

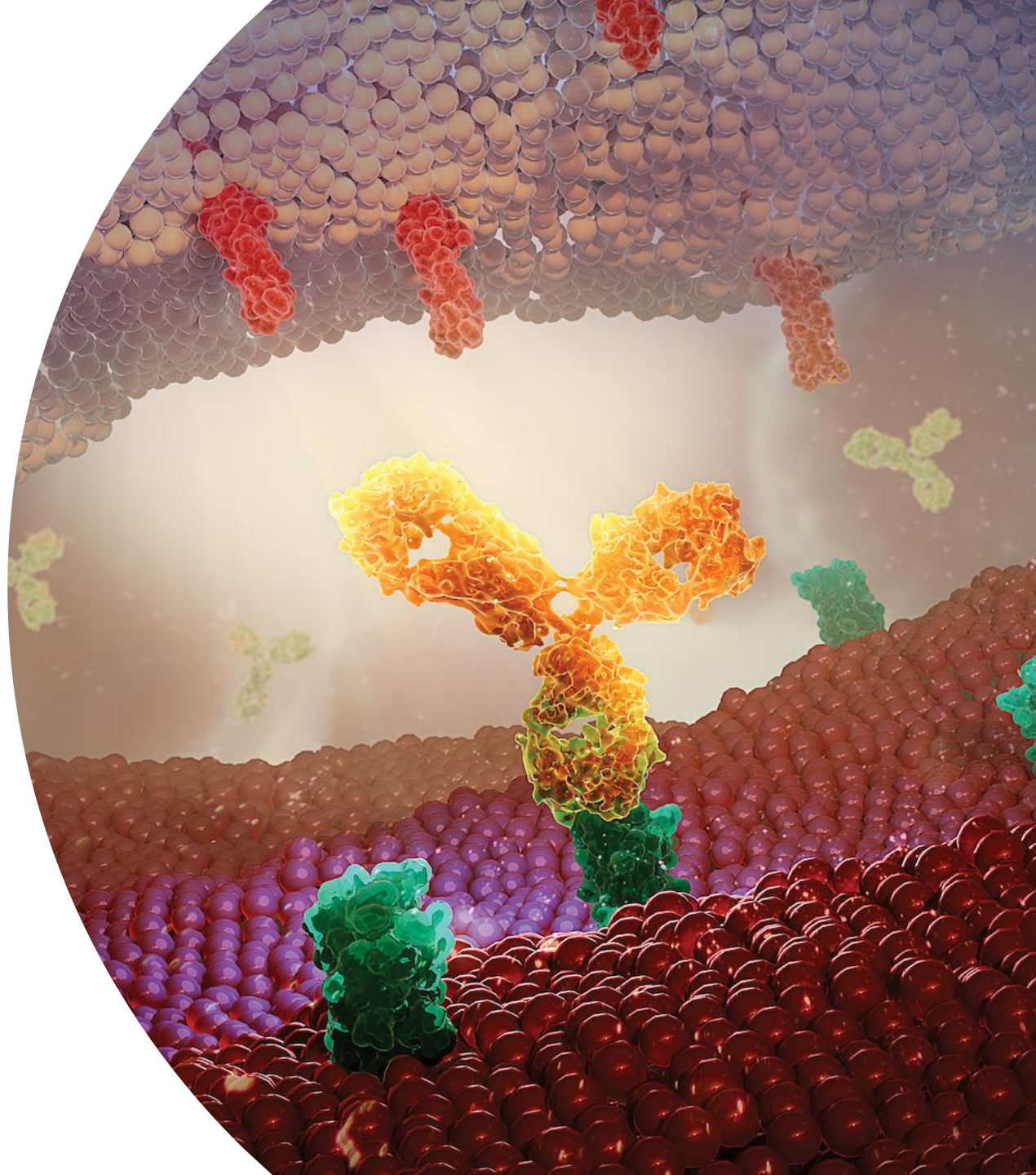


Case studies in cIEF and CE-SDS analytical transfers in a GMP environment

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27Jul23

Strictly Confidential



Global Technical Operations – Technical Transfer and Validation

- Global group
- Owners of analytical methods for all commercial products
 - Strategy, documentation, execution, etc.
 - Life-cycle management
- Responsible for analytical method validation for late-stage products
 - Bridge between development and operations (QC)
- Responsible for analytical technical transfers world-wide for commercial products
 - QC, CTLs, Government labs, etc.



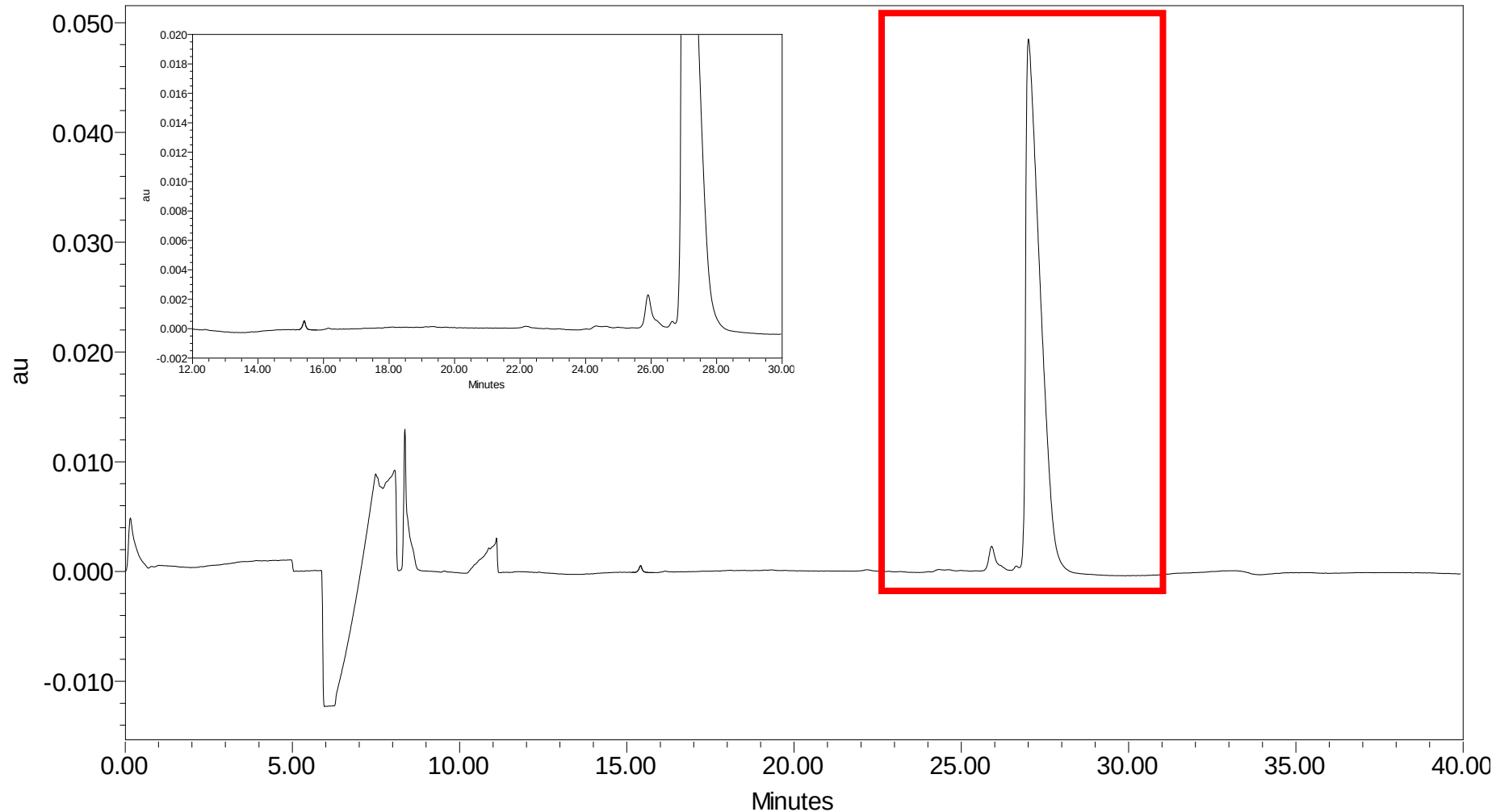
Method Validation – Background – CE-SDS and cIEF

- Global SOPs
- Co-validation with quality control groups
- 5 level, 4 runs per lab design using samples representative of release-level impurities (S1) and specification-limit impurities (S5) as well as blended samples
 - Demonstrates method performance across entire range of anticipated impurity

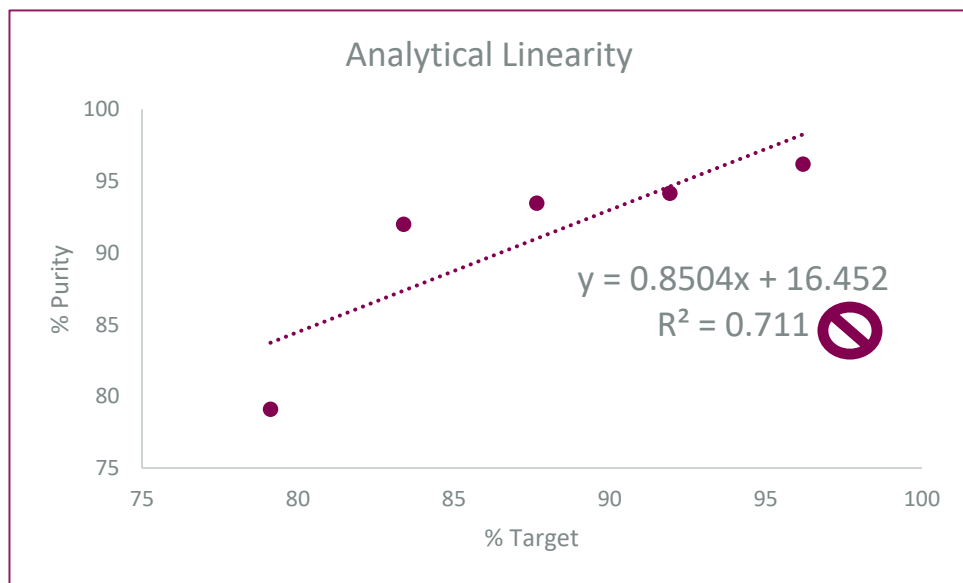
Run	Site	Instrument	Analyst	Sample				
				S1	S2	S3	S4	S5
1	GTO-A	1	1	2x	2x	2x	2x	2x
2			1	2x				2x
3		2	1	2x	2x	2x	2x	2x
4			1	2x				2x
5	QC	3	2	2x	2x	2x	2x	2x
6			2	2x				2x
7		4	2	2x	2x	2x	2x	2x
8			2	2x				2x



