



Determination of Charge Heterogeneity for Monoclonal Antibodies by iCE: Method Development, Validation and Application in QC Environments

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Outlines



- iCE Technology
- A Generic iCE Method for mAbs
 - The Method
 - Validation Performance
- Application in GMP-fashion Analysis

iCE Technology



- iCE: Imaged Capillary Electrophoresis
 - Imaged cIEF
 - Charge-based separation in a pH gradient

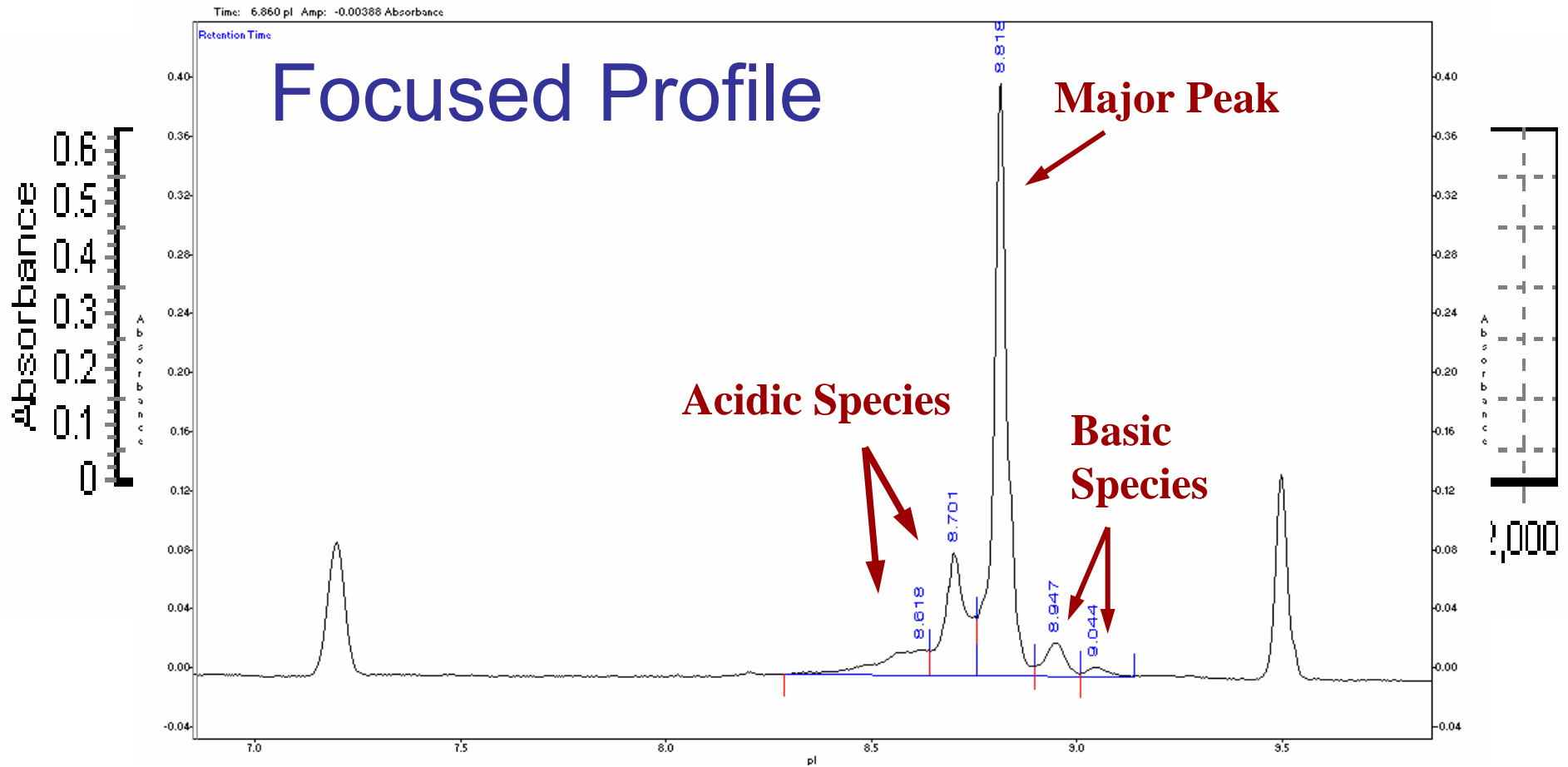
- Applications: Charge Heterogeneity Determination for Proteins
 - Characterize charge profile
 - Monitor acidic species

Why iCE ?



	Gel IEF	cIEF/CZE	iCE
Time/labor	4-8 hrs, gel handling	30 – 90 min	5 – 10 min
Quantitation /automation	Semi/no	Yes	Yes
Mobilization	N/A	Yes	No
Throughput	Low	Intermediate	High

