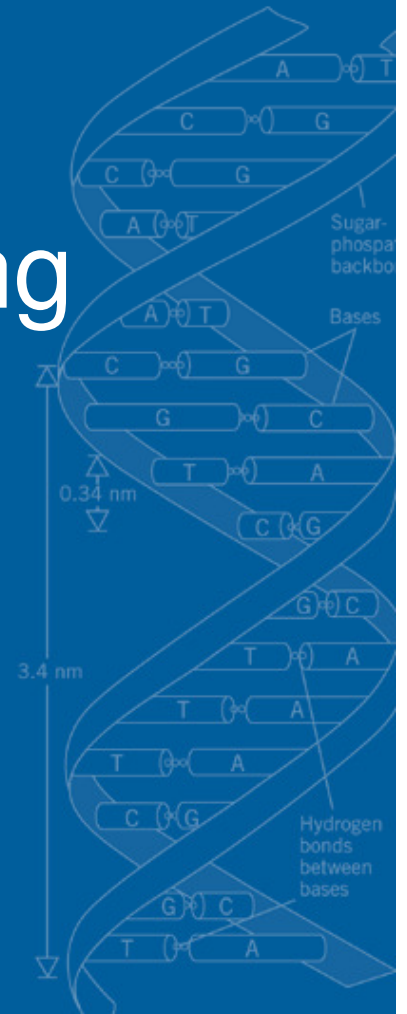


CEPharm 2007

Implementation of Imaged Capillary Isoelectric Focusing in Quality Control

Zara Safarian, Ph.D.
QC Scientist, Genentech Inc.
Miami 2007



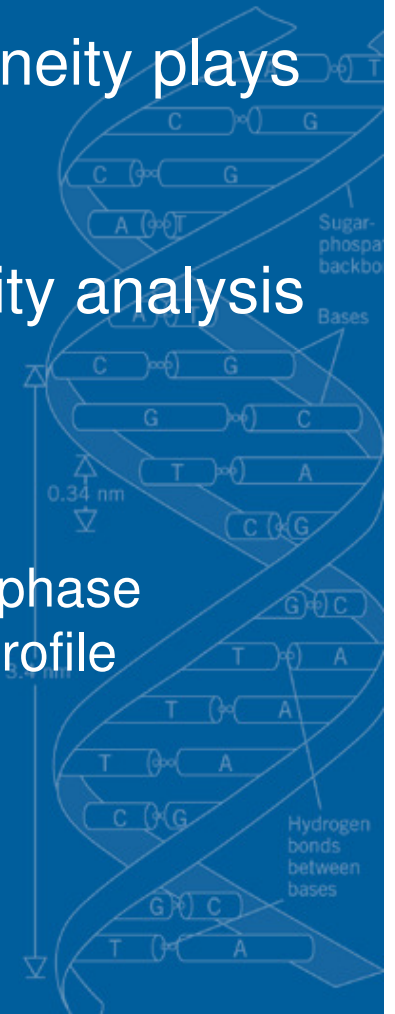
OUTLINES

1. Drivers of quantitative imaged CIEF in Quality Control
2. Challenges: *peak profile; temperature control; data management*
3. Method Robustness
4. Method Validation
5. System Suitability: *Instrument and Sample related*
6. Stability-indicating Properties
7. Conclusions
8. Acknowledgments



DRIVERS OF quantitative imaged CIEF IN QC (1)

1. Quantitative purity method for charge heterogeneity plays key role in control systems
2. Conventional technique for charge heterogeneity analysis is IEC:
 - Product –specific development and training
 - Complex separation parameters: column, mobile phase composition and pH, salt, temperature, gradient profile
 - Often complex separation profile for MAbs
 - Run time is 1 – 1.5 hours



DRIVERS OF quantitative imaged CIEF IN QC (2)

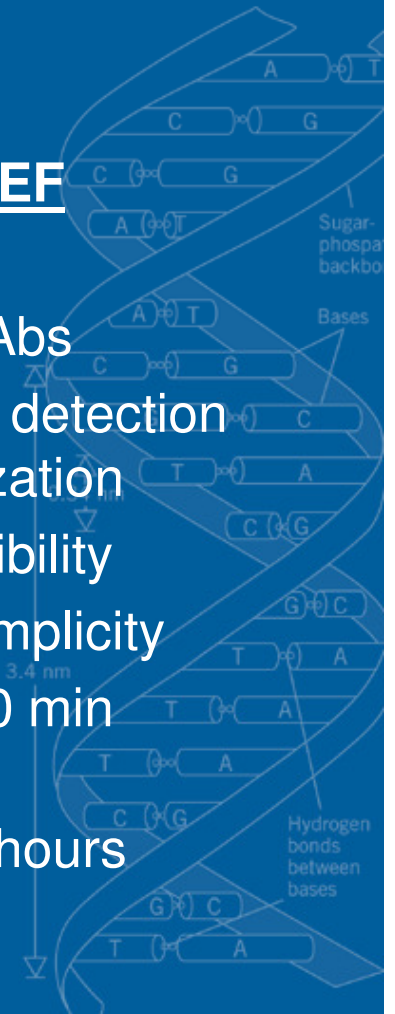
2. Alternative techniques: CIEF and imaged CIEF

CIEF

- Single-point detection with mobilization
- Poor reproducibility
- Long analysis time: 1-2 hours
- Long method development: days – weeks