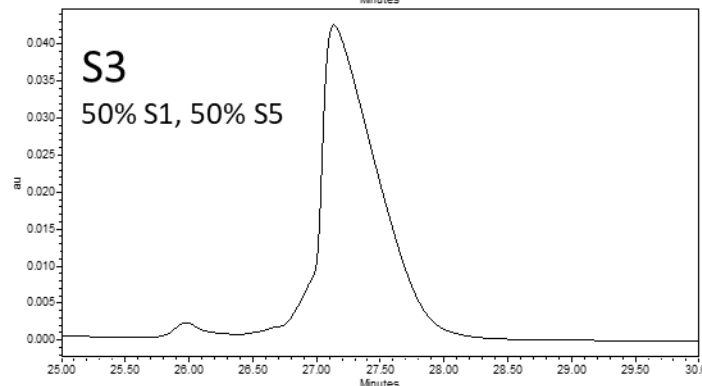
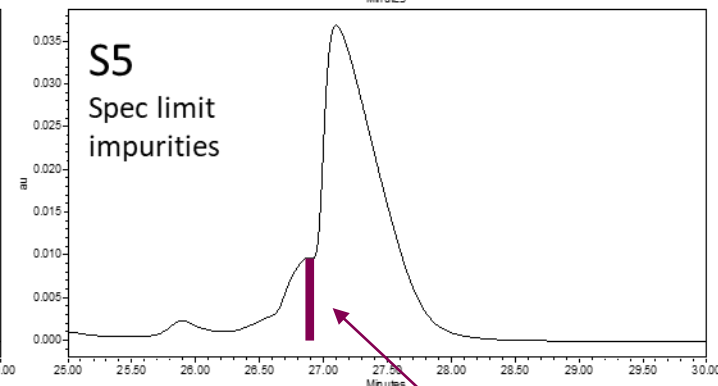
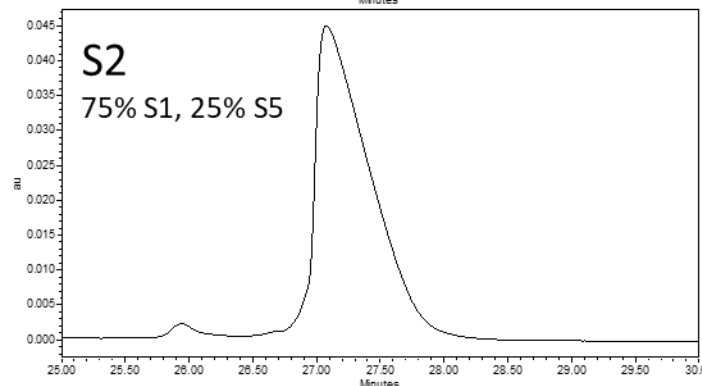
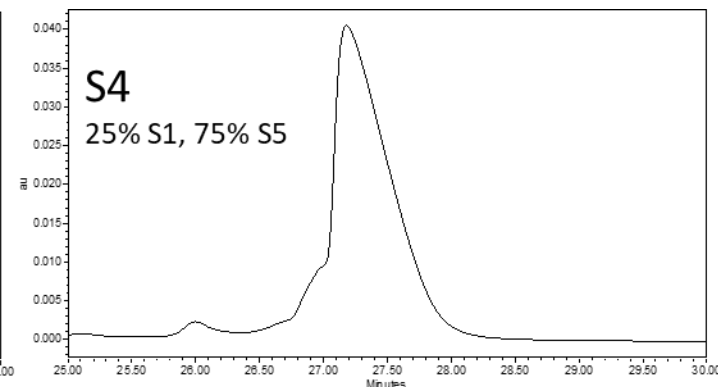
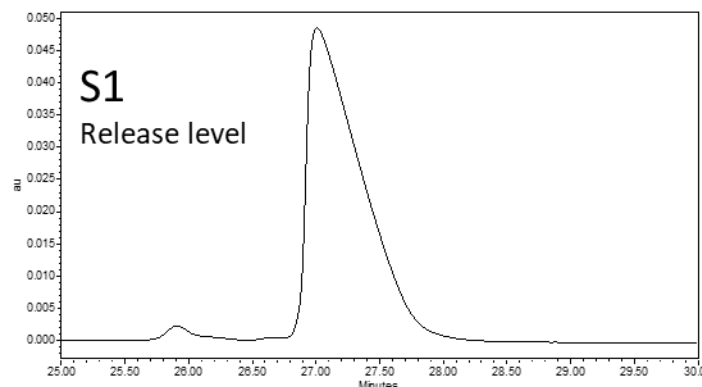
Method Validation – CE-SDS Case Study: Typical mAb Profile



Method Validation – CE-SDS Case Study: Shoulder Peaks



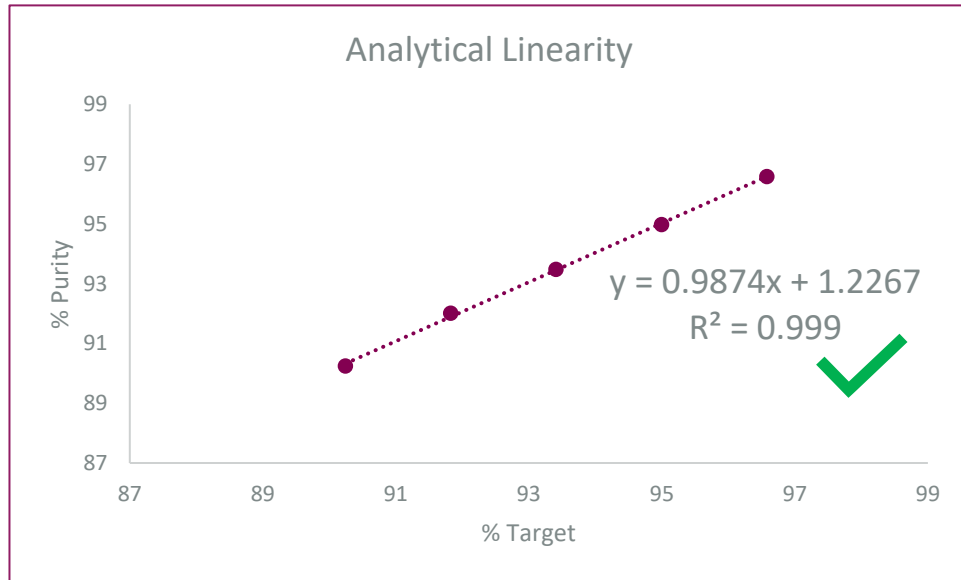
Sample	% Recovery
S2	102
S3	107 ❌
S4	110 ❌



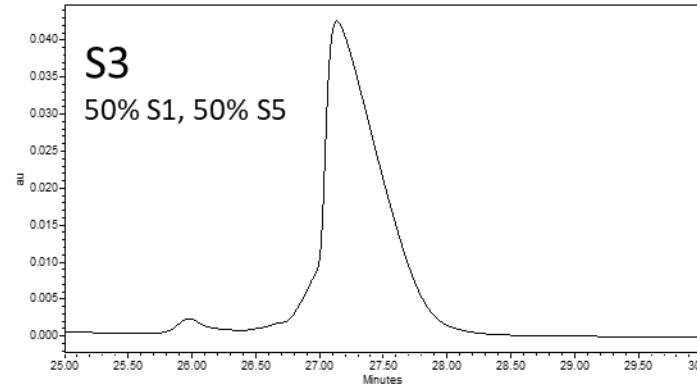
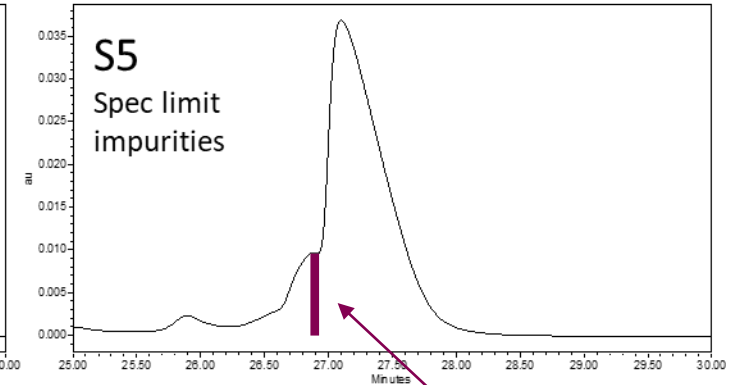
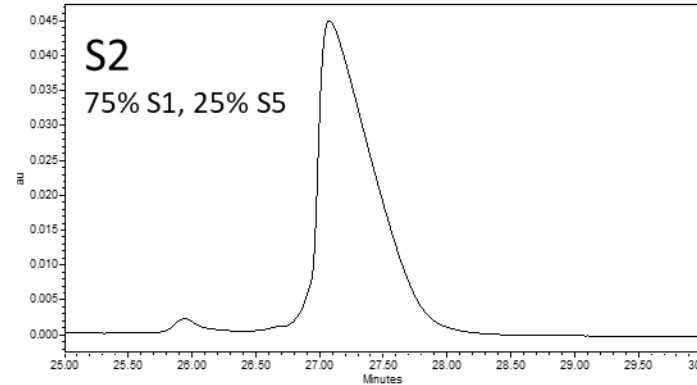
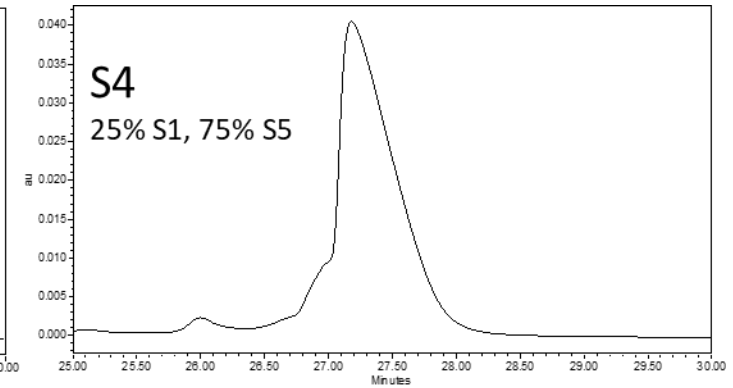
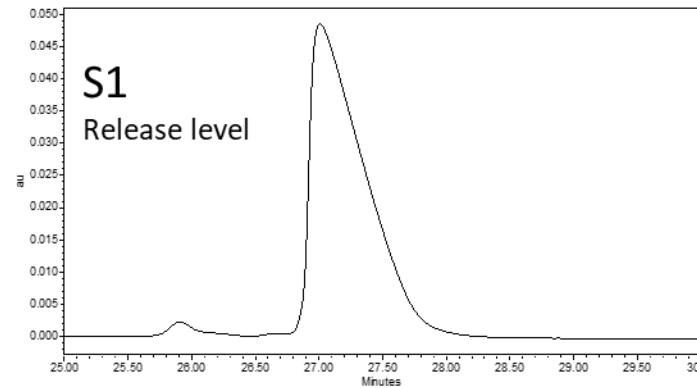
Integrate shoulder
Exclude from purity