iCE Focusing Process

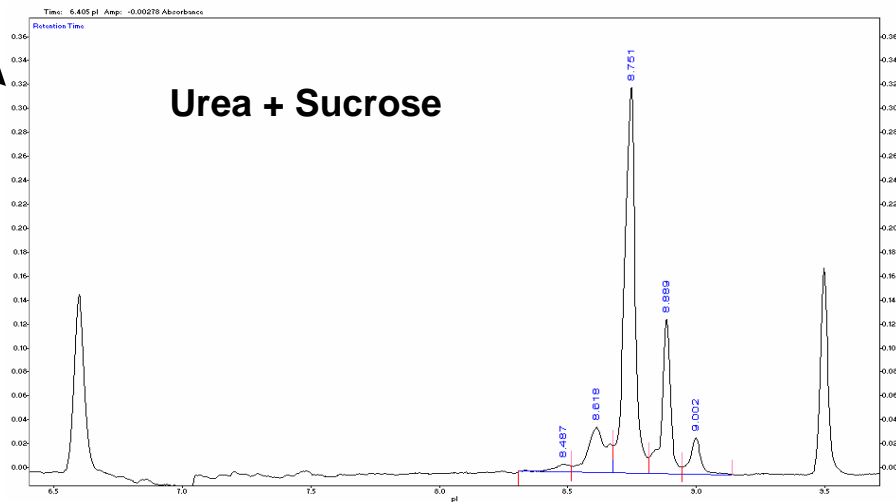
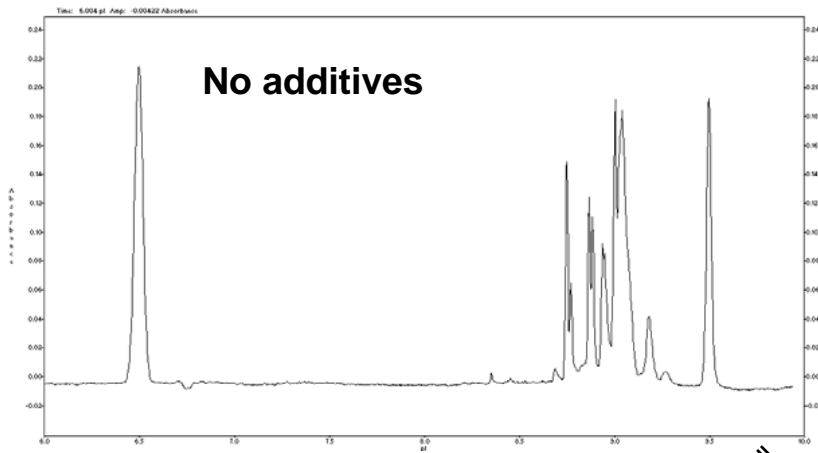


Method Development: Factors Attributing to iCE Separation



- **Ampholytes: pH range/strength**
- **Analytes:**
 - Work concentration
 - Ion composition and strength
- **Methylcellulose level**
- **Focusing voltage and time**
- **Sample transfer/delay time**
- **Additives**

Example: Additives Improve Separation



iCE Method Developed for mAbs



➤ Scope of the method:

- Drug substance
- Drug product
- Stability testing
- In-process characterization or formulation study

➤ Throughput:

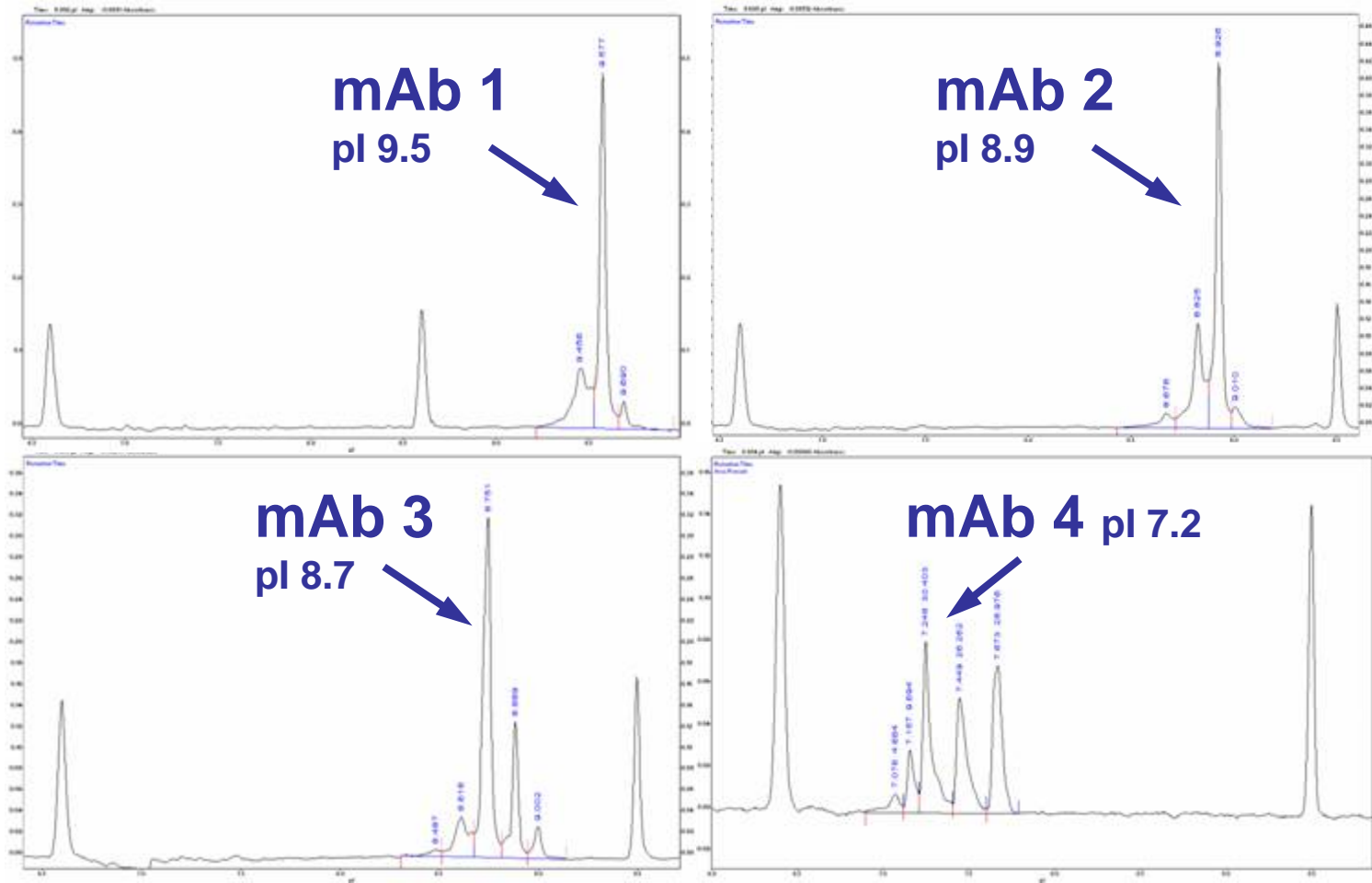
- ~10 minutes turn-around time per sample
- 2 x 40 samples/injections per day/per instrument

