Critical Reagents: Addressing the Global Pharmalyte Shortage

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Lilly

Overview

- Carrier Ampholyte Background
- Problem & Industry Impact
- Identifying Equivalents
- Comparing Pre- vs Post-change Pharmalyte
- Future Mitigation Plan & Conclusion

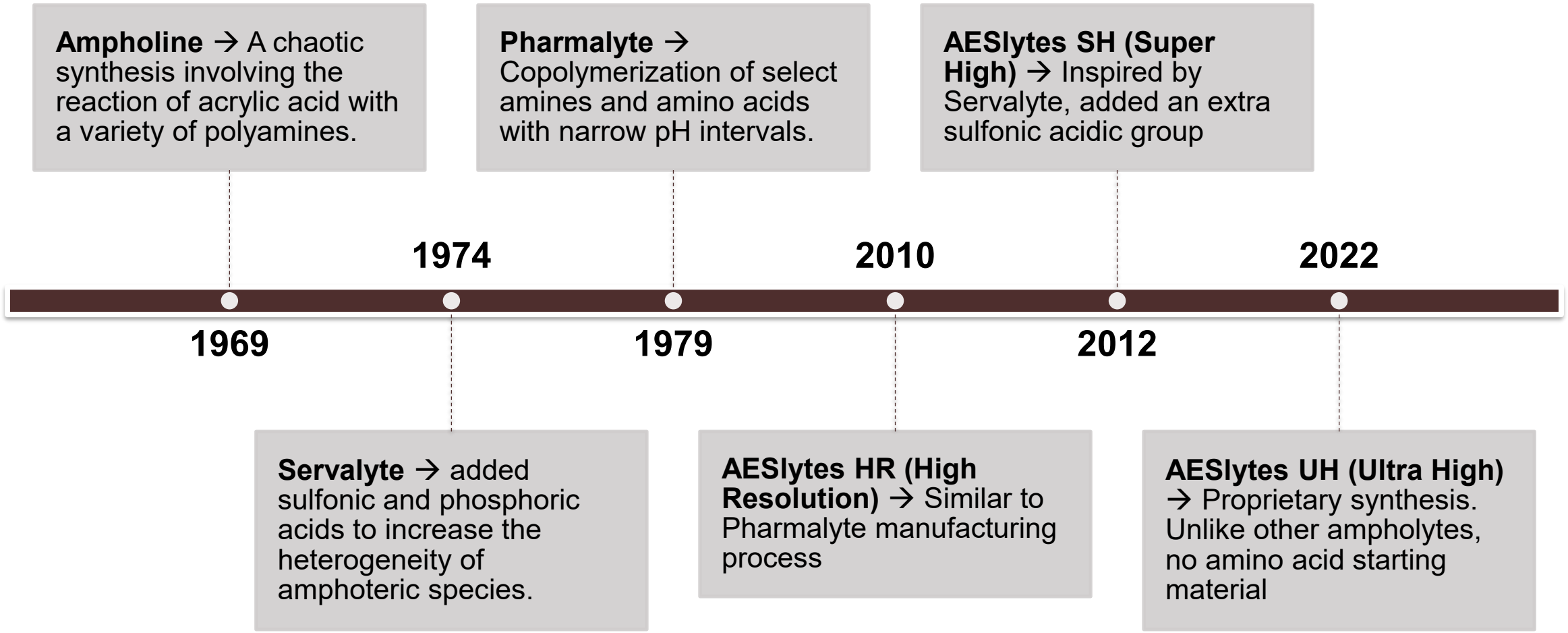
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Carrier Ampholytes - icIEF Critical Reagents

- Carrier ampholytes are mixtures of amphoteric molecules that migrate to their pI in an electrical field → creates a pI gradient in the capillary
- Ampholytes are critical regents for icIEF because they drive the separation of charged variants during analysis
- Multiple companies manufacture ampholytes, each using their own proprietary process
  - Cytiva → Pharmalyte
  - Advanced Electrophoresis Solutions → AESlyte (HR, SH & UH)
  - Serva Electrophoresis → Servalyte
  - Bio-Rad → Biolyte

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Evolution of Carrier Ampholytes



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Global Pharmalyte Shortage: Problem & Industry Impact