Method Validation – CE-SDS Case study: Shoulder Peaks



Sample	% Recovery
S2	100 ✓
S3	100 ✓
S4	100 ✓



Method Validation – CE-SDS – Lessons Learned

- Include stressed samples in development studies
 - Consider characterizing peaks of interest
- Prior to validation...
 - Identify potentially problematic peaks/shoulders
 - Identify potential integration problems
 - Shoulders, dips, common vs split baselines, etc.
 - Define integration practices (shoulders, LOD, etc.)
- *Integration must be consistent in QC globally*



Method Transfer – Background – CE-SDS and cIEF

- Transfer Unit (TU) and Receiving Unit (RU) data
 - TU data (and samples) may be generated during method validation
- RU: 2 analysts, 3 runs per analyst design using samples representative of release-level impurities (S1) and a degraded sample (S3)
 - Demonstrates RU performance for expected samples

Run	Site	Analyst	Sample	
			S1	S3
1	RU	1	2x	2x
2			2x	2x
3			2x	2x
4		2	2x	2x
5			2x	2x
6			2x	2x



Method Transfer – Background – CE-SDS and cIEF

- RU data is evaluated for equivalence (EAC) and intermediate precision
 - EAC is usually 3 times the 95% confidence bound of TU data standard deviation*
 - 90% confidence interval on mean RU data must be within EAC of mean TU data

Example:

Equivalence Test Results						
Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
% Purity Nominal	99.511	99.454	0.057	[-0.038, 0.153]	0.754	Equivalent

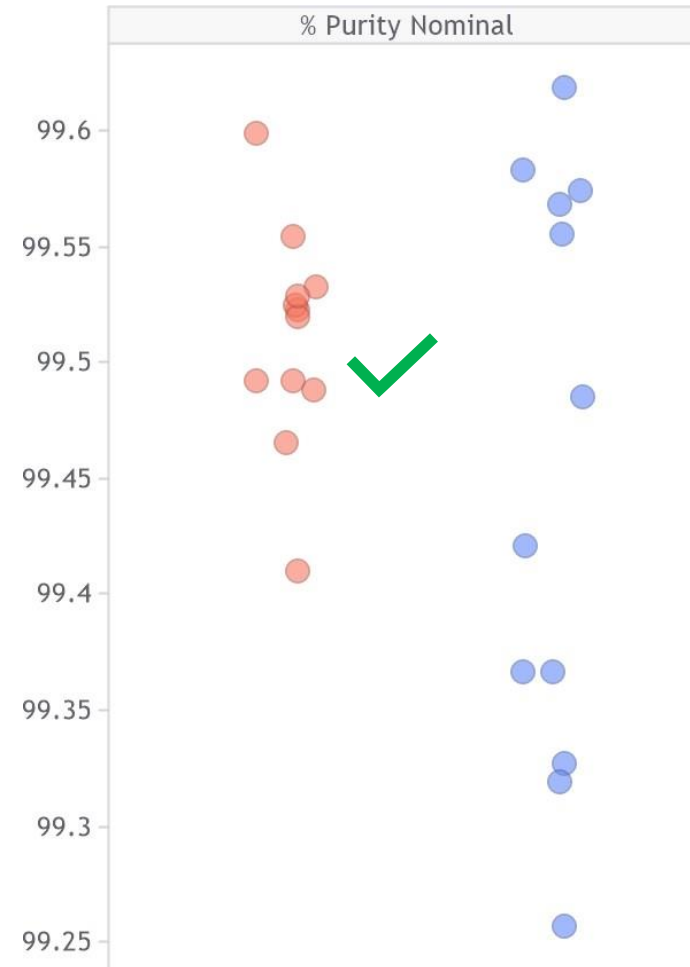
Intermediate Precision Results - RU			
Sample ID	Intermediate Precision SD	Acceptance Criterion	Conclusion
% Purity Nominal	0.0	0.6	Pass



Method Transfer – Background – CE-SDS and cIEF

Example:

RU – red
TU – blue



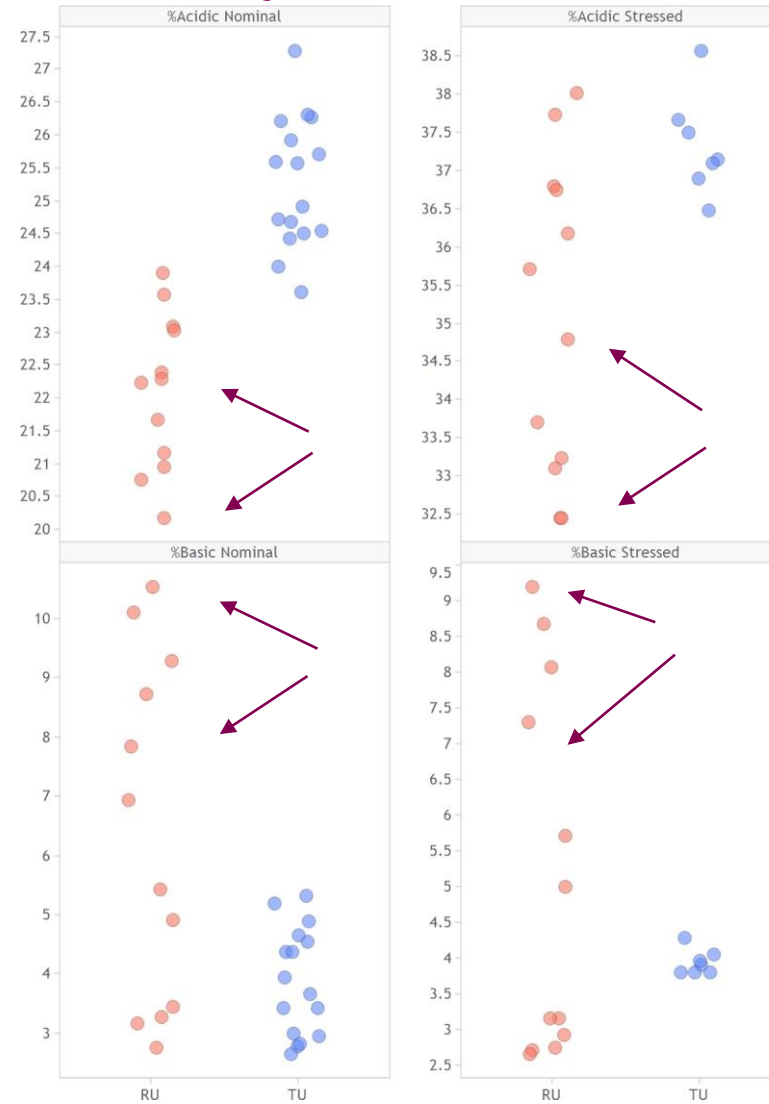
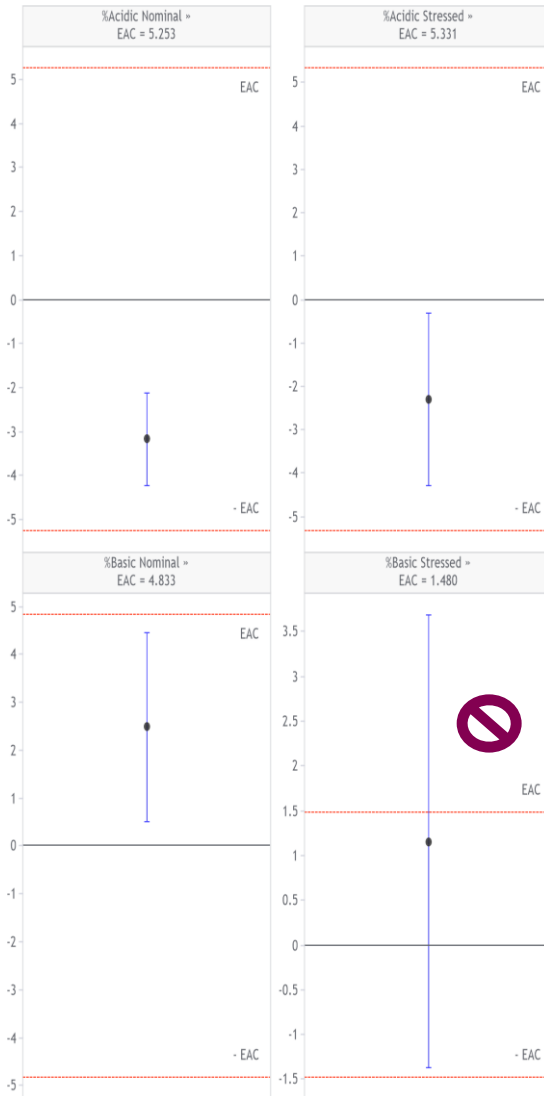
Method Transfer – cIEF Case study – Resolution

Equivalence Test Results						
Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
% Acidic Nominal	22.102	25.267	-3.165	[-4.210, -2.120]	5.253	Equivalent
% Acidic Stressed	35.078	37.371	-2.293	[-4.284, -0.302]	5.331	Equivalent
% Basic Nominal	6.371	3.876	2.494	[0.520, 4.469]	4.833	Equivalent
% Basic Stressed	5.114	3.955	1.159 ←	[-1.372, 3.689] ←	1.480 ←	Inconclusive
% Main Nominal	71.527	70.873	0.654	[-1.010, 2.317]	8.304	Equivalent
% Main Stressed	59.808	58.693	1.115	[-0.091, 2.321]	6.664	Equivalent

Intermediate Precision Results - RU			
Sample ID	Intermediate Precision SD	Acceptance Criterion	Conclusion
% Acidic Nominal	1.2	5	Pass
% Acidic Stressed	2.1	5	Pass
% Basic Nominal	3.0	5	Pass
% Basic Stressed	2.7	5	Pass
% Main Nominal	2.0	5	Pass
% Main Stressed	1.3	5	Pass