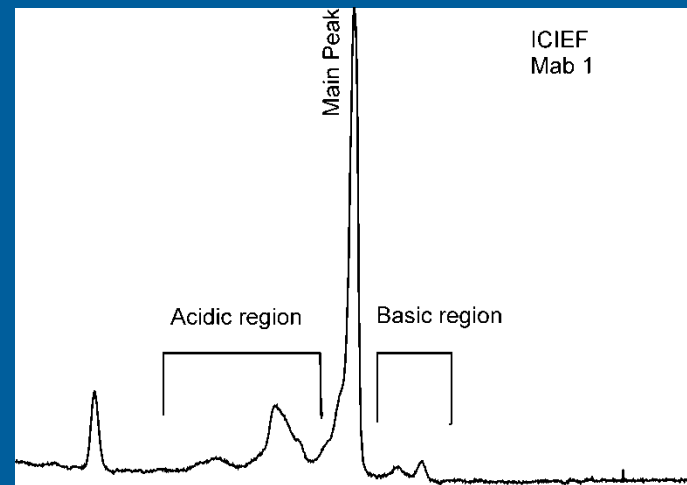
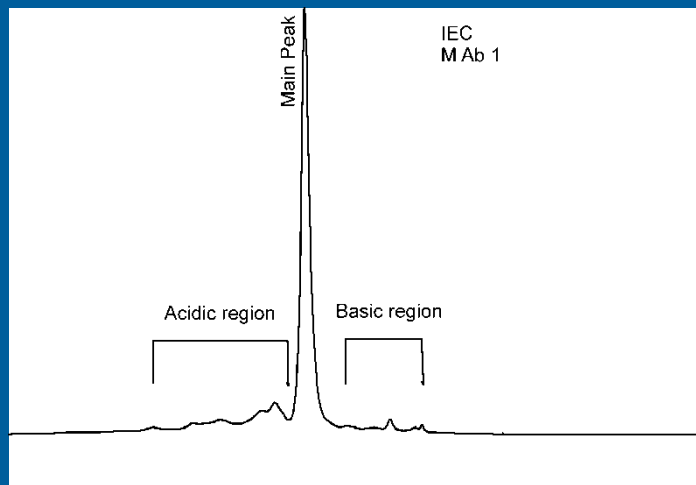
Imaged CIEF

- Generic for MAbs
- Whole column detection with no mobilization
- High reproducibility
- Operational simplicity
- Short run: 5-10 min
- Fast method development: hours

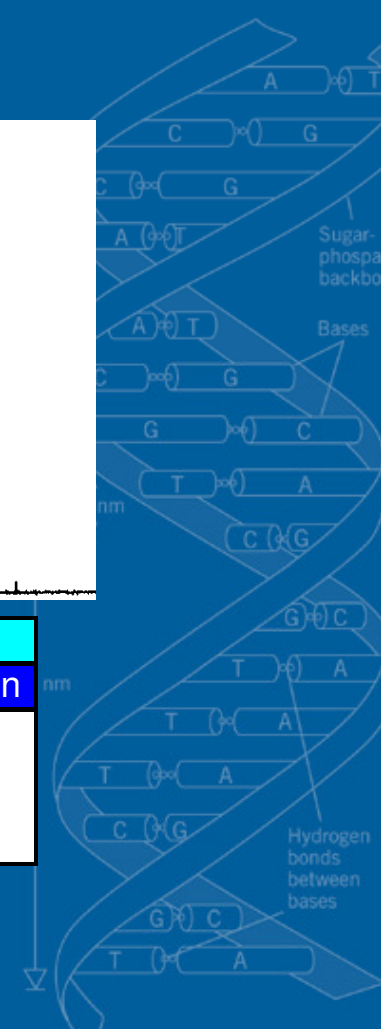


DRIVERS OF imaged CIEF IN QC (3)

Gain in simplicity without loss in information

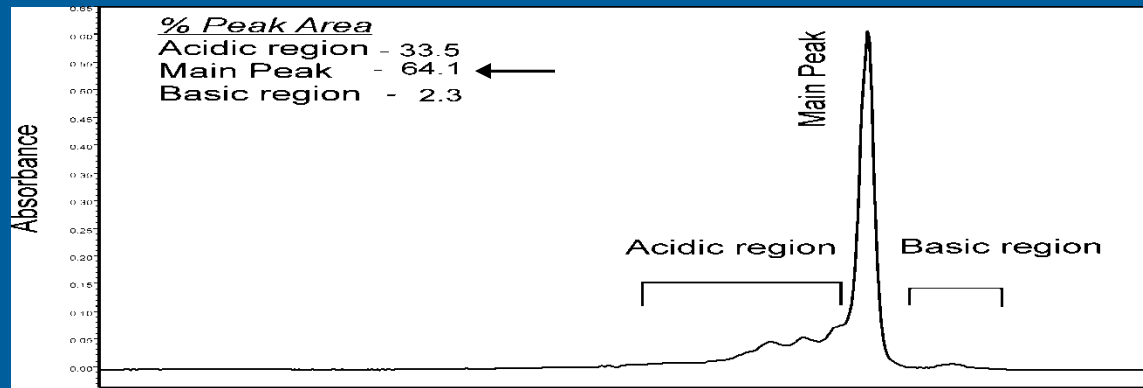


Method	Analysis Time (min.)	Percent Peak Area		
		Acidic Region	Main Peak	Basic Region
IEC	60	26.4	65.0	8.7
iCIEF	10	27.0	67.7	4.8