Generics of the iCE Method



- Suitable for > 20 monoclonal antibodies
- From various sources
- For different therapeutic areas
- Wide charge ranges
 - pI range: 6.9 - 9.7
 - Total acidic species: 15 - 45%

Example of mAbs Charge Profiles by iCE



ICH Guidelines for Analytical Method Validation



Type of Analytical Procedures	Identification	Testing for Impurities		Assay
		Quantitation	Limit	Dissolution; Content/Potency
Characteristics:				
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Intermediate Precision	-	+	-	+
Specificity	+	+	+	+
Detection Limit	-	-	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

ICH-Q2A, March 1995

Validation Elements and Material for the iCE Method



Elements:

1. Accuracy
2. Precision
3. Specificity
4. Quantitation Limit (QL)
5. Linearity and Range
6. Robustness

Materials:

1. A mAb with 5 charge species
2. Purified parent species (PS)
3. Purified Mono-deamidated species (AS1)

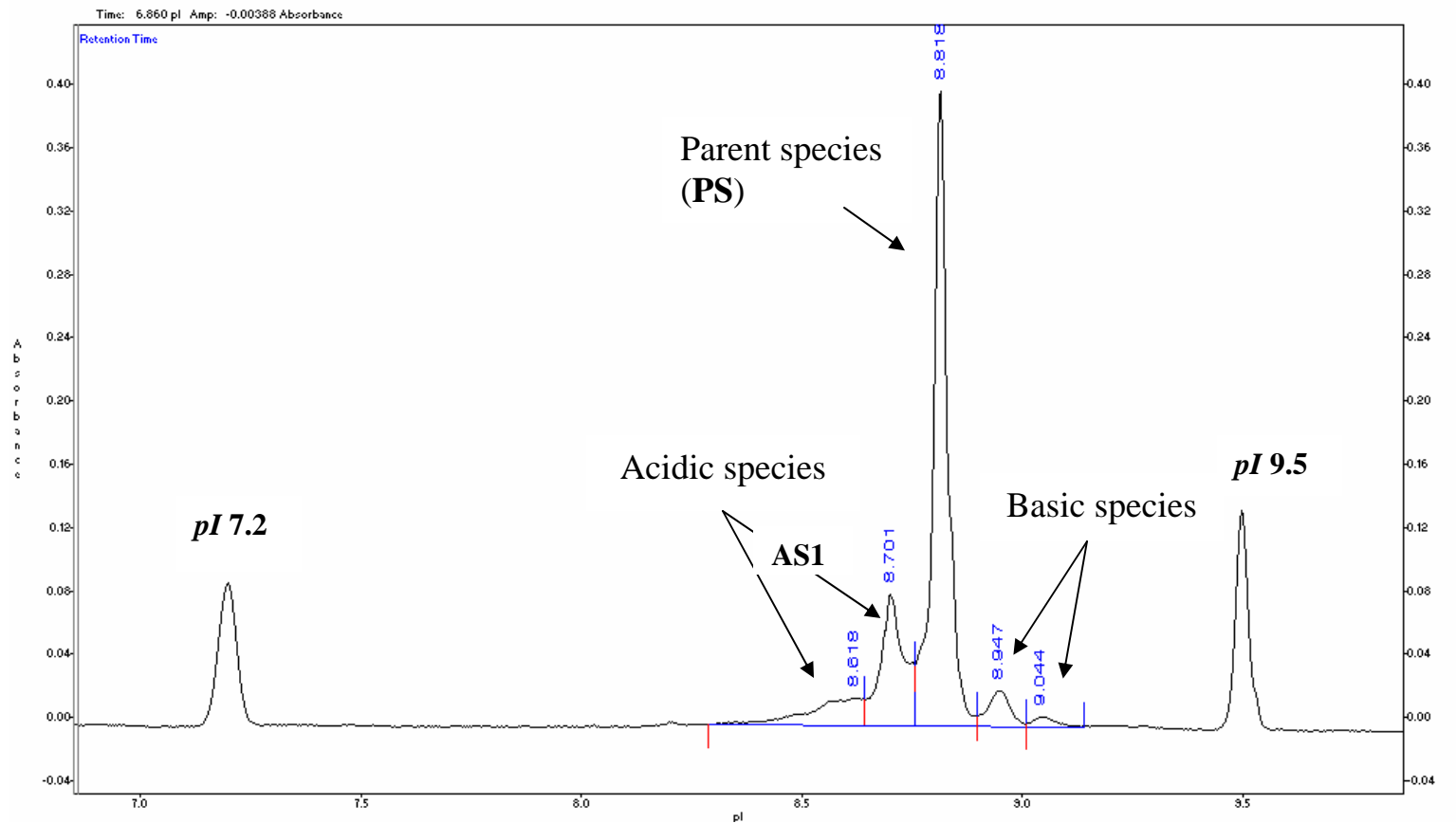
1. Specificity



➤ Results:

- Separation of five species differing by one charge from their neighbors
- Separation of spiked mono-deamidated acidic species from parent species

Demonstrated Separation of Different Charge Species



Demonstrated a Resolving Power of One Single Charge



AS1 Level	pI Value	
	Mono-deamidated Species (AS1)	Parent Species (PS)
4% (N = 3 x 3)	8.59	8.69
45% (N = 3 x 3)	8.59	8.69
94% (N = 3 x 3)	8.59	8.69
Average (N = 3 x 3 x 3)	8.59	8.69
SD	0.015	0.022
RSD	0.18%	0.26%

One single charge = ~0.1 pI unit for mAbs

2. Precision



➤ Criterion:

- $RSD \leq 5\%$ for Repeatability (inter-injections)
- $RSD \leq 10\%$ for Intermediate Precision
 - Multiple sample preparations / days
 - Between analysts

➤ Results:

- Repeatability: $RSD = 0.78 - 1.05\%$ (%AS1), $0.06 - 0.09\%$ (pI)
- Intermediate Precision:
 - At 45% level, $RSD = 1.49\%$ (AS1), 0.23% (pI), N=18, 3 preps;
 $RSD = 1.34\%$ (AS1), 0.25% (pI), N=24, 2 analysts
 - At 4% level, $RSD = 1.55\%$ (AS1), 0.21% (pI), N=9, 3 preps

Repeatability for %Peak Area of Mono-deamidated Acidic Species AS1



Injection	Prep # 1	Prep #2	Prep #3
	% Mono-deamidated Acidic Species AS1		
1	44.3	45.3	45.5
2	44.6	44.5	45.9
3	45.3	44.8	45.0
4	44.4	45.3	45.7
5	44.3	45.3	46.3
6	44.1	45.2	46.3
Average (N=6)	44.5	45.1	45.8
SD	0.40	0.40	0.50
RSD	0.92%	0.78%	1.05%

Repeatability for pI Value of Mono-deamidated Acidic Species AS1



Injection	Prep # 1	Prep #2	Prep #3
	pI Value of Parent Species		
1	8.70	8.70	8.66
2	8.71	8.71	8.66
3	8.71	8.71	8.68
4	8.71	8.70	8.68
5	8.70	8.70	8.66
6	8.70	8.70	8.66
Average (N=6)	8.71	8.70	8.66
SD	0.00	0.00	0.01
RSD	0.06%	0.06%	0.09%

Intermediate Precision

Multiple Sample Preparations at **45% AS1 Level**



Sample Preparations	% AS1	pI value of PS
Prep #1	44.5	8.71
Prep #2	45.1	8.70
Prep #3	45.8	8.66
Average (N=18)	45.1	8.69
SD	0.70	0.02
RSD	1.49%	0.23%

Intermediate Precision



Multiple Sample Preparations at 4% AS1 Level

Sample Preparations	% AS1	pI value of PS
Prep #1 (N=3)	3.93	8.71
Prep #2 (N=3)	3.94	8.70
Prep #3 (N=3)	3.98	8.67
Average (N=9)	3.95	8.69
SD	0.06	0.018
RSD	1.55%	0.21%

Intermediate Precision

Between Analysts at 45% AS1 Level



Analysts	% AS1	pI value of PS
Analyst #1 (N=3 x 6)	45.1	8.69
Analyst #2 (N=1 x 6)	45.0	8.71
Average (N=24)	45.1	8.70
SD	0.60	0.02
RSD	1.34%	0.25%

Repeatability and intermediate precision met the acceptance criteria of $RSD \leq 5\%$ and 10% .



3. Accuracy

➤ Criterion:

- Quantitatively spiking AS1 at levels of 4, 10, 18, 27, 36, 45, 54, and 94%
- Accuracy as %AS1 recovery within $100 \pm 10\%$, $100 \pm 15\%$, $100 \pm 20\%$, $100 \pm 25\%$ for AS1=94-45%, 36-27%, 18%, and 10- 4%, respectively

➤ Results:

- At 4% AS1 level, 98.7% recovery, RSD=1.55%
- At 10% AS1 level, 116.8% recovery, RSD=2.42%
- At 18% AS1 level, 107.9% recovery, RSD=3.59%
- At 27%-94% AS1 level, $100 \pm 2\%$ recovery, RSD=0.11%-1.07%

Recovery of iCE Analysis Met Acceptance Criteria



Theoretical AS1% of Spiked Sample	Measured AS1%	Recovery	Acceptance Criteria	RSD% (3 Preps)
4%	3.95%	98.7%	100% ± 25%	1.55%
10.3%	12.03%	116.8%	100% ± 25%	2.42%
18.4%	19.85%	107.9%	100% ± 20%	3.59%
27.4%	27.95%	102.0%	100% ± 15%	0.63%
36.4%	36.38%	100.0%	100% ± 15%	1.07%
45.4%	45.12%	99.4%	100% ± 10%	0.91%
54.3%	53.29%	98.0%	100% ± 10%	0.44%
94%	93.82%	99.8%	100% ± 10%	0.11%