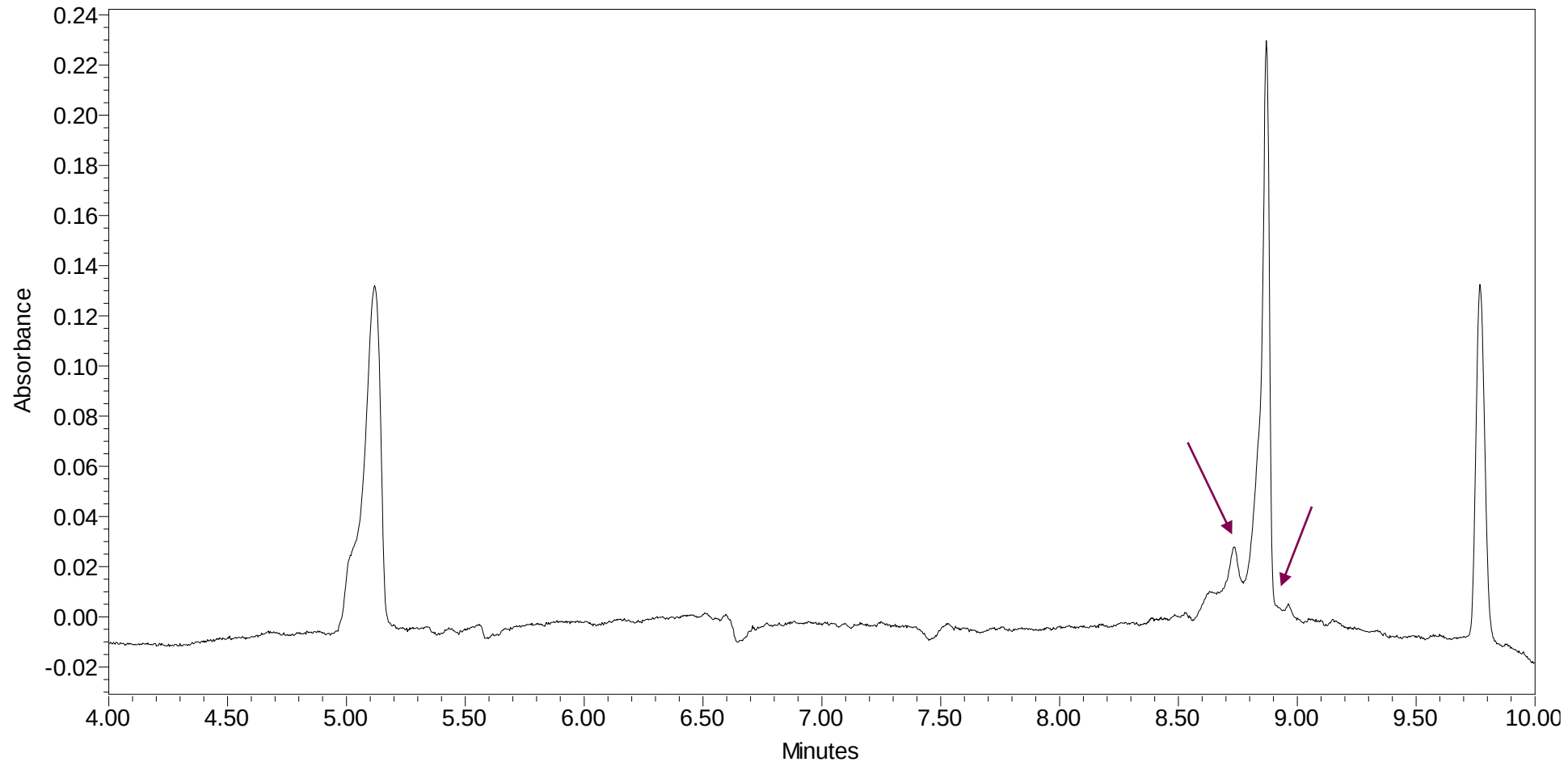


Method Transfer – cIEF Case study – Resolution



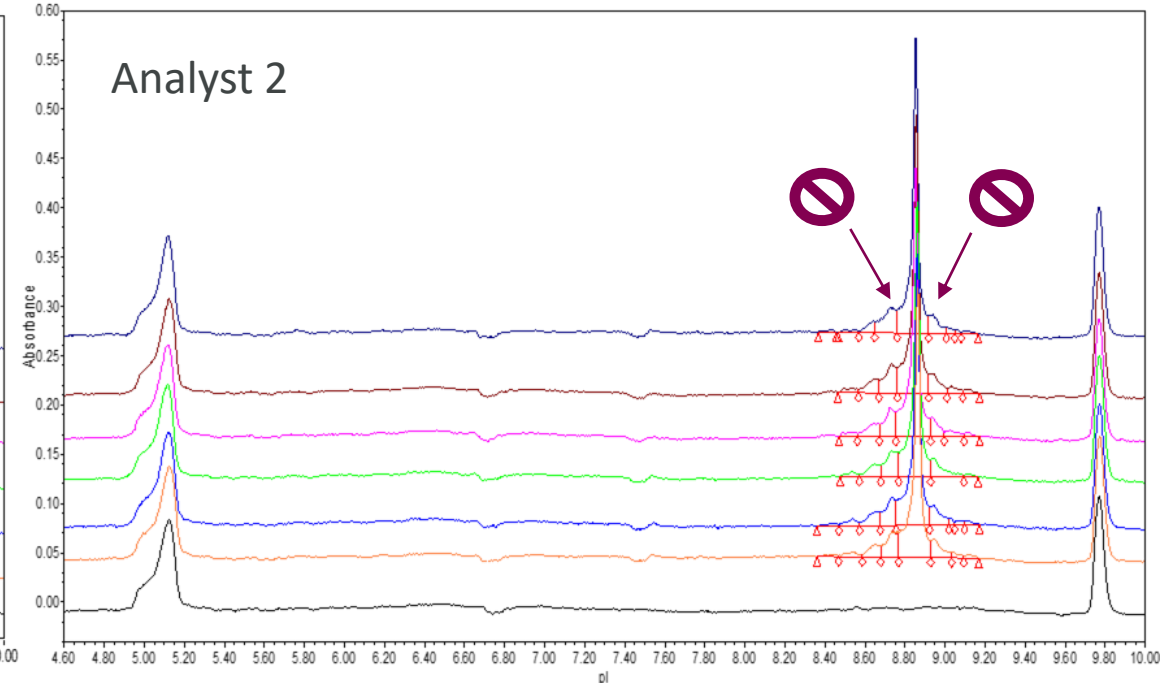
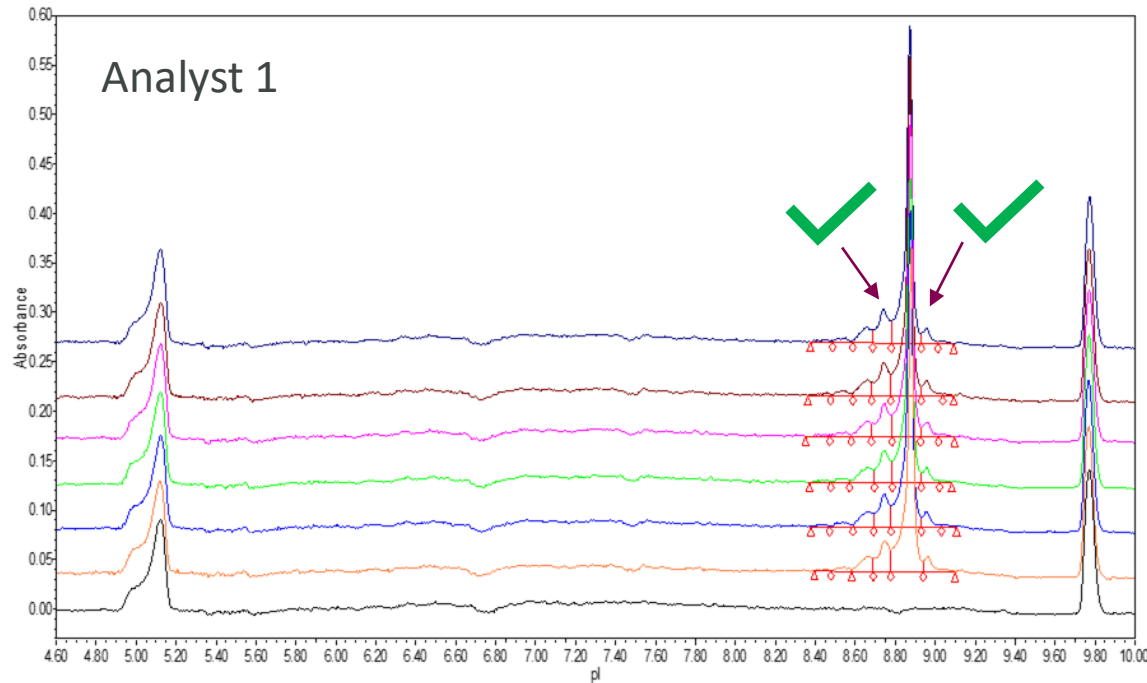
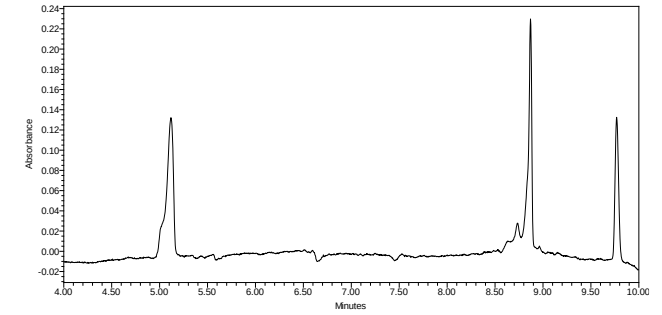
Method Transfer – cIEF Case study – Resolution