Problem

- Pharmalyte was first commercialized in 1979 and has maintained the same manufacturing equipment for 46 years
- Late 2024, Cytiva began updating their manufacturing equipment to improve their manufacturing consistency
- The new manufacturing equipment installation created a +5 month backlog in fulfilling orders
- Cytiva did not communicate that there would be an extended period of time where no new product was being manufactured – testing labs did not have a chance to prepare for the shortage

Industry Impact

- Pharmalyte 3-10 is a critical reagent in the industry wide platform method for icIEF
  - Almost all Lilly methods developed prior to the shortage use Pharmalyte
- The shortage impacted icIEF testing support from pre-clinical development through commercial

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Risks Associated with the Shortage

1. Running out of material before the re-supply → How can testing continue?

2. Comparability of post-change Pharmlayte to pre-change supply → Will the post-change pharmalyte perform like the pre-change or will icIEF results be impacted?

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Immediate Impact Mitigation Plan

Commercial

- Created an inventory across all Lilly testing labs to determine how long the existing Pharmalyte supply could support testing
- Designated the existing supply for commercial testing
- Immediately began evaluating equivalents for commercial assets

Development

- Early phase projects were switched to an equivalent AESlyte
- If an equivalent could not be identified, reduced testing to only critical testing
  - Existing supply was reserved for commercial assets and BLA supporting studies

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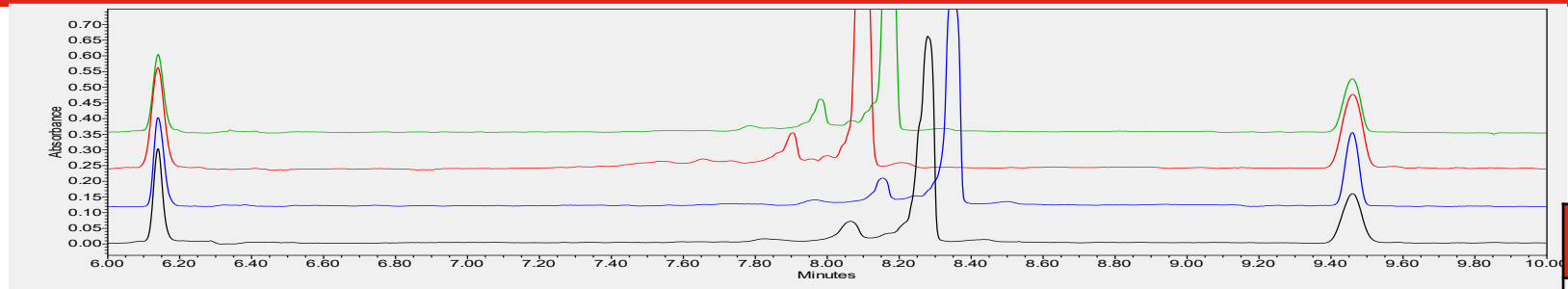
Identifying Alternate Ampholytes