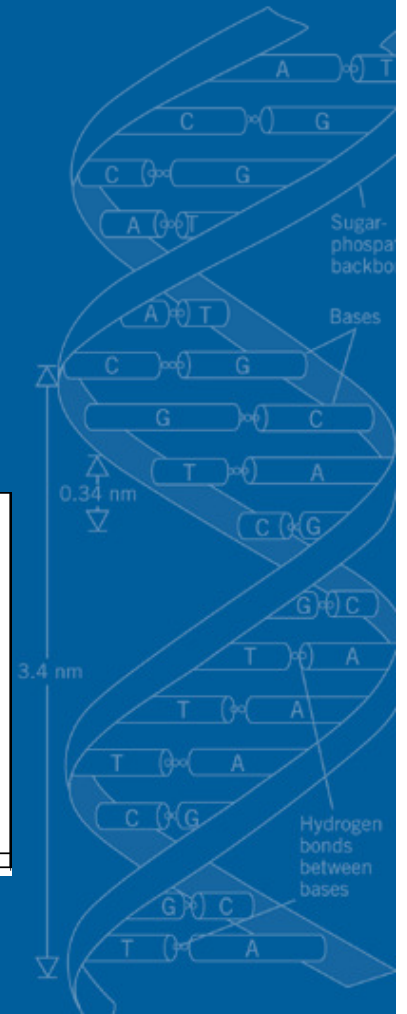
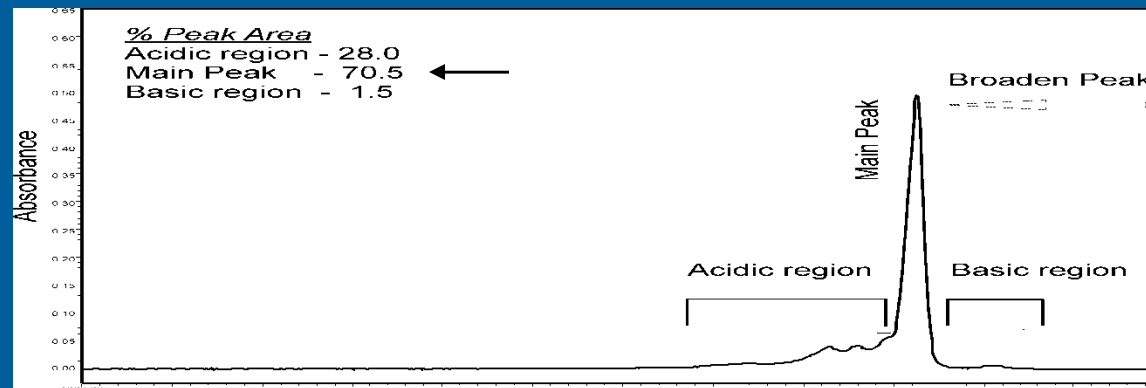


CHALLENGES: Peak profile (1)

Expected Profile

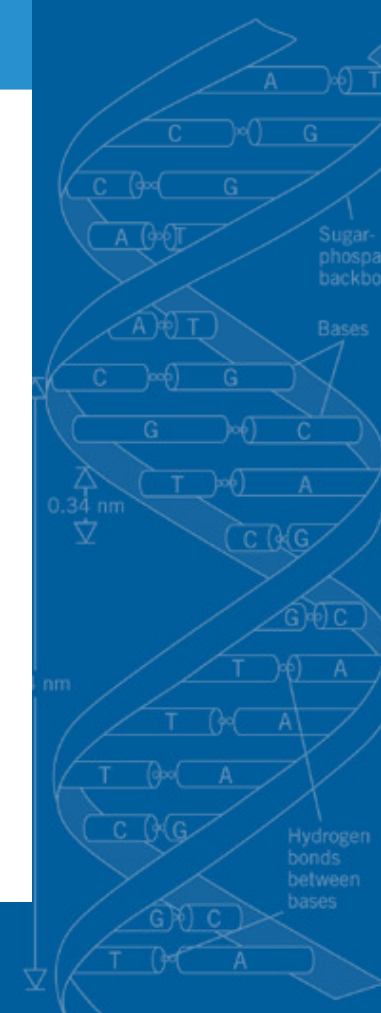


Observed Profile



CHALLENGES: Peak Profile (2)

iCE280 IEF Analyzer with PrinCE MicroInjector



CHALLENGES: Peak Profile (3)

Cause:

- Disturbed equilibrium due to the hydrodynamic flow
- Hydrodynamic flow induced by improper balance between analyzer and autosampler vials

Resolution:

- Convergent replaced autosampler and analyzer vials with marked vials to control level of liquids – *temporary solution*
- Limited number of injections to avoid overflow of waste vial
- Convergent included balancing check in their OQ/IQ protocols



CHALLENGES: Temperature Control

Problem Source:

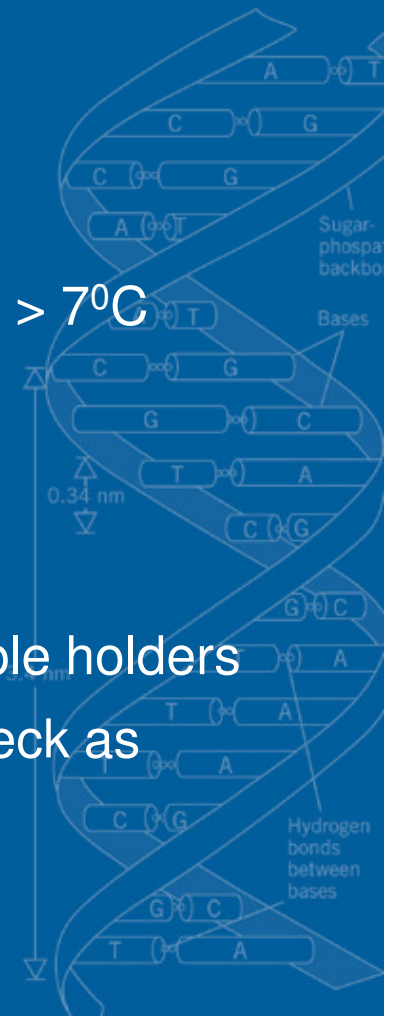
- Autosampler Sample Stability

Cause:

- Difference between actual and set temperatures $> 7^{\circ}\text{C}$

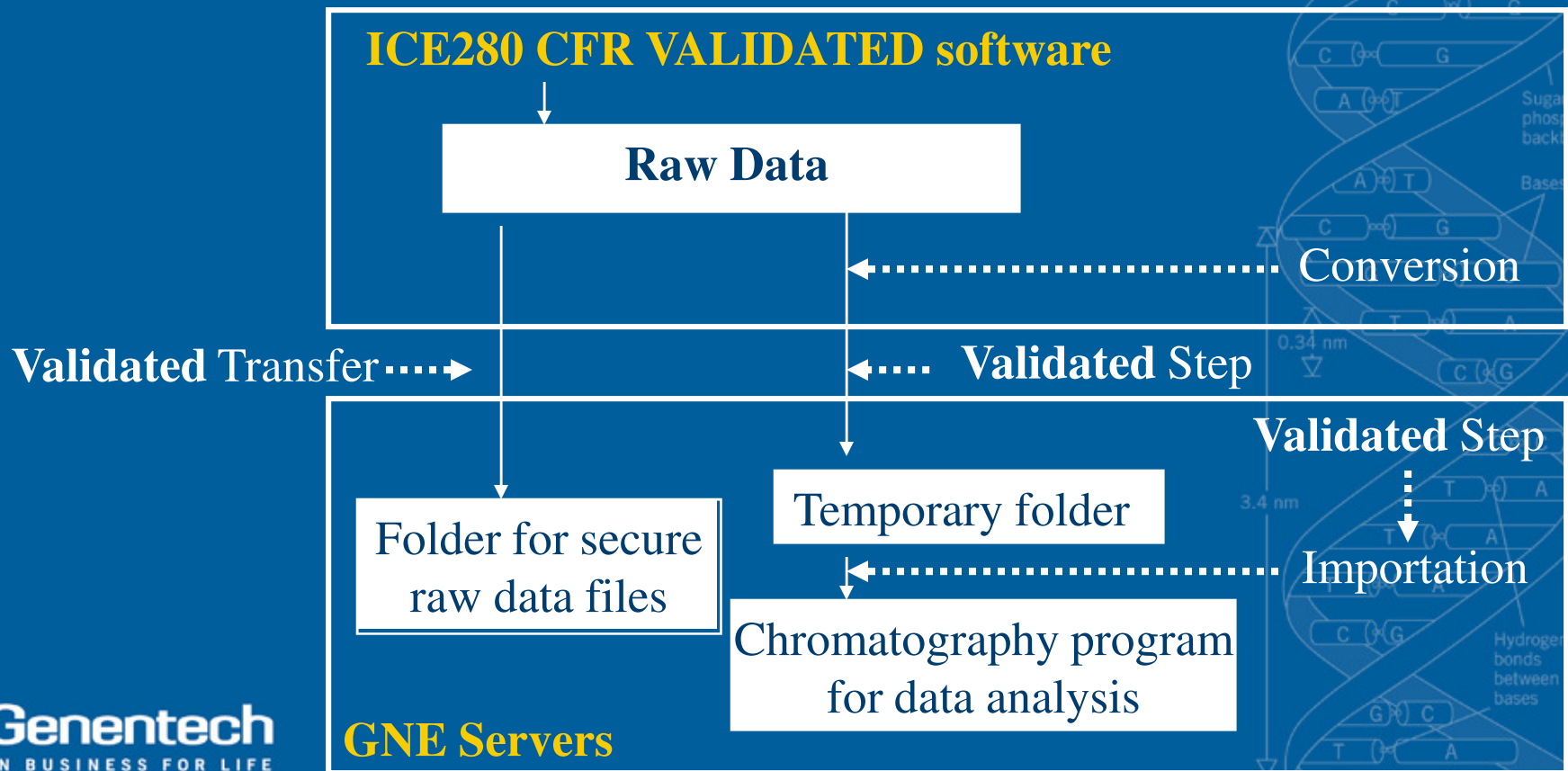
Resolution:

- Set analysis at ambient temperature
- Limited number of injections per sequence
- Convergent is ready to test new design for sample holders
- Convergent will use sample vial temperature check as qualification test