- Target profile for release-level sample



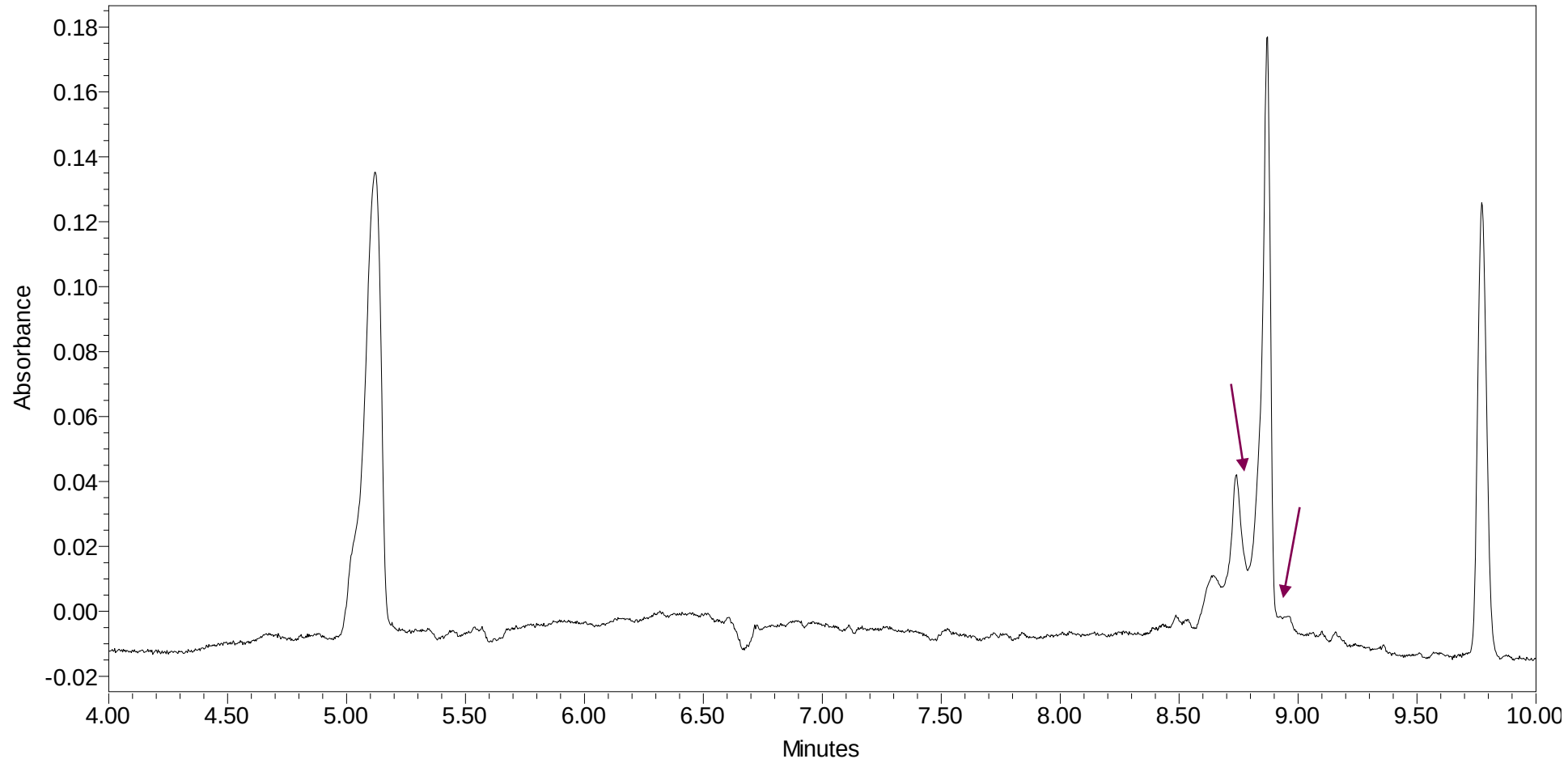
Method Transfer – cIEF Case study – Resolution

- Initial RU profile for release-level sample
 - 3 runs, 2 results per run
- Analyst 2 fails visual acceptance criterion



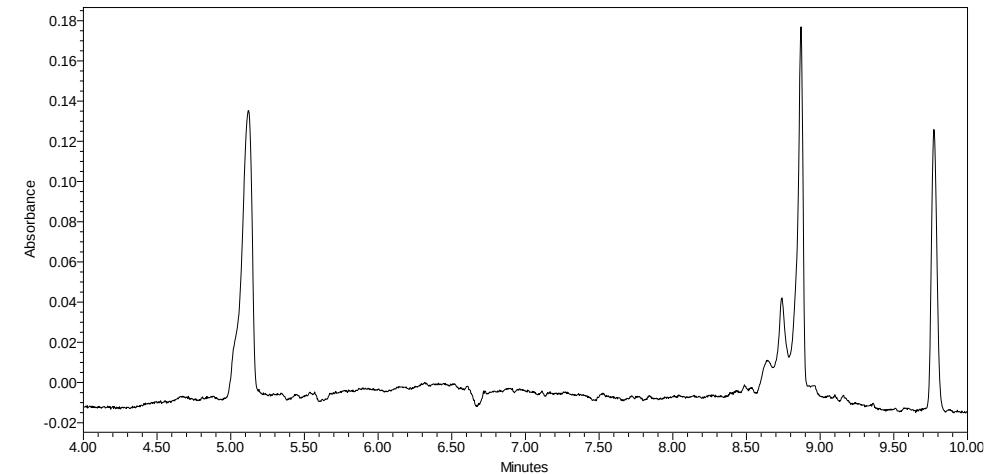
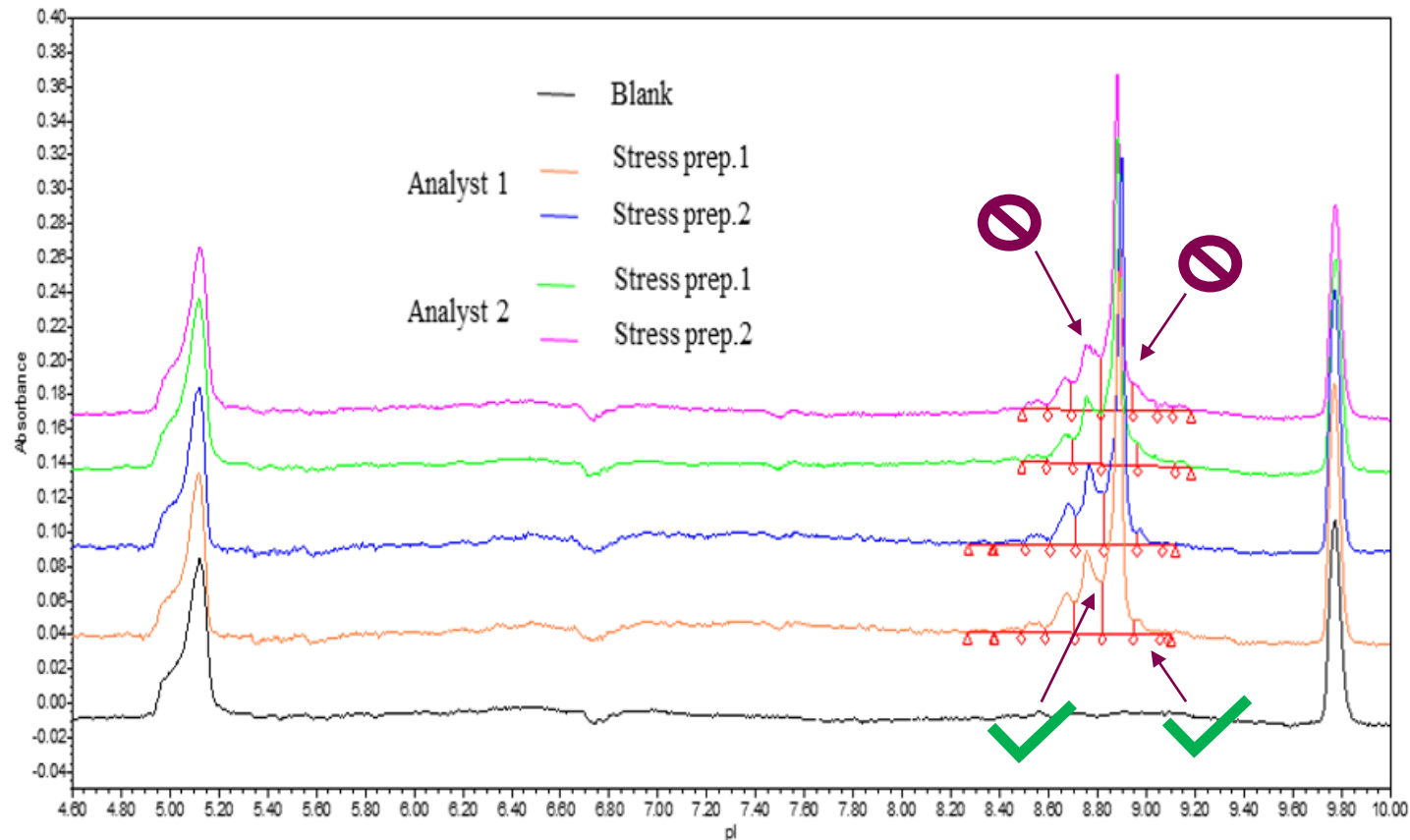
Method Transfer – cIEF Case study – Resolution

- Target profile for degraded sample



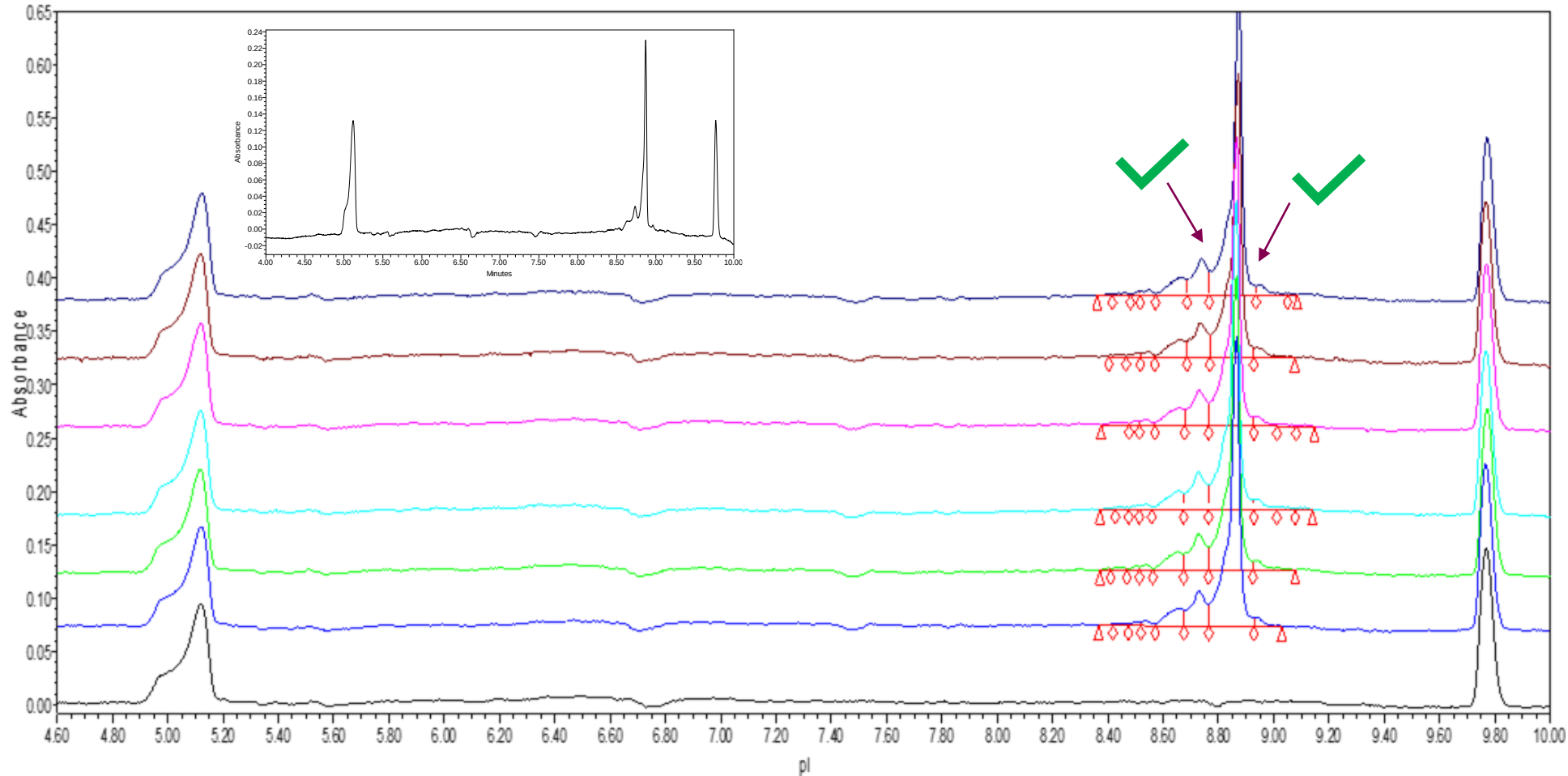
Method Transfer – cIEF Case study – Resolution

