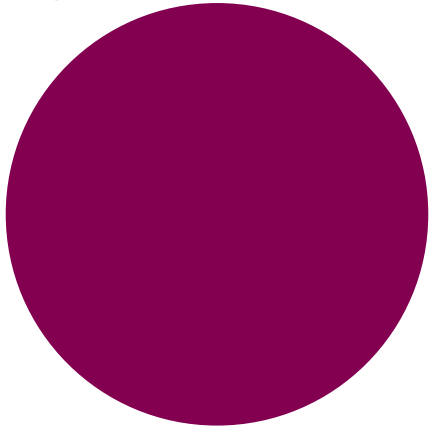


- In-process materials ran to observe variation during conjugation
- General trends in peak area as expected
- No buffer interference in profiles

Sample	% Acidic	% Main	% Basic	Main Peak MT
Material 1	53.8	40.6	5.6	9.0
In Process Step 1	43.0	41.8	15.1	9.0
In Process Step 2	52.8	37.5	9.7	9.0

Method Conclusions

- While profiles are stable and reproducible, irreversible ring opening quickly convolutes profile and limits observable PTMs



- All profiles repeatable for across multiple analysts and material sources
- Acid treated ADC profile has some similarities to mAb intermediate

Next Steps

- Move away from a 30cm effective length capillary
  - BioPhase method should be viable?
- Confirm robustness of Coated Capillaries, Acid Treatment

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And Viewers Like You!

Extended References

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