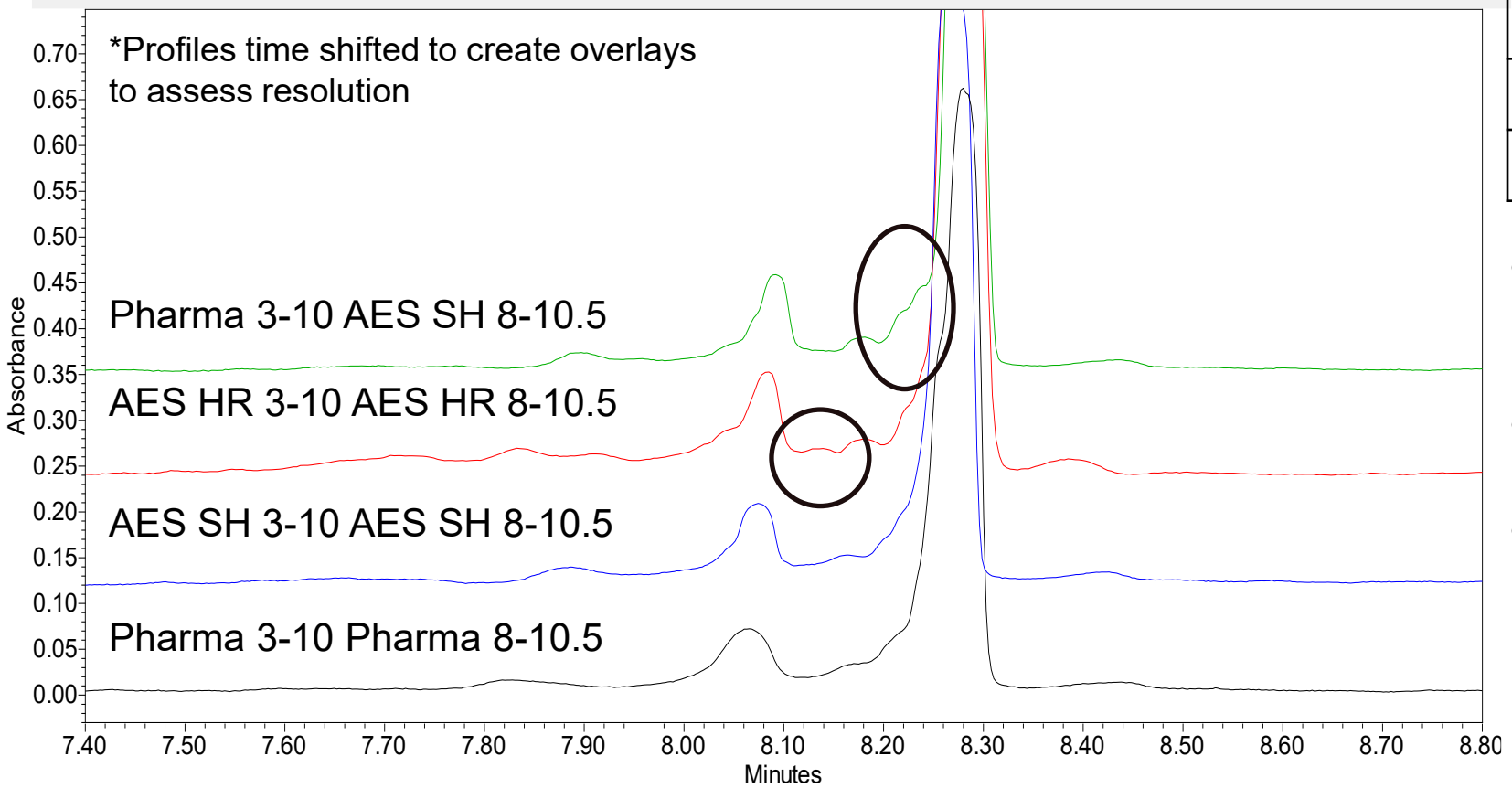
Pharmalyte Range	Potential Equivalents
3-10	• AESlyte HR or SH 3-10 • BioRad 3-10
4-6.5	• AESlyte HR or SH 4-6 • BioRad 4-6
5-8	• AESlyte HR 5-8 • BioRad 5-8
8-10.5	• AESlyte HR or SH 8-10.5

- Pharmalyte 4-6.5 was replaced with an alternate manufacturers 4-6 range (4-6.5 not available)
- AES UH (ultra high) & Servalyte were not included in the alternate ampholyte evaluations → previous evaluations determined they are not equivalent to pharmalyte
- For methods using 2 ampholytes – Potential alternates were combined with each other and Pharmalyte

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mAb1 – Pharmalyte 3-10 & 8-10.5 Alternates Ampholytes





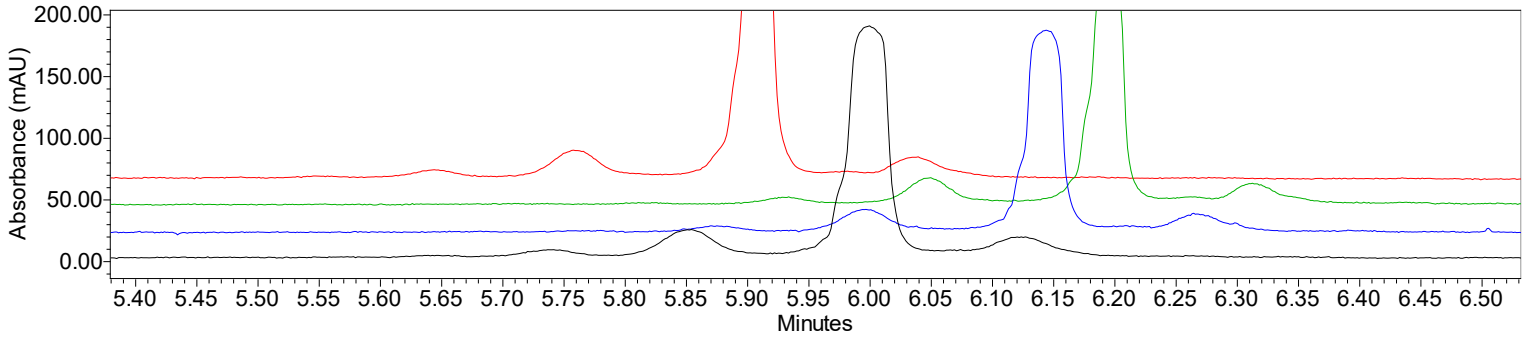
Carrier Ampholytes → 2:1 Pharmalyte 8-10.5 and Pharmalyte 3-10