- Initial RU profile for degraded sample
- Analyst 2 fails visual acceptance criterion



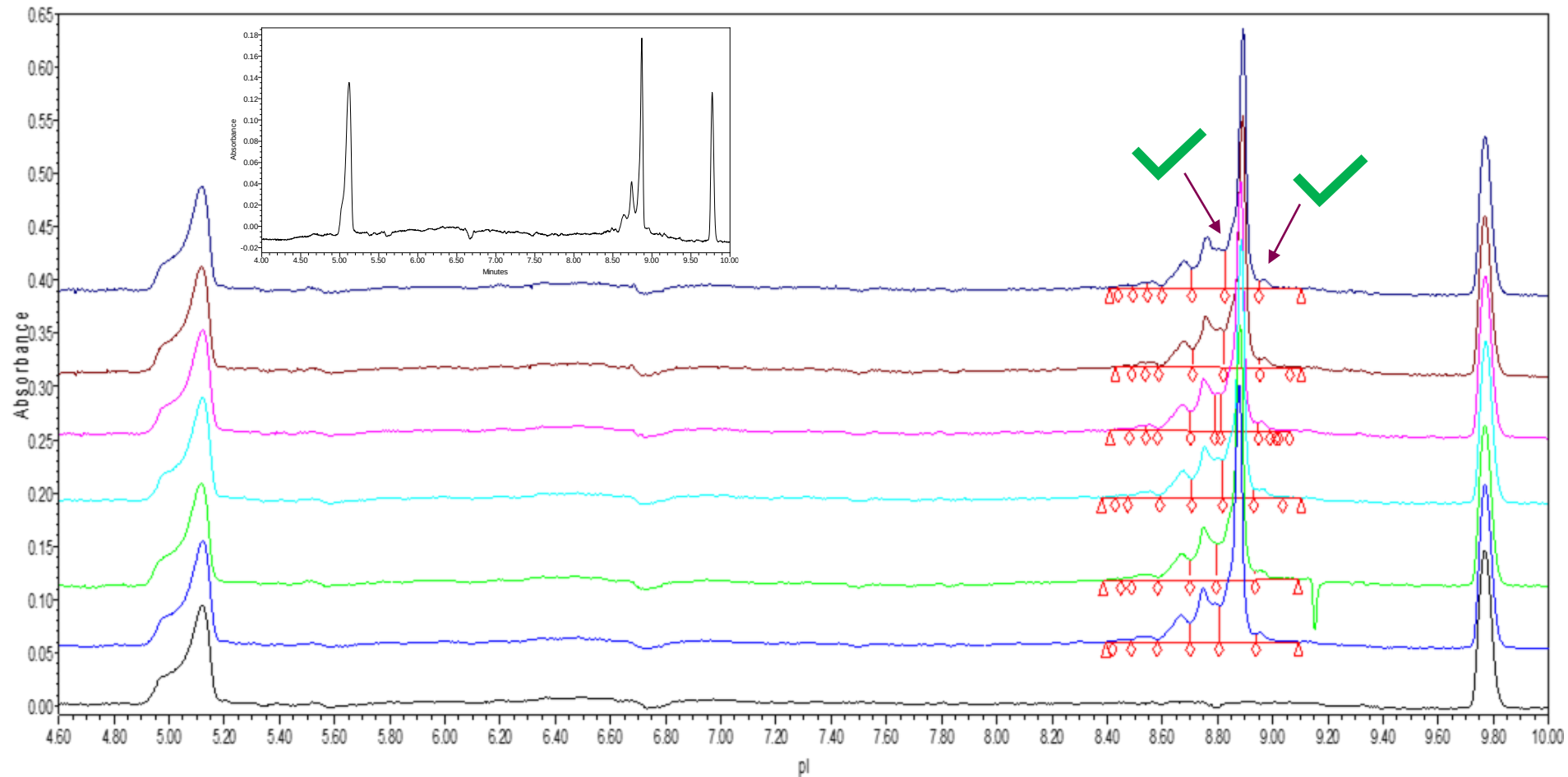
Method Transfer – cIEF Case study – Resolution

- RU release-level sample profile after remediation (Analyst 2: 3 runs, 2 results per run)



Method Transfer – cIEF Case study – Resolution

- RU degraded sample profile after remediation (Analyst 2: 3 runs, 2 results per run)



Method Transfer – cIEF Case study – Resolution

Equivalence Test Results

Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
% Acidic Nominal	22.870	25.267	-2.397	[-3.216, -1.578]	5.253	Equivalent
% Acidic Stressed	36.715	37.347	-0.632	[-1.782, 0.518]	5.331	Equivalent
% Basic Nominal	3.514	3.876	-0.362	[-1.257, 0.533]	4.833	Equivalent
% Basic Stressed	2.890	3.953	-1.063	[-1.307, -0.819]	1.480	Equivalent
% Main Nominal	73.615	70.873	2.742	[1.454, 4.030]	8.304	Equivalent
% Main Stressed	60.395	58.705	1.689	[0.528, 2.850]	6.664	Equivalent



Intermediate Precision Results - RU

Sample ID	Intermediate Precision SD	Acceptance Criterion	Conclusion
% Acidic Nominal	0.6	5	Pass
% Acidic Stressed	1.4	5	Pass
% Basic Nominal	1.0	5	Pass
% Basic Stressed	0.3	5	Pass
% Main Nominal	1.0	5	Pass
% Main Stressed	1.4	5	Pass



Method Transfer – cIEF Case study – Resolution

Final Results

Equivalence Test Results

Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
% Acidic Nominal	22.870	25.267	-2.397	[-3.216, -1.578]	5.253	Equivalent
% Acidic Stressed	36.715	37.347	-0.632	[-1.782, 0.518]	5.331	Equivalent
% Basic Nominal	3.514	3.876	-0.362	[-1.257, 0.533]	4.833	Equivalent
% Basic Stressed	2.890	3.953	-1.063	[-1.307, -0.819]	1.480	Equivalent
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Intermediate Precision Results - RU

Sample ID	Intermediate Precision SD	Acceptance Criterion	Conclusion
% Acidic Nominal	0.6	5	Pass
% Acidic Stressed	1.4	5	Pass
% Basic Nominal	1.0	5	Pass
% Basic Stressed	0.3	5	Pass
% Main Nominal	1.0	5	Pass
% Main Stressed	1.4	5	Pass

Initial Results

Equivalence Test Results

Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
% Acidic Nominal	22.102	25.267	-3.165	[-4.210, -2.120]	5.253	Equivalent
% Acidic Stressed	35.078	37.371	-2.293	[-4.284, -0.302]	5.331	Equivalent
% Basic Nominal	6.371	3.876	2.494	[0.520, 4.469]	4.833	Equivalent
% Basic Stressed	5.114	3.955	1.159	[-1.372, 3.689]	1.480	Inconclusive
% Main Nominal	71.527	70.873	0.654	[-1.010, 2.317]	8.304	Equivalent
% Main Stressed	59.808	58.693	1.115	[-0.091, 2.321]	6.664	Equivalent

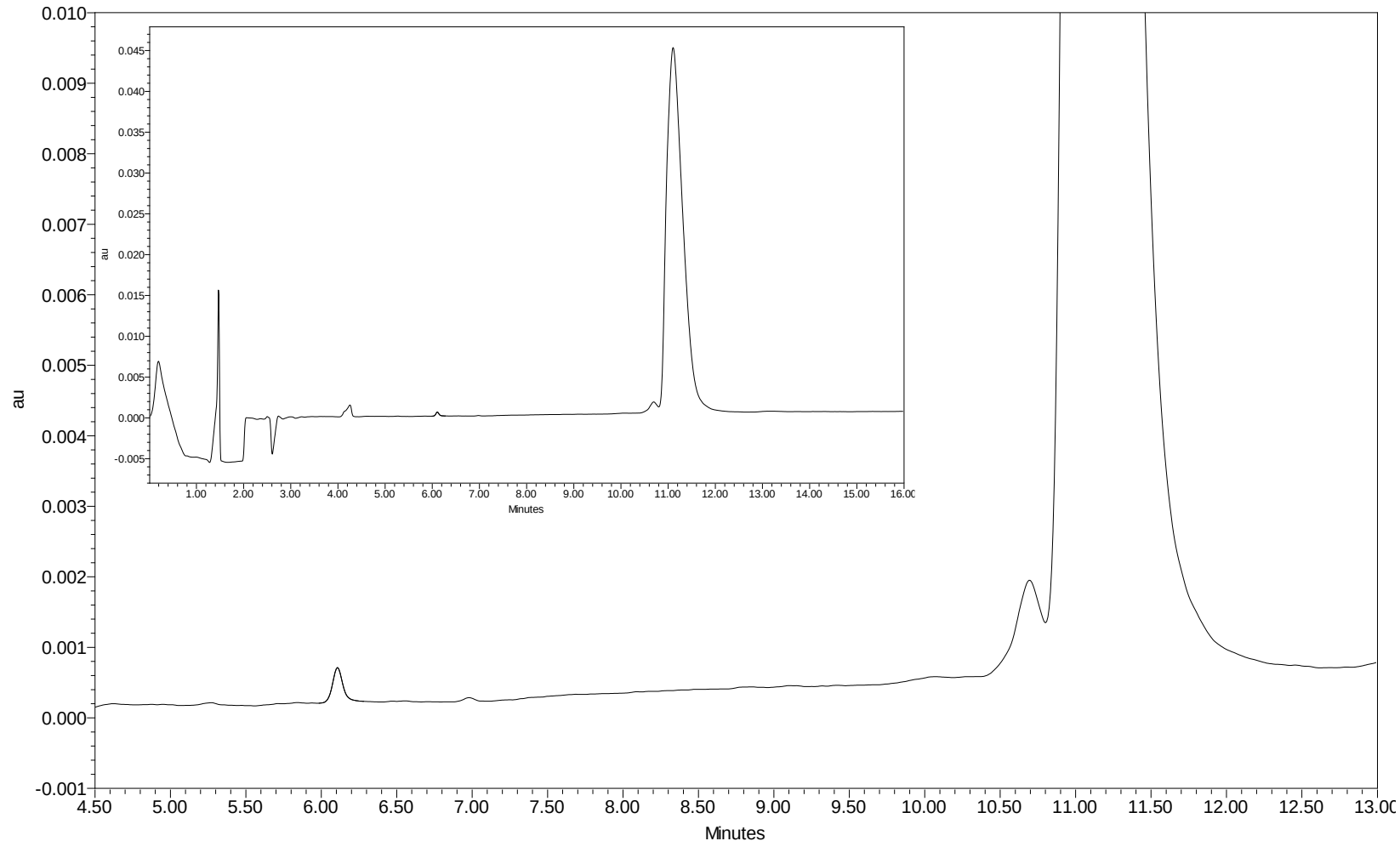
Intermediate Precision Results - RU

Sample ID	Intermediate Precision SD	Acceptance Criterion	Conclusion
% Acidic Nominal	1.2	5	Pass
% Acidic Stressed	2.1	5	Pass
% Basic Nominal	3.0	5	Pass
% Basic Stressed	2.7	5	Pass
% Main Nominal	2.0	5	Pass
% Main Stressed	1.3	5	Pass