4. Linearity and Range



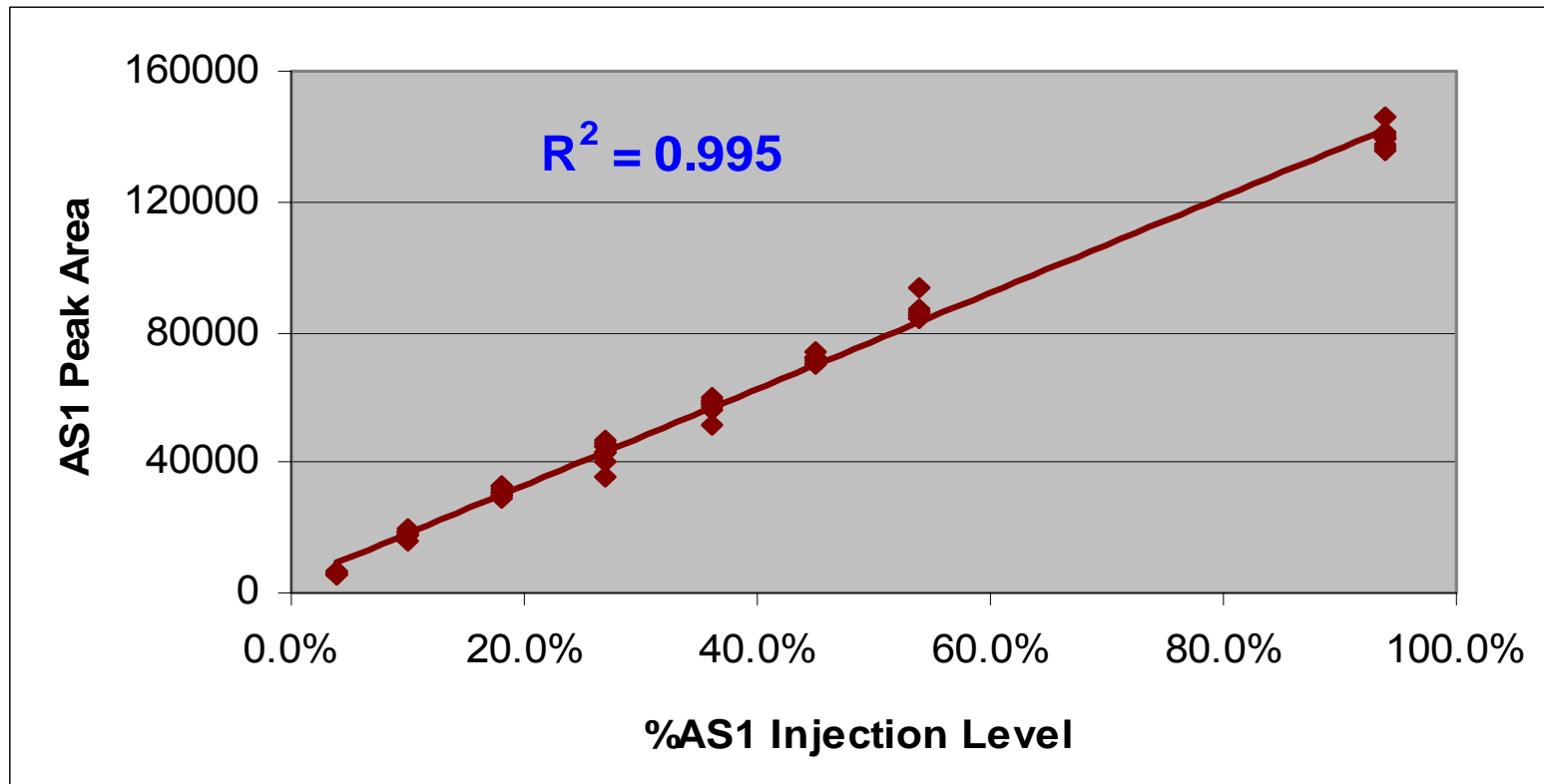
➤ Criterion:

- Range: QL to 45% or higher
- Linearity over Range:
 - Determination coefficient (r^2) ≥ 0.98

➤ Results:

- Range=4% to 94%
- $R^2 = 0.995$ over the range

Linear Plots



9 data points, 9 injections, 3 preparations were included at each of the eight AS1 levels.

5. Quantitation Limit (QL)



➤ Criterion:

- $QL \leq 5\%$

➤ Results:

- $QL = 4\%$
 - At 4% AS1
 - Accuracy = 98.7%
 - Precision = 1.55% (N = 3 preps x 3 injections)
 - Resolution (R) = 1.34



6. Robustness

1. Prepared sample stability
2. Cartridge to cartridge variation
3. Consistency of AS measurements over time: system suitability results from multiple analysts using different iCE280 units and separation cartridges