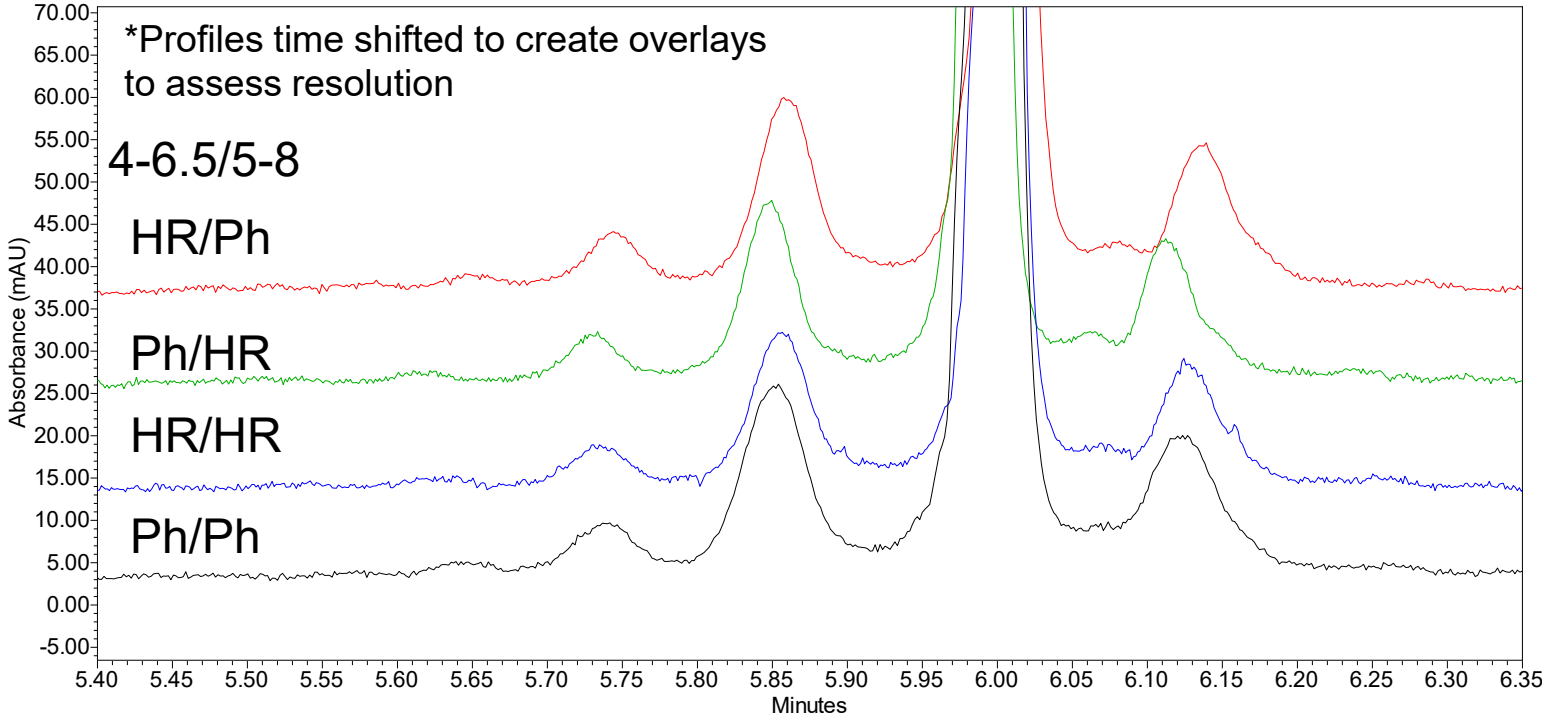
Method	%MP	%TAV	%TBV
Validated	78.34	19.55	2.11
AES SH	76.83	21.05	2.12
StDev	**0.87**	**0.87**	**0.11**