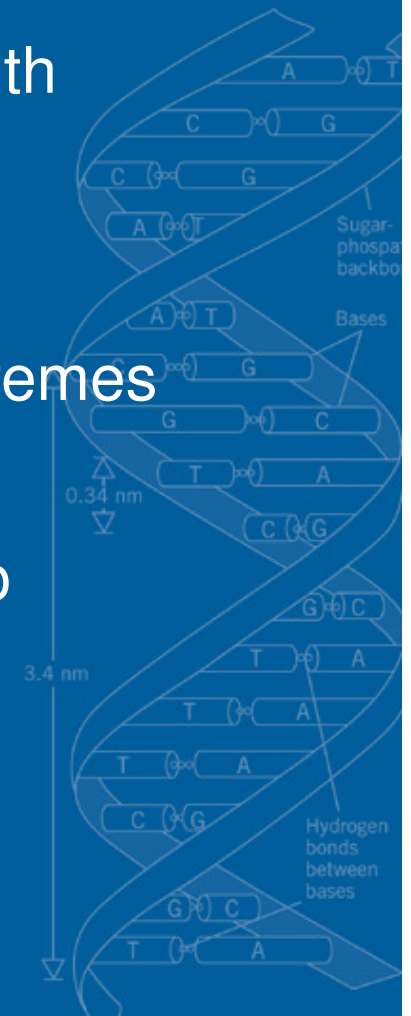
CHALLENGES: Archiving Electronic Records

21 CFR part 11 compliance for archiving electronic records



METHOD ROBUSTNESS (1): Approach

1. Perform extensive testing to define method with meaningful system suitability
2. Identify critical method parameters
3. Compare reproducibility of quantitation at extremes with target
4. Show that method is clear and precise prior to validation



METHOD ROBUSTNESS (2)

Studies:

Precision: intra- and inter-day, sample preparation and injections repeatability

Stability: sample, methylcellulose, electrolytes

Variations in: MC concentration, ampholytes concentration, ratio of broad range and narrow range ampholytes

Electrolytes depletion

Cartridge-to-Cartridge (5)

Instrument-to-Instrument (3)

LOQ

Alternative instrument: Beckman PA800

Sample Recovery: control and stressed samples admixtures



METHOD ROBUSTNESS (3): Inter-Day Precision

2 instruments, 2 cartridges, 8 sample preparations,
7 days, 1 analyst.

% Peak Area

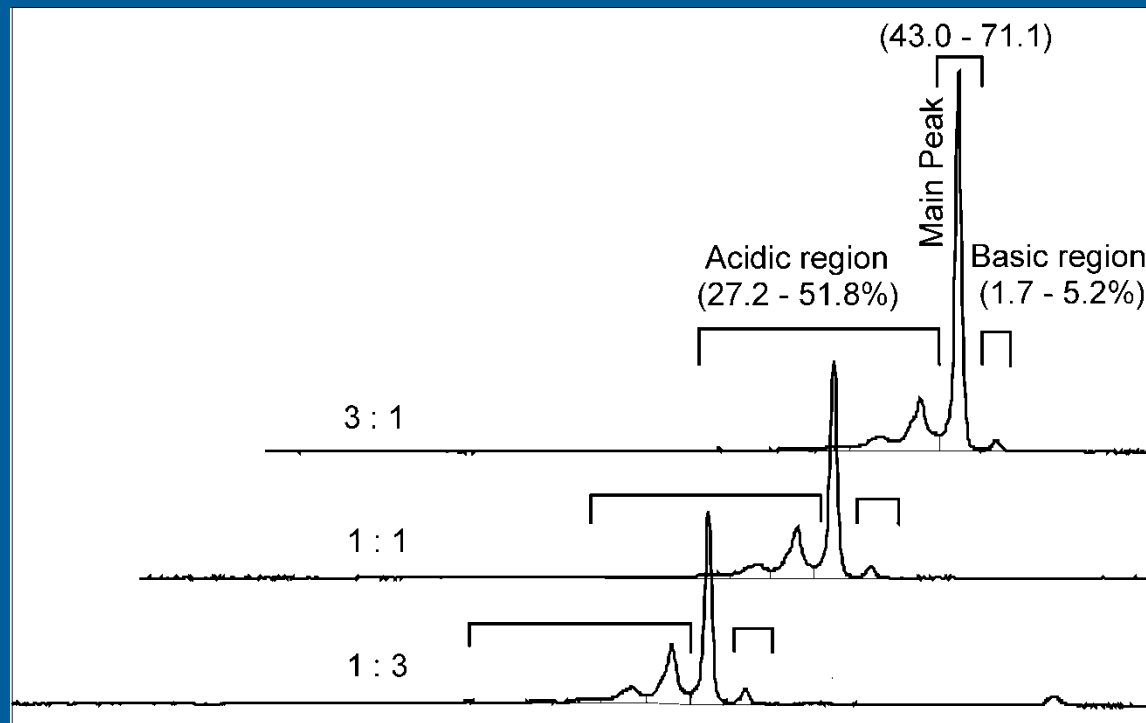
	Acidic Region	Main Peak	Basic Region
Mean	27.0	71.1	2.0
SD	0.5	0.6	0.2
%RSD	2.0	0.9	11.0