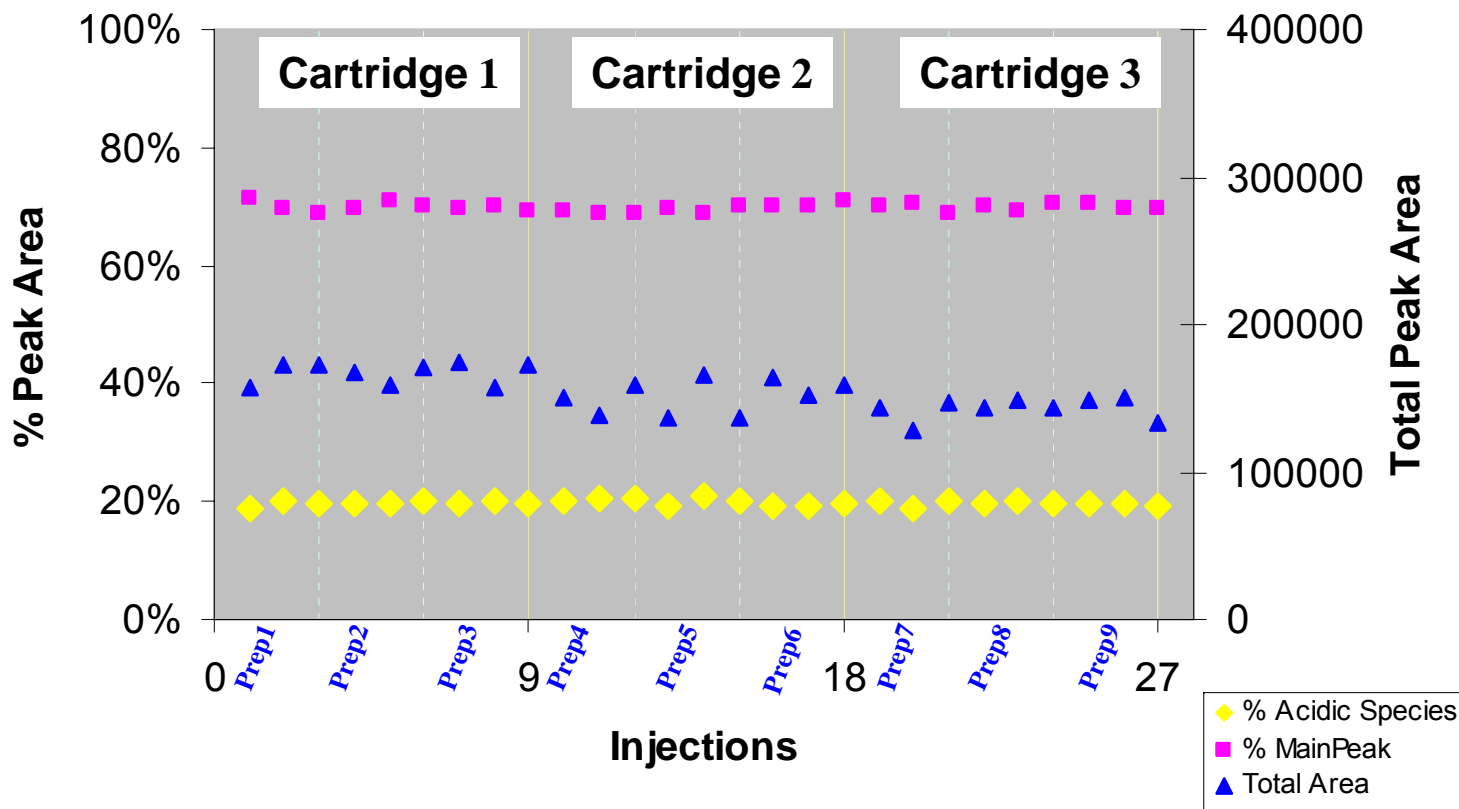
6.1. Prepared Sample Stability



Prepared Sample Conditions	%AS1	<i>pI</i> value of PS	Total Peak Area
Time Zero (N=3)	46.5	8.68	189961
24-hour at 2-8°C (N=3)	46.8	8.68	193598
6-hour at RT (N=3)	46.7	8.68	193946
Average (N=9)	46.7	8.68	191969
SD	0.20	0	3146
RSD	0.43%	0.0%	1.82%

No Significant Changes Observed for Samples Prepared in Injection Solutions over a Period of 24-hour in Autosampler

6.2. Cartridge to Cartridge Variations



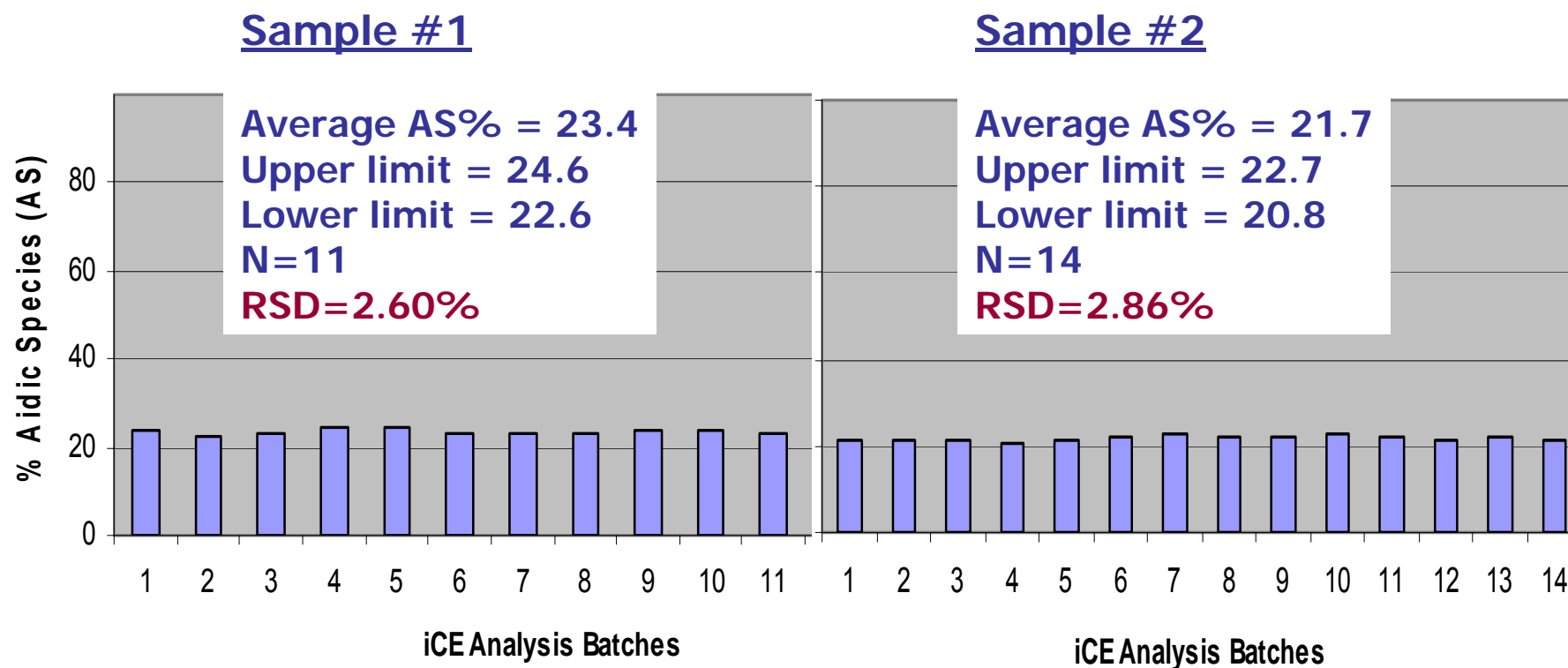
RSD=2.2% for %AS, 0.14% for main peak pl value over 27 data points, 27 injections, 9 preps, 3 columns/3 days

6.3. Reproducibility of % Acidic Species Measurements Over Time



- SS injections from iCE runs over 6 months
- Two mAb control samples
- Two analysts
- Three iCE 280 instruments
- 8 capillary cartridges

Reproducibility Demonstrated Over Time



Validation Summary



- **Specificity:** Resolution of one single charge, ~0.1 pl unit
- **Precision**
 - Repeatability: RSD \leq 1.05%
 - Intermediate Precision: RSD \leq 1.49% (45%AS1)
RSD \leq 1.55% (4%AS1)
- **Accuracy (by spiking mono-deamidated species AS1)**

<u>AS1 Level</u>	<u>Recovery%</u>	<u>RSD</u>
4%	98.7	0.52%
10%	116.8	2.42%
18%	107.9	3.59%
27-94%	100.0 \pm 2	0.11-1.07%