- **AESlyte SH 3-10 and 8-10.5 produces a profile that is equivalent to the current method**
- Slight bias in results → 1.5% increase in TAV and 0.1 pI shift
- Combining AESlyte SH with Pharmalyte does NOT produce an equivalent profile

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mAb 2 – Pharmalyte 4-6.5 & 5-8 Alternate Ampholytes





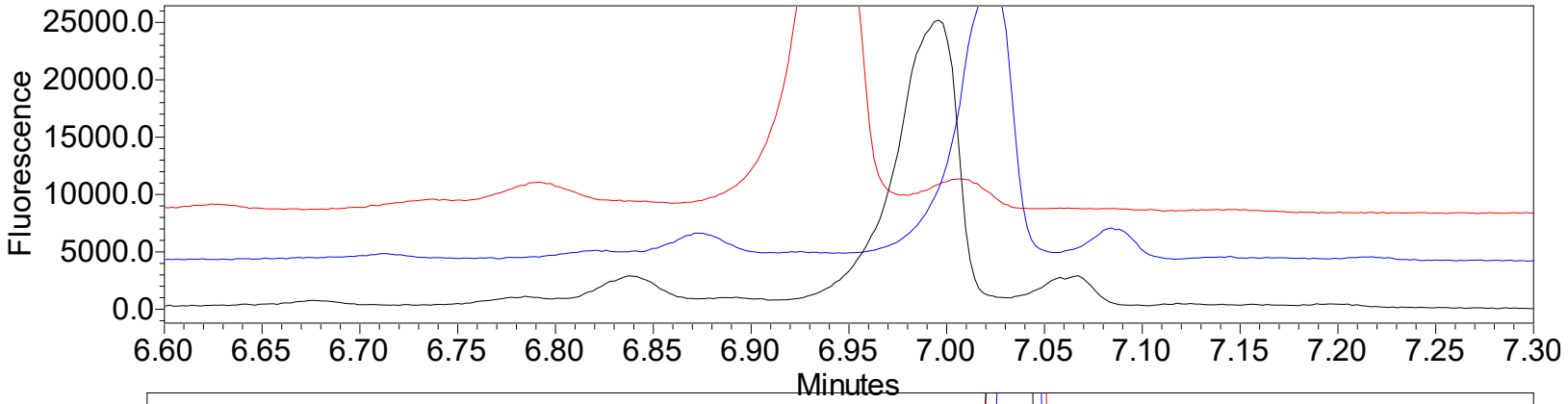
Carrier Ampholytes → 1:1 Pharmalyte 4-6.5 & Pharmalyte 5-8