N = 56

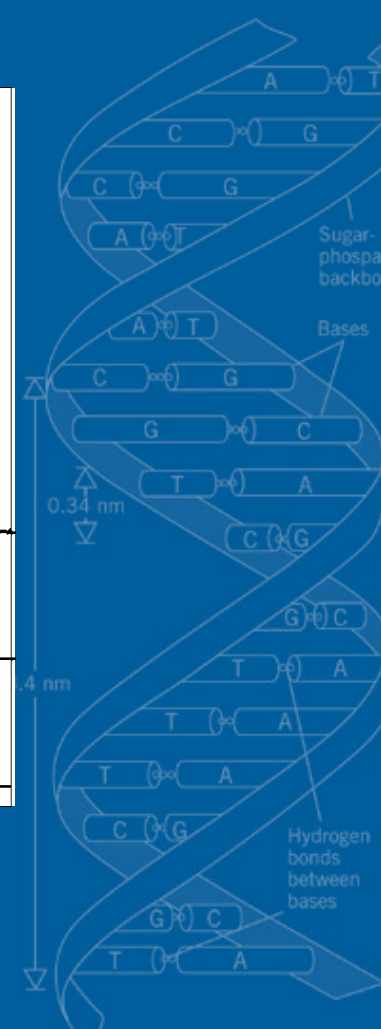
Method demonstrates good Inter-Day Precision

METHOD ROBUSTNESS (4): Samples Recovery

Sample: Admixture – Control: Stressed (%)



Recovery is within 96 – 107%



METHOD ROBUSTNESS: Summary

Combined RSD from all robustness experiments:
3 instruments, 5 lots of cartridges, 2 analysts and different
conditions.

% Peak Area

	Acidic Region	Main Peak	Basic Region
Mean	26.8	71.1	2.1
SD	0.7	0.8	0.3
%RSD	2.6	1.1	12.8