Validation Summary - continued



➤ **Linearity/Range**

- 4 - 94% range, $R^2 = 0.995$

➤ **Quantitation Limit (QL)**

- QL= 4%

➤ **Robustness**

- Sample stability (24h): RSD=0.0%(pl), 0.43%(%AS1)
- Cartridge variation: RSD=0.14%(pl), 2.2%(%AS)
3 columns/days, 9 preps, 27 data points
- Long term reproducibility (6 mo) :
 - Sample#1: RSD=2.86%, N=14
 - Sample#2: RSD=2.60%, N=11

iCE Validation Conclusion



- Validated for mAb charge variants determination according to the ICH guidelines:
 - Acceptable specificity, precision, accuracy, and linearity/range
 - Adequate QL to support wide range of impurity specifications
 - Sufficient Robustness

iCE Applications



➤ **Product Release**

- Quality Control for API and DP

➤ **Stability test**

- Deamidation monitor

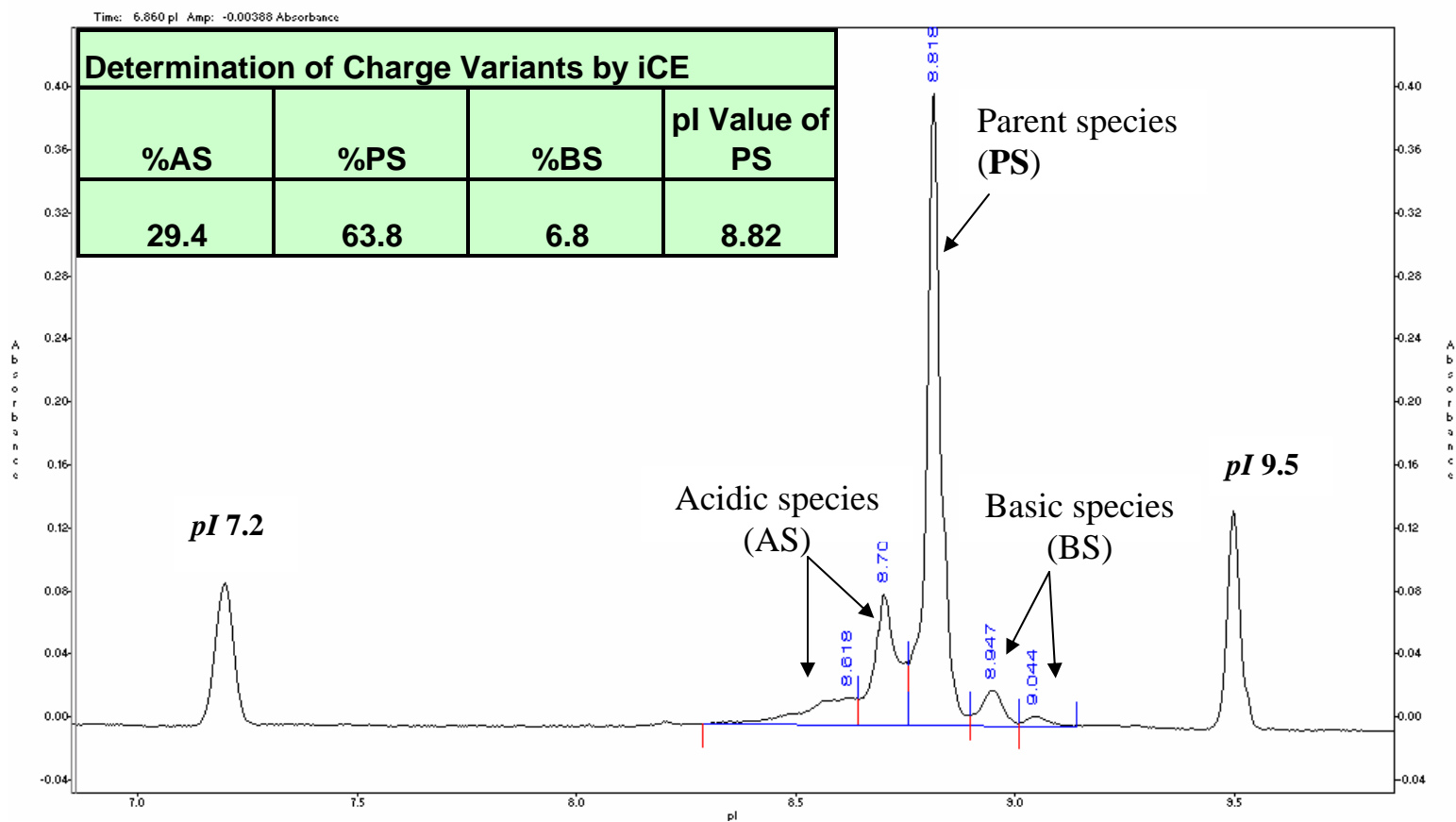
➤ **Comparability study**

- Consistent performance between cell lines, formulations, scales