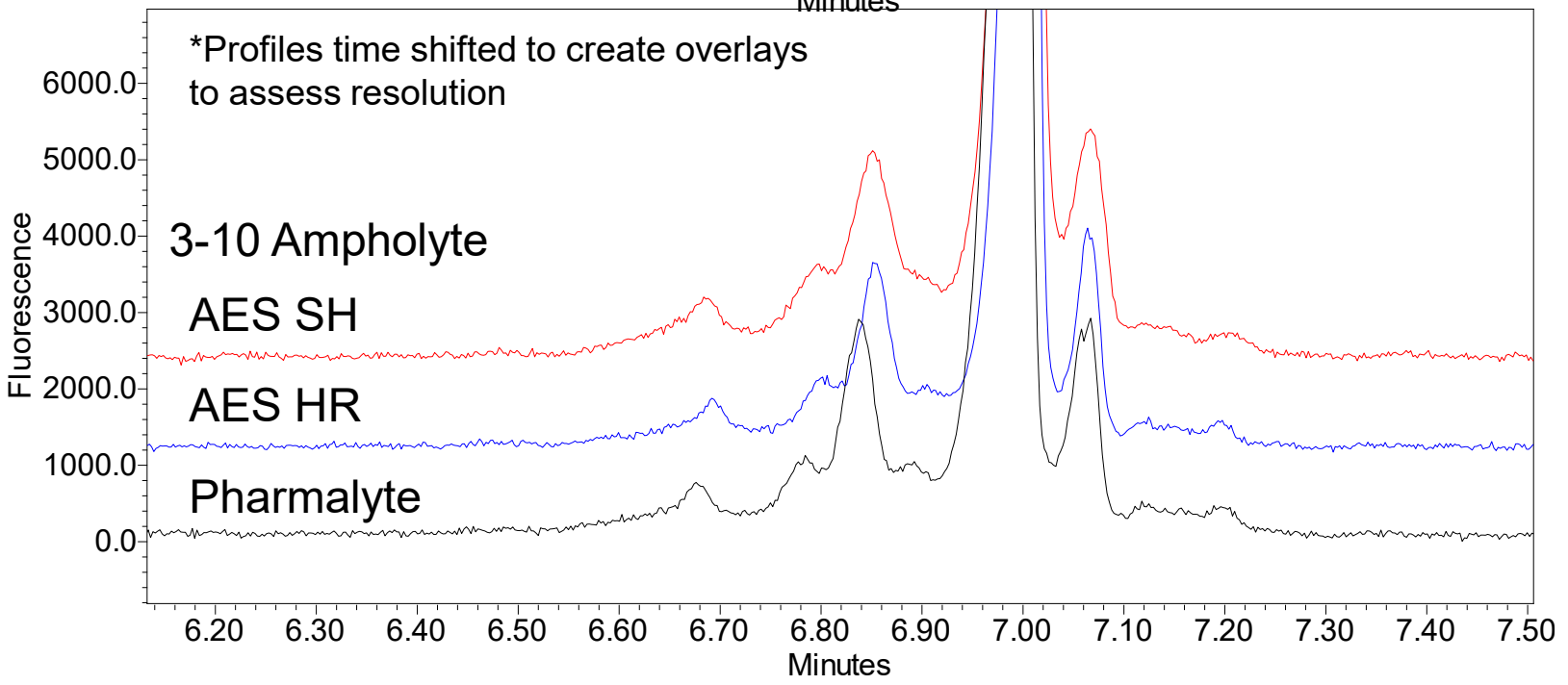
Master Mix	%MP	%TAV	%TBV	MP pI
Ph/Ph	69.3	16.6	14.1	6.00
HR/HR	70.2	16.6	13.2	6.14
Ph/HR	69.8	16.9	13.3	6.19
HR/PH	71.2	16.4	12.4	5.91
StDev	**0.81**	**0.21**	**0.70**	**0.13**

- **AESlyte HR 4-6 & 5-8 are equivalent to Pharmalyte 4-6.5 & 5-8 for mAb 2**
- Combining AESlyte with Pharmalyte produces equivalent profiles
- Biolyte was evaluated but was not comparable

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mAb 3 – Pharmalyte 3-10 Alternate Ampholyte





Method Carrier Ampholytes → 1:1 Pharmalyte 3-10 & AESlyte SH 6-9

AES SH 6-9 drives the separation for this method

3-10 Ampholyte	MP%	TAV%	TBV%	AV1 end p/v
Pharmalyte	72.5	17.6	9.9	1.3
AES HR	71.7	18.3	10.0	1.2
AES SH	71.3	18.4	10.4	1.3
StDev	**0.75**	**0.52**	**0.31**	**0.10**

N=6, 2 Analysts/Setups

Pharmalyte, AESlyte HR and AESlyte SH 3-10 are ALL equivalent for mAb 3

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Alternate Ampholyte Evaluation Summary

- Equivalent ampholytes were successfully identified for all Lilly methods, except one molecule
- AESlytes HR or SH were shown to be the most comparable to Pharmalyte
- Ability to mix ampholytes from different manufacturers was shown to be molecule specific (mAb 1 & 2)
- pI shifting was the most notable change for mAb 2 but remained within the method acceptance criteria
  - Most likely related to replacement of 4-6.5 with 4-6
- mAb 3 separation is driven by the narrow range ampholyte leading to less impact observed with alternate 3-10 ampholytes