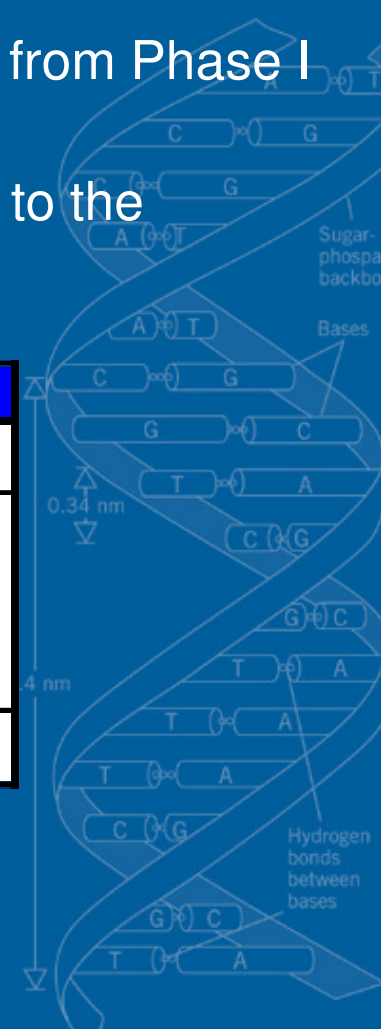
N = 229

Method is Robust

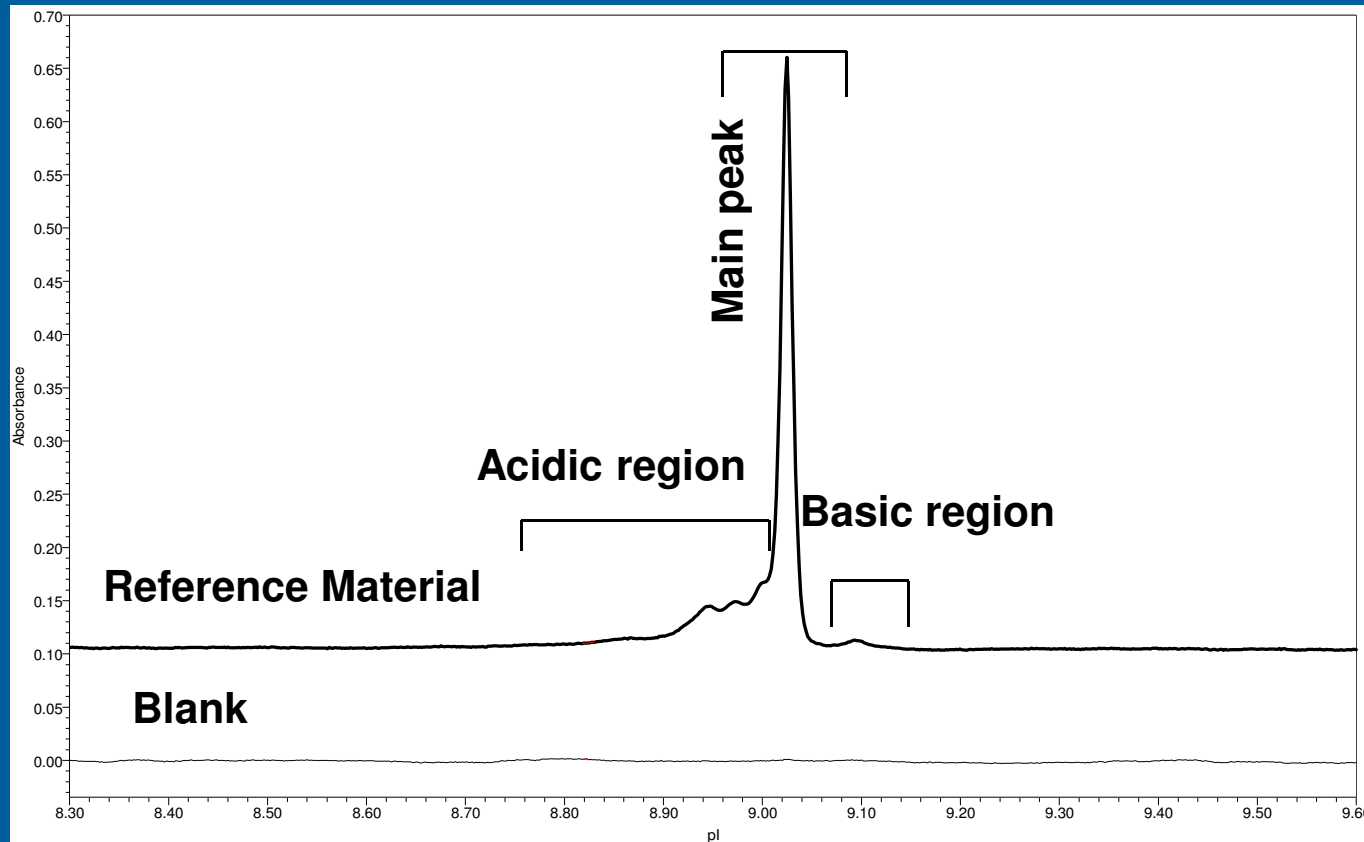
METHOD VALIDATION

- Method Validation follows Life Cycle Approach starting from Phase I
- Validation is performed for Phase I Clinical Testing
- Focuses on the characteristics that are deemed critical to the method performance

Characteristics	Acceptance Criteria
Accuracy	80-120%
Precision Repeatability Intermediate	RSD values for % peak area of acidic region and basic region $\leq 20\%$ and main peak $\leq 10\%$
Specificity	No Interference from blank



METHOD VALIDATION. Specificity



**Method is Specific.
No Interference from Blank**

METHOD VALIDATION. Precision

Intermediate Precision (N=12)

% Peak Area

	Acidic Region	Main Peak	Basic Region
Average	31.12	66.15	2.73
RSD (%)	3.5	1.7	9.2

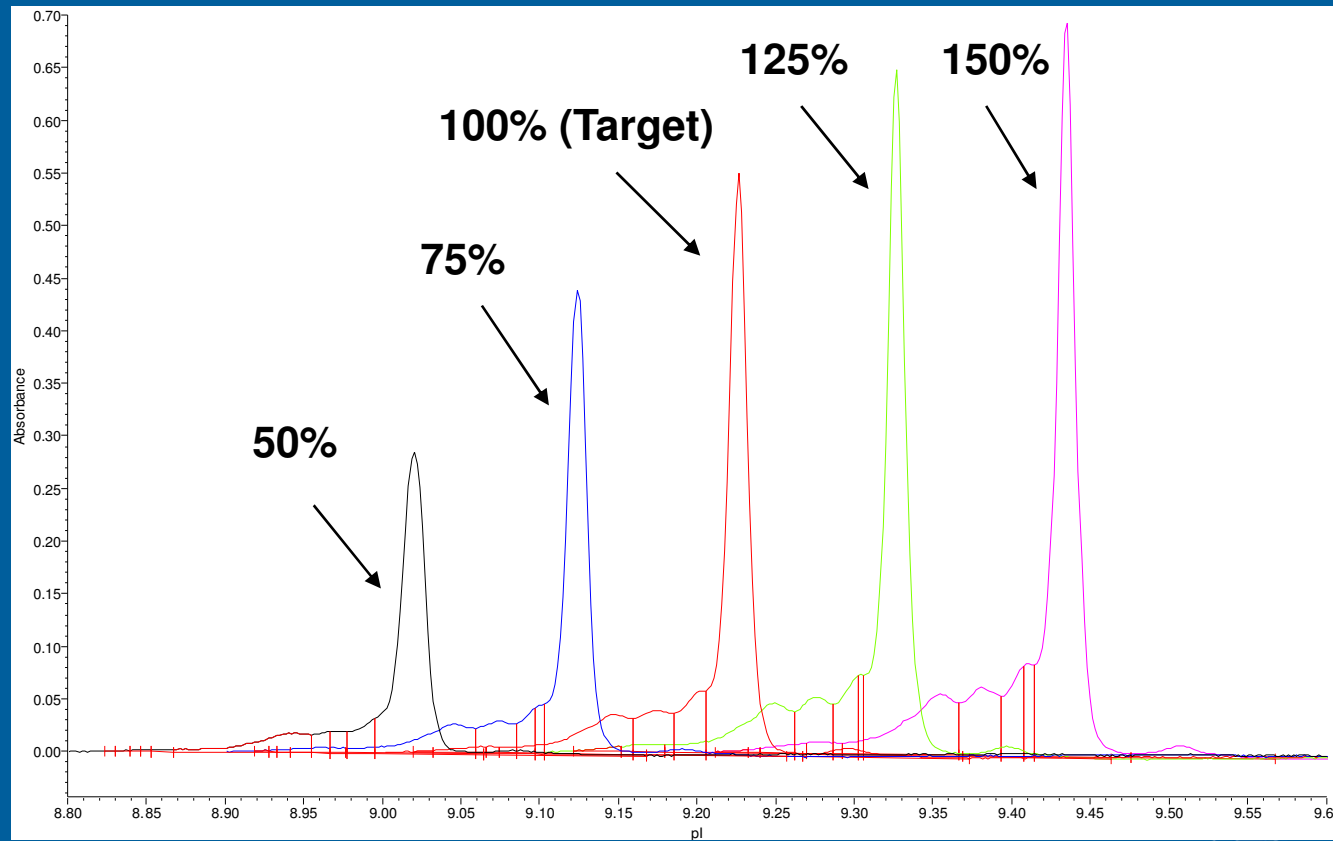
Repeatability (N=6)