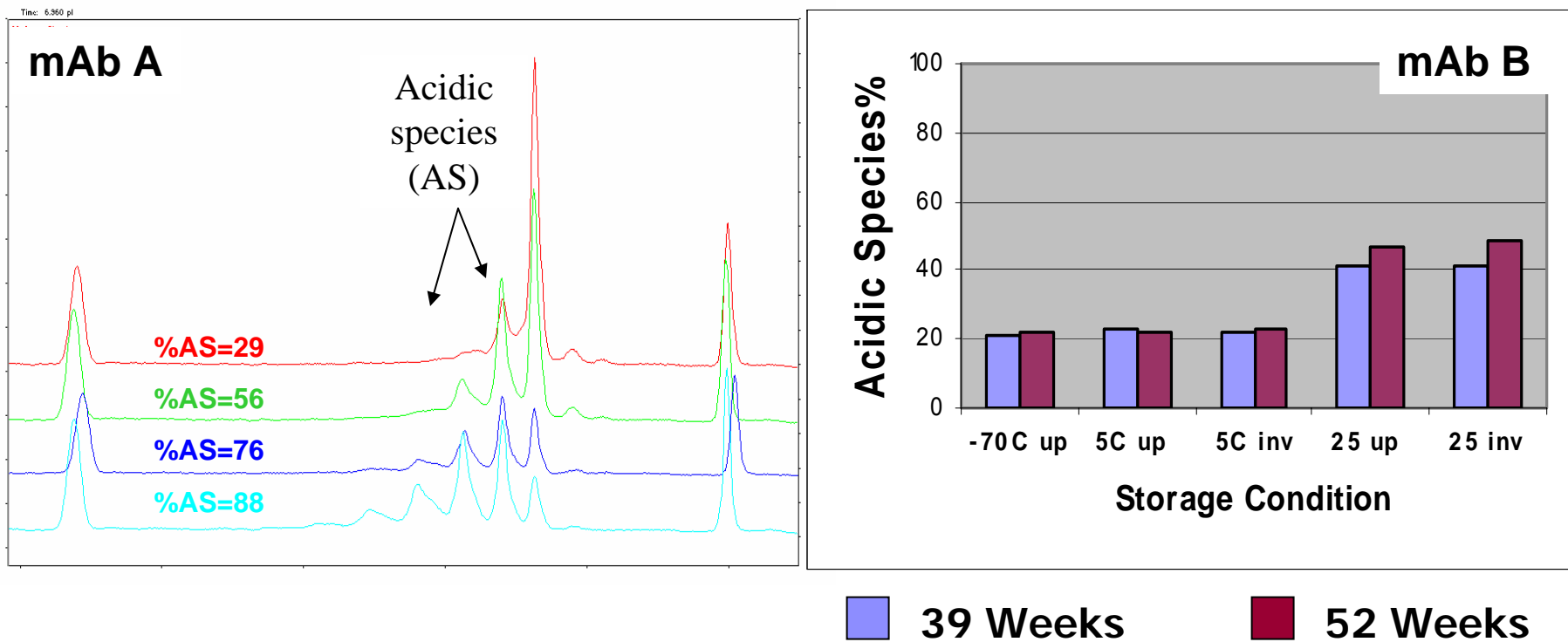
➤ **In-process characterization**

- Understand of attributes that cause charge variation

Determining Charge Variants for Product Release



Monitoring Deamidation in Stability Testing



Charge Heterogeneity Profiling by iCE

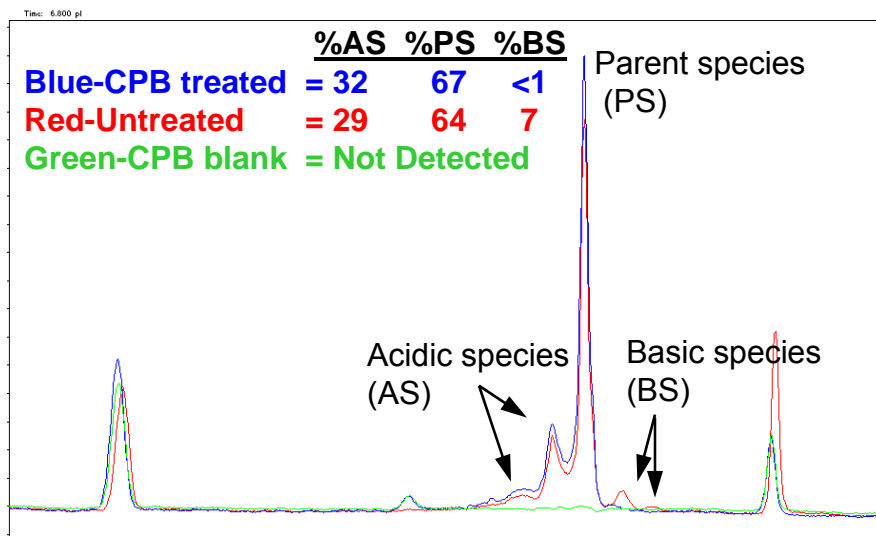


- Glycosylation
 - Deglycosylation with PNGase
 - Desialylation with sialidase
- Deamidation
 - Stress: elevated temperature, high pH
- C-terminus Lys process variants
 - CPB treatment

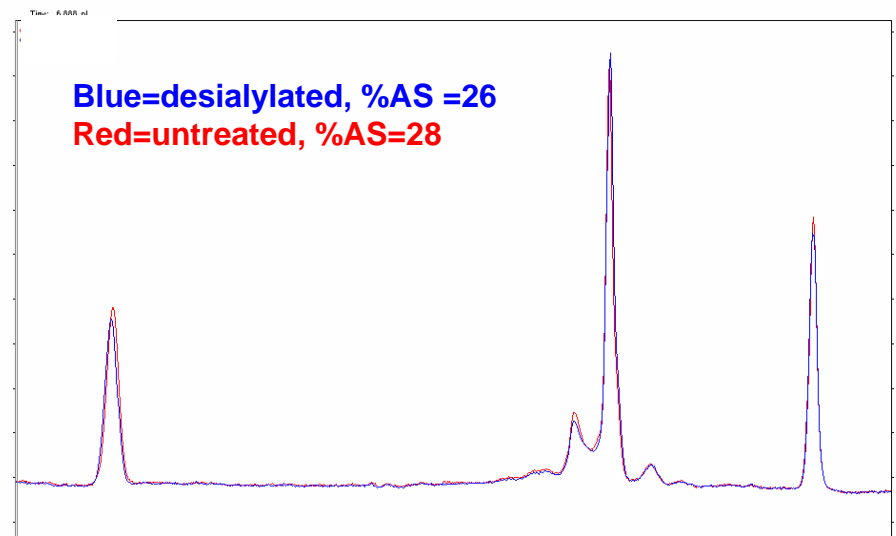
Examples of iCE Charge Heterogeneity Profiling



C-terminus Lys Variants



Desialylation



Conclusion



- Validated a generic iCE method for quantitation of charge variants for mAbs
- The method has unique advantages for separation of charge species
- The method is suitable for release, stability, and comparability testing

Acknowledgements



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