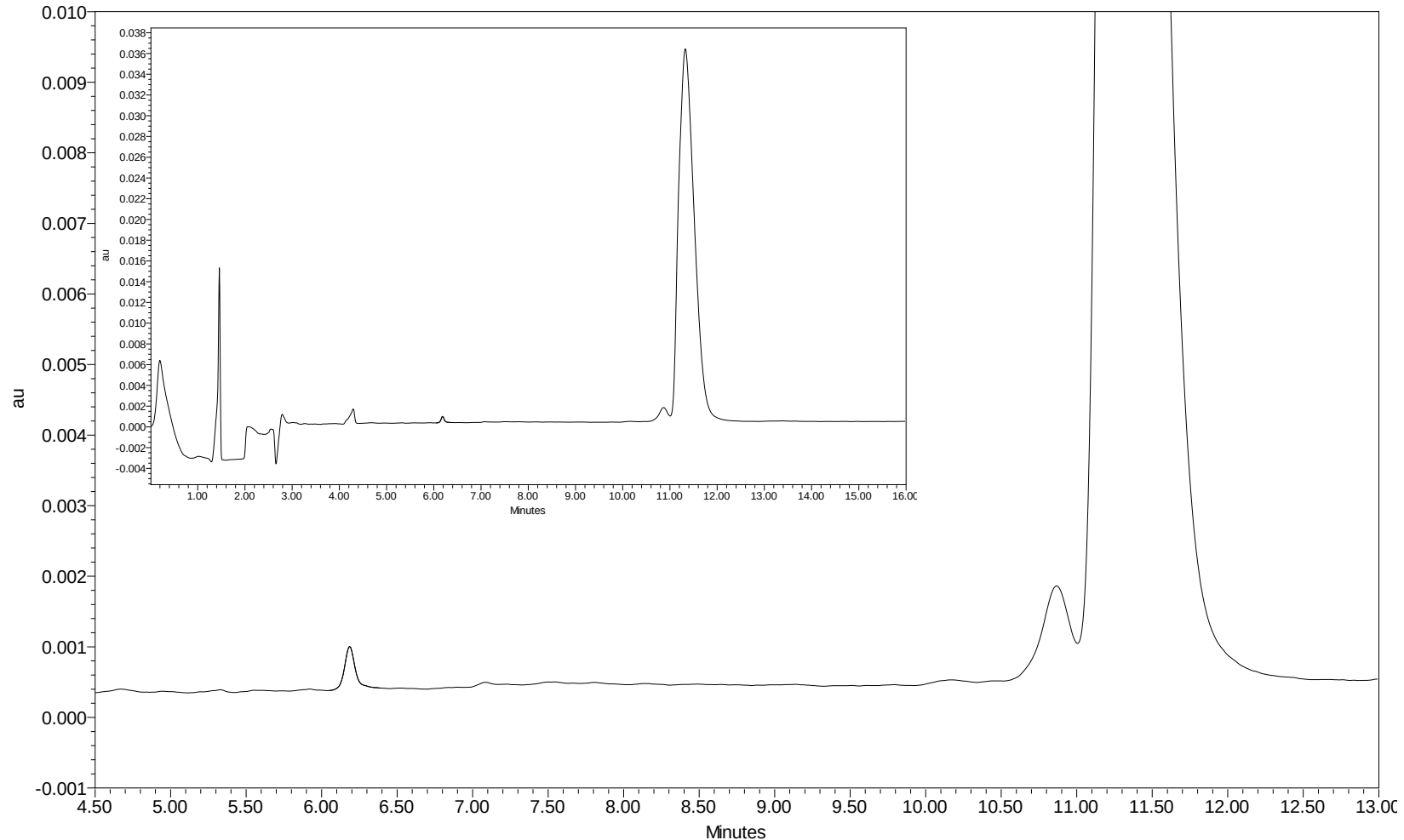
Method Transfer – CE-SDS Case study – Reagents

- Target profile for release-level sample



Method Transfer – CE-SDS Case study – Reagents

- Initial RU profile for release-level sample



Method Transfer – CE-SDS Case study – Reagents

Equivalence Test Results

Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
High Molecular Weight-NR Stressed	1.162	1.222	-0.060	[-0.194, 0.074]	0.949	Equivalent
IgG Main Peak-NR Nominal	97.517	98.228	-0.711 ←	[-1.195, -0.228]	0.766	Inconclusive
IgG Main Peak-NR Stressed	93.687	94.342	-0.655 ←	[-1.363, 0.053]	1.148	Inconclusive
NR Fragment Nominal	2.483	1.772	0.711 ←	[0.228, 1.195]	0.766	Inconclusive
NR Fragment Stressed	4.987	4.130	0.857 ←	[0.172, 1.541]	0.841	Inconclusive

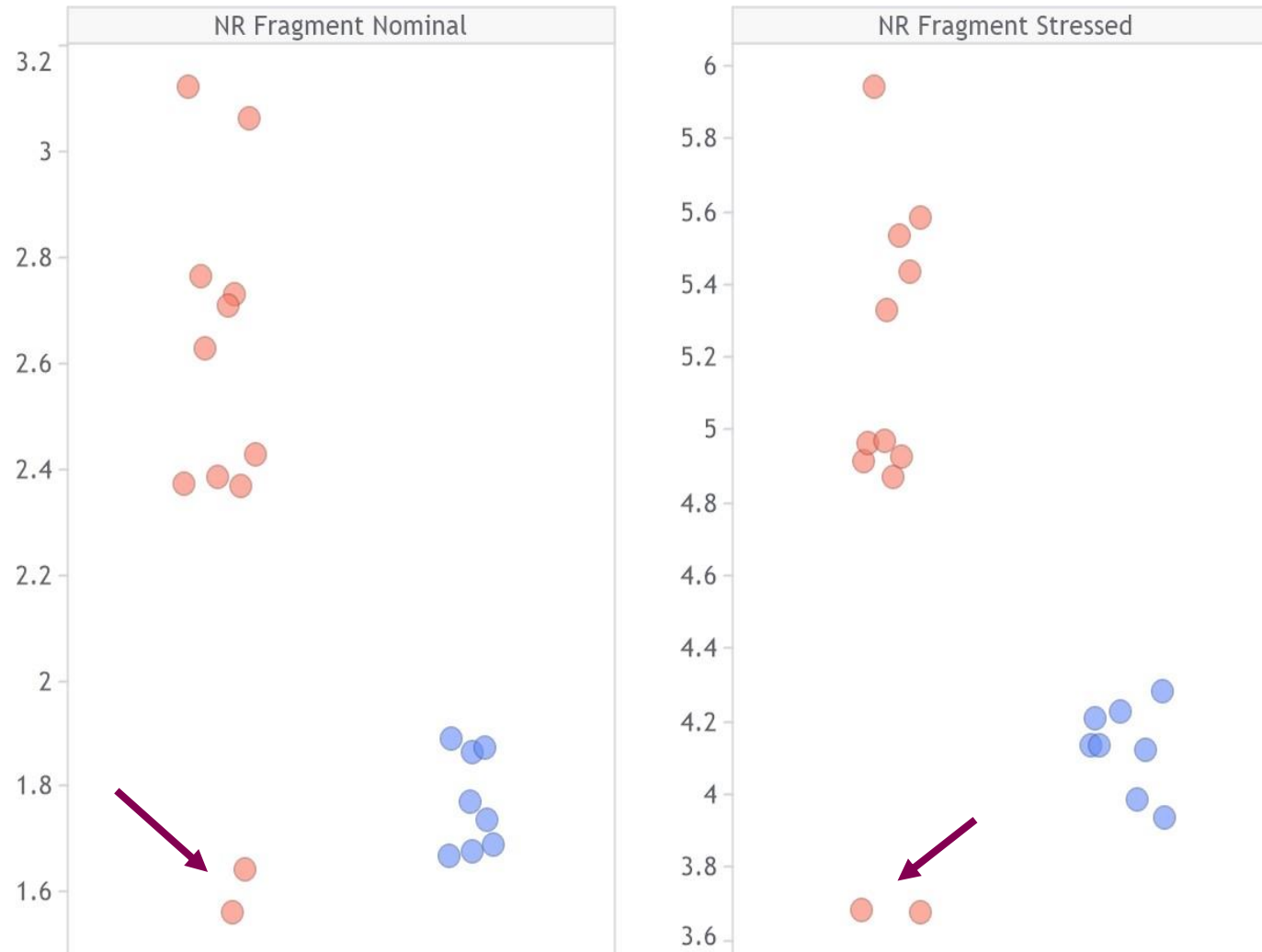
Intermediate Precision Results - RU

Sample ID	Intermediate Precision % CV	Acceptance Criterion	Conclusion
High Molecular Weight-NR Nominal	N/A	30	Pass
High Molecular Weight-NR Stressed	13	30	Pass
IgG Main Peak-NR Nominal	0.5	3.0	Pass
IgG Main Peak-NR Stressed	0.8	3.0	Pass
NR Fragment Nominal	20 ←	20	Pass
NR Fragment Stressed	15 ←	20	Pass