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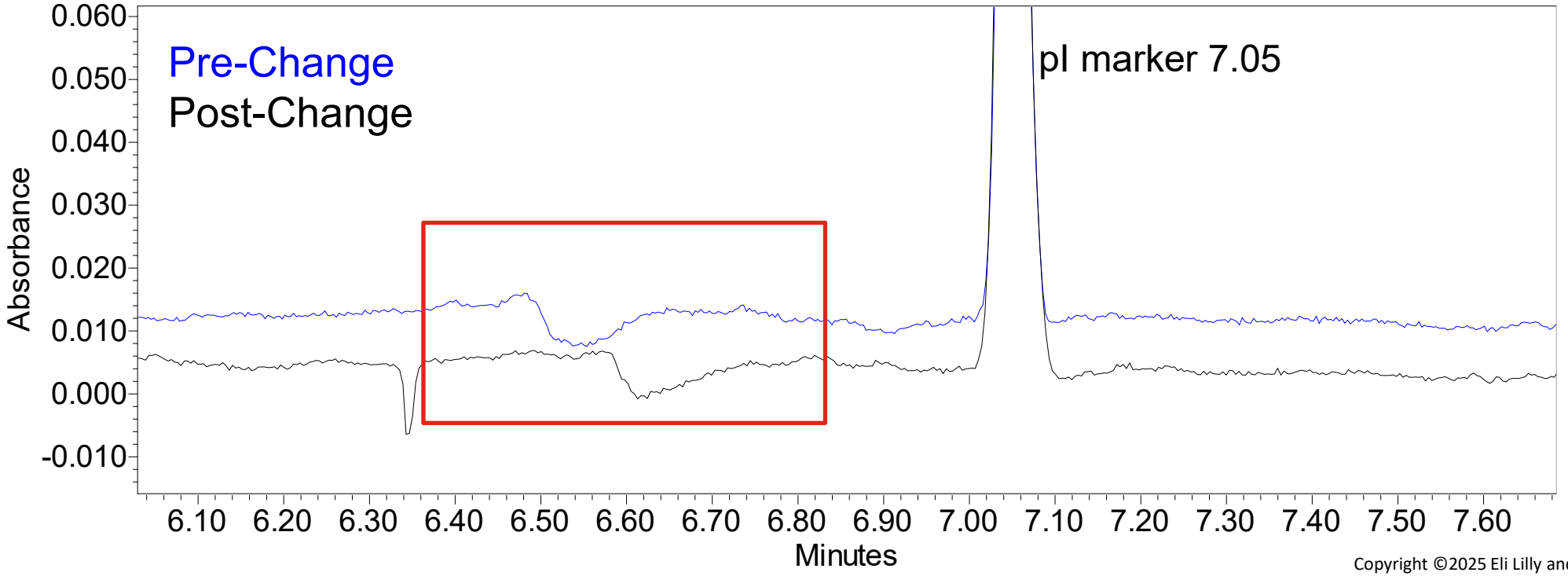
Alternate Ampholyte Implementation Plan

- A statistical analysis was completed leveraging data from previous method validation and historical testing to determine appropriate acceptance criteria for establishing alternates as validated equivalents
- Study will be executed under a formal protocol with the following requirements
  - Minimum of 2 labs
  - Minimum of 12 factors (e.g. minimum of 2 instruments, 2 analysts, 2 ampholyte batches, 2 cartridges, and 4 days)
- Ampholytes would be considered validated equivalents upon completion of the study
- Issue a change control to revise the global method to include an alternate manufacturer

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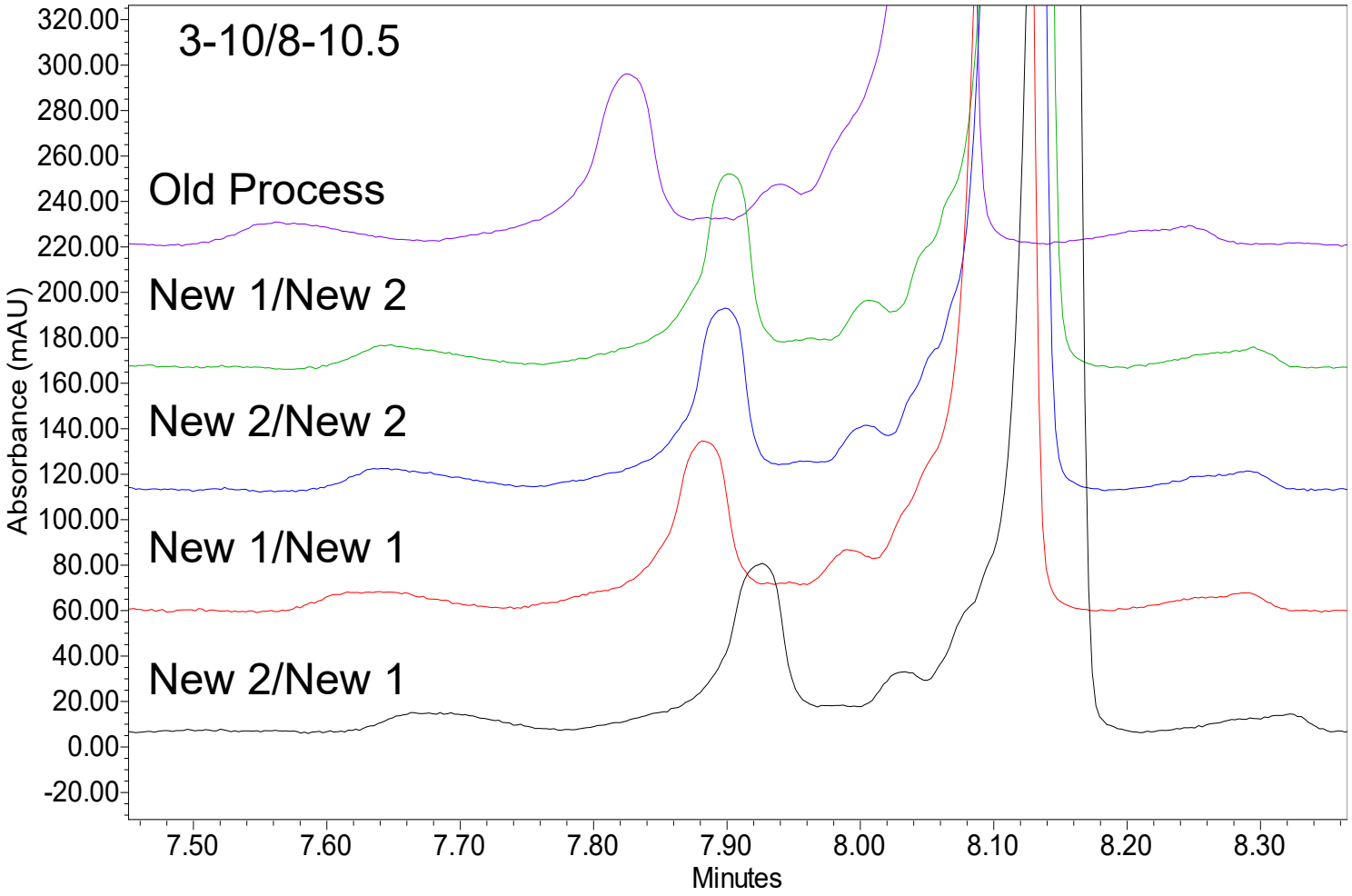
Post-Change Pharmalyte

- Received post-change Pharmalyte 3-10 in March 2025 and began comparing it to the pre-change lots
- One of the goals of updating the manufacturing equipment was to improve manufacturing consistency and remove the know dip ~6.6 pI in Pharmalyte 3-10



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mAb 1 - Evaluation of Post-Change Pharmalyte



*Data generated by Alex Antevska

Ampholyte lot (3-10/8-10.5)	% MP	% TAV	%TBV
Old	79.36	19.08	1.56
New 1/New 1	79.57	18.89	1.54
New 1/New 2	79.41	18.99	1.60
New 2/New 2	79.54	18.96	1.50
New 2/New1	79.33	19.20	1.47
Average	79.44	19.03	1.53
StDev	0.19	0.14	0.11

N=3 Injections

The post-change pharmalyte 3-10 and 8-10.5 showed to be comparable to the pre-change manufacturing process for mAb 1

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Post-Change Pharmalyte Implementation Plan

Development

- After initial evaluation, the post-change manufactured ampholytes can be approved for use in the development space
  - Must remain consistent with previous numerical results and profile appearance
- As new post-change lots are received, control charting will be used to monitor any changes

Commercial

- A study similar to the alternative manufacturer plan will be executed to allow use in commercial labs
  - Reduce risk of problems arising during commercial testing
  - This study was successfully completed for mAb1 and another commercial asset

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Conclusion

- The global Pharmalyte shortage had broad impact across the industry
- Equivalent AESlyte ampholytes were successfully identified for 3 commercial and 4 development molecules
  - AESlyte HR or SH was dependent on the molecule
- Post-change Pharmalyte has not shown any impact across multiple molecules
- Long term mitigation plan was established due to the shortage
  - Alternate manufacturers will be added to the current commercial methods
  - It is an expectation to evaluate backup manufacturers during method development
  - Continue to explore alternate manufacturesr and custom ampholyte services

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Questions?