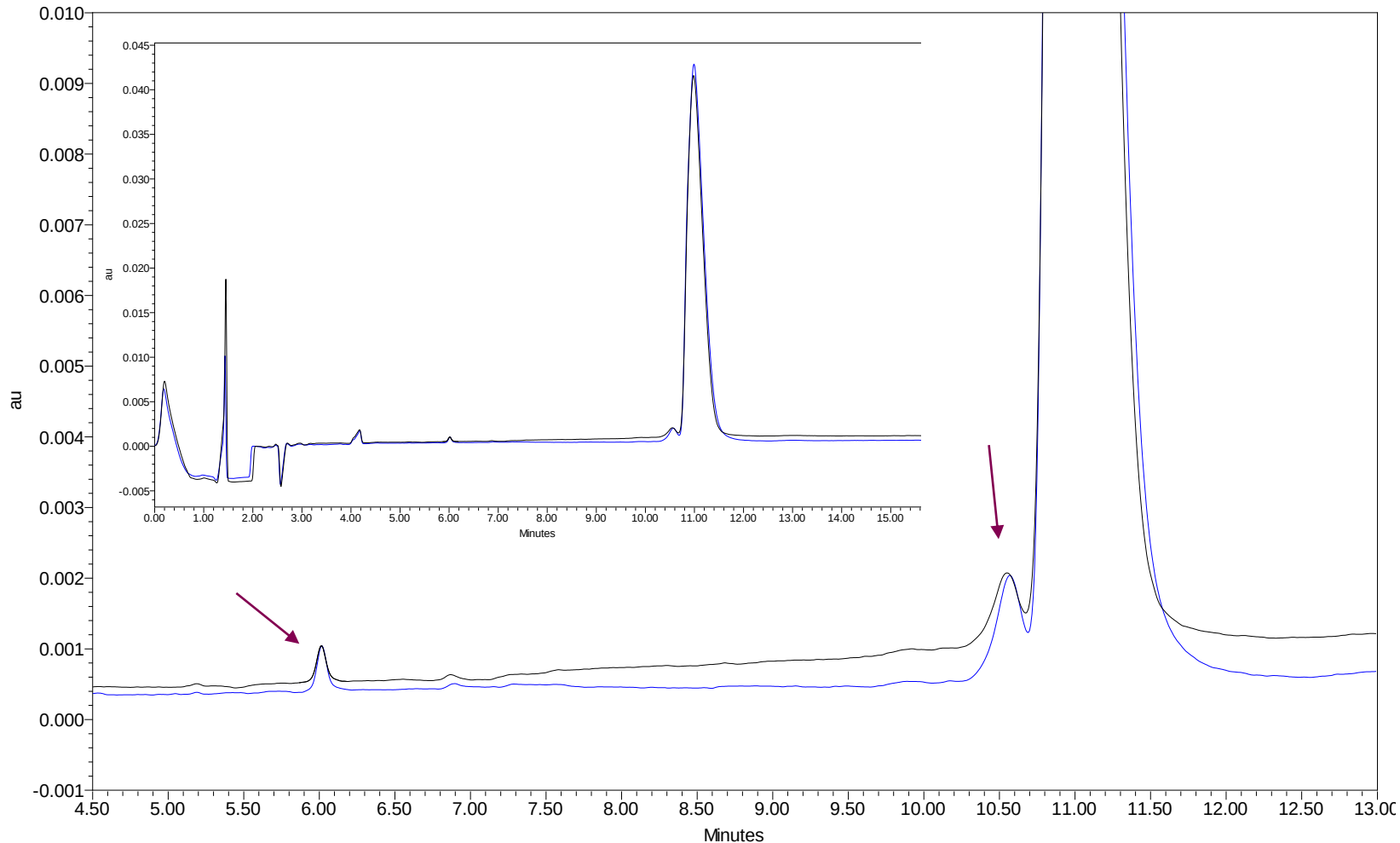
Method Transfer – CE-SDS Case study – Reagents

RU – red
TU – blue



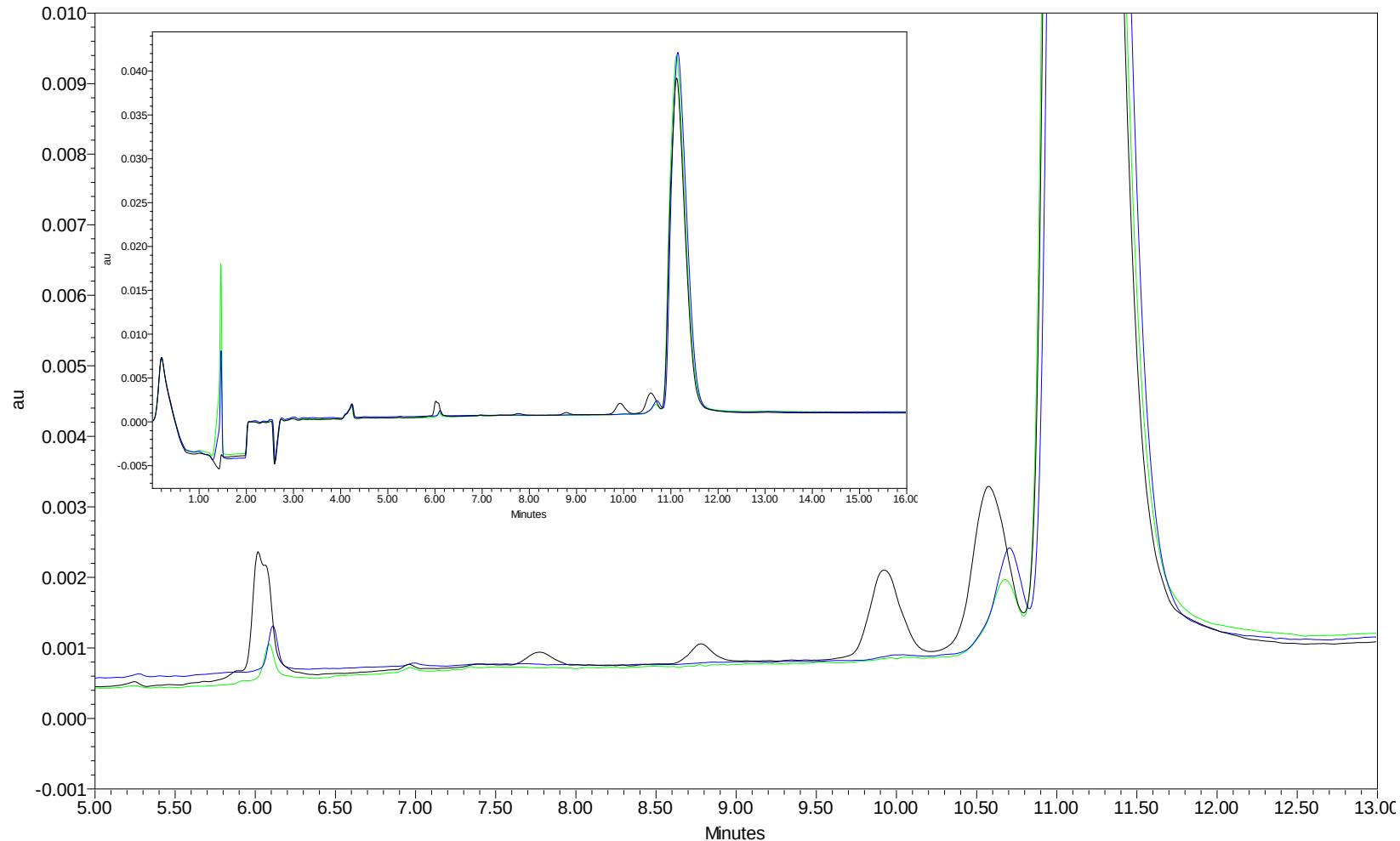
Method Transfer – CE-SDS Case study – Reagents

- Target RU profile (black) vs Initial RU profile (blue) for release-level sample



Method Transfer – CE-SDS Case study – Reagents

- Black – No IAM; Blue – Partially dissolved IAM; Green – Fully dissolved IAM



Method Transfer – CE-SDS Case study – Reagents

Equivalence Test Results

Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
High Molecular Weight-NR Stressed	1.136	1.222	-0.087	[-0.224, 0.051]	0.949	Equivalent
IgG Main Peak-NR Nominal	98.155	98.228 ←	-0.073	[-0.183, 0.036]	0.766	Equivalent
IgG Main Peak-NR Stressed	94.309	94.342 ←	-0.033	[-0.180, 0.113]	1.148	Equivalent
NR Fragment Nominal	1.845	1.772 ←	0.073	[-0.036, 0.183]	0.766	Equivalent
NR Fragment Stressed	4.336	4.131 ←	0.206	[0.064, 0.348]	0.841	Equivalent



Intermediate Precision Results - RU

Sample ID	Intermediate Precision % CV	Acceptance Criterion	Conclusion
High Molecular Weight-NR Nominal	N/A	30	Pass
High Molecular Weight-NR Stressed	10	30	Pass
IgG Main Peak-NR Nominal	0.1	3.0	Pass
IgG Main Peak-NR Stressed	0.1	3.0	Pass
NR Fragment Nominal	5 ←	20	Pass
NR Fragment Stressed	3 ←	20	Pass



Method Transfer – CE-SDS Case study – EACs

Equivalence Test Results						
Sample ID	Mean RU [%]	Mean TU [%]	Mean RU - Mean TU [%]	90% Confidence Interval [%]	+/- EAC [%]	Conclusion
Reduced Fragment Nominal	0.491	0.546	-0.055	[-0.091, -0.018]	0.401	Equivalent
Reduced Fragment Stressed	0.770	0.859	-0.090	[-0.156, -0.023]	0.091 *	Inconclusive
Reduced Light Chain + % Heavy Chain Nominal	99.369	99.277	0.092	[0.000, 0.184]	0.370	Equivalent
Reduced Light Chain + % Heavy Chain Stressed	97.377	97.280	0.096	[-0.069, 0.262]	0.498	Equivalent
Reduced Non-heavy/Light Chain Nominal	0.491	0.546	-0.055	[-0.091, -0.018]	0.401	Equivalent
Reduced Non-heavy/Light Chain Stressed	2.413	2.362	0.051	[-0.094, 0.196]	0.908	Equivalent



Method Transfer – Importance of Using EACs

- Using EACs ensures consistency between laboratories
 - Bias between laboratories can be observed and corrected
 - Intermediate precision (and linearity) may “pass” while EAC fails
 - Electropherograms may look similar but have elevated peaks
 - Electropherograms may be similar but integration differences exist
- *EACs must be evaluated for fit-for-purpose (especially with precise TU data)
 - Should be set before RU transfer but after TU data generation (i.e. use method validation)
 - Consider “platform” EACs



Method Transfer – Lessons Learned

- Importance of using degraded samples
 - May show inconsistencies between laboratories that may not be detected using release-level samples
 - Challenges integration practices
 - Challenges system/laboratory performance
- Sometimes resolution differences will exist with cIEF
- Be clear with target profile expectations and integration guidance
 - Include specifics when necessary



CE-SDS and cIEF Transfers – General Lessons Learned

- Avoid heat blocks
- Verify electropherograms and integration during training
- Videos are helpful, particularly for complicated steps
- SOPs:
 - Allow flexibility where possible
 - Provide examples (integration, ghost peaks, etc.)
- CE-SDS:
 - Verify vial tray configurations at receiving unit
 - Use peak height for detection metrics rather than signal to noise