% Peak Area

	INJECTION			SAMPLE PREPARATION		
	Acidic Region	Main Peak	Basic Region	Acidic Region	Main Peak	Basic Region
Average	30.83	66.18	2.77	30.70	66.72	2.58
RSD (%)	2.8	1.1	5.8	2.1	1.1	6.7

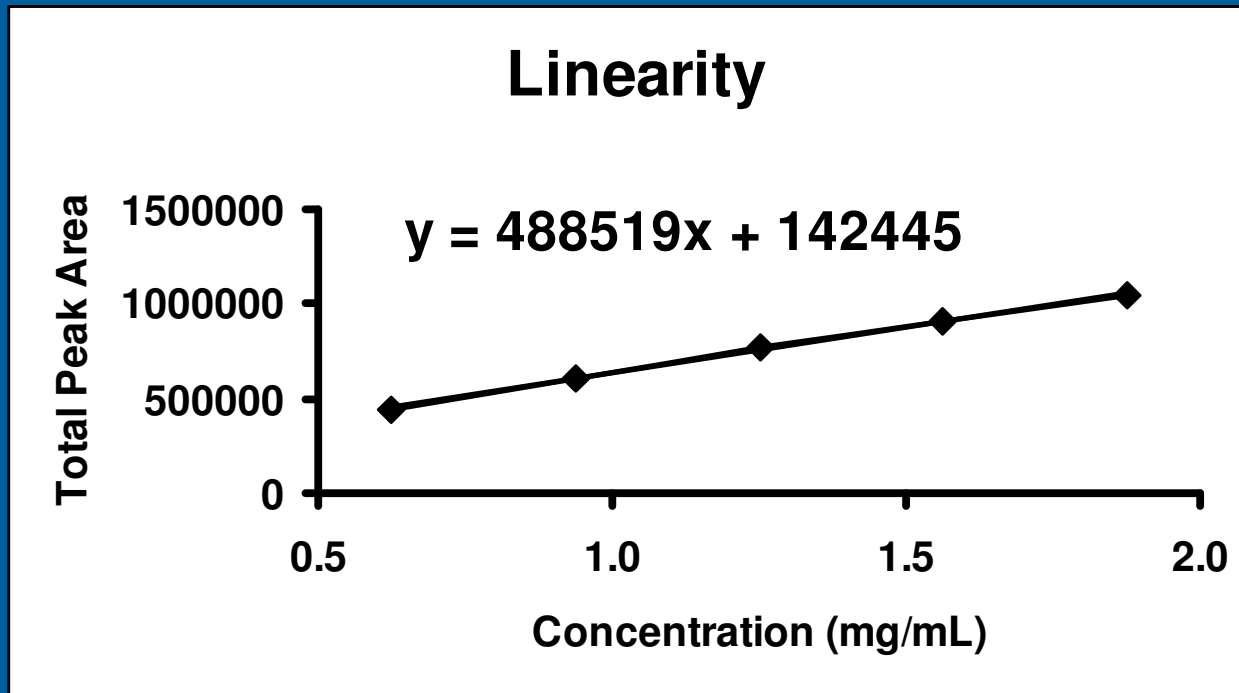
Method is Precise

METHOD VALIDATION. Accuracy



Recovery over 50 – 150% of target concentration
is within 90 - 113%

METHOD VALIDATION. Linearity



Method is linear in concentration range 0.6 – 1.9 mg/mL
Pearson correlation coefficient = 1.0

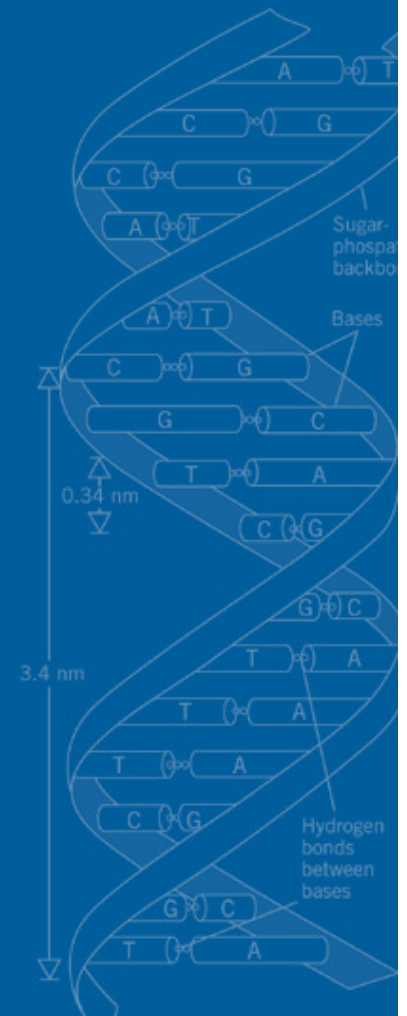
SYSTEM SUITABILITY – Instrument related

1. To check plugged capillary:

- Typical Current vs Time Plot
- Start of the plateau < 150 sec
- Spike height > 5 μA

2. To check whole column UV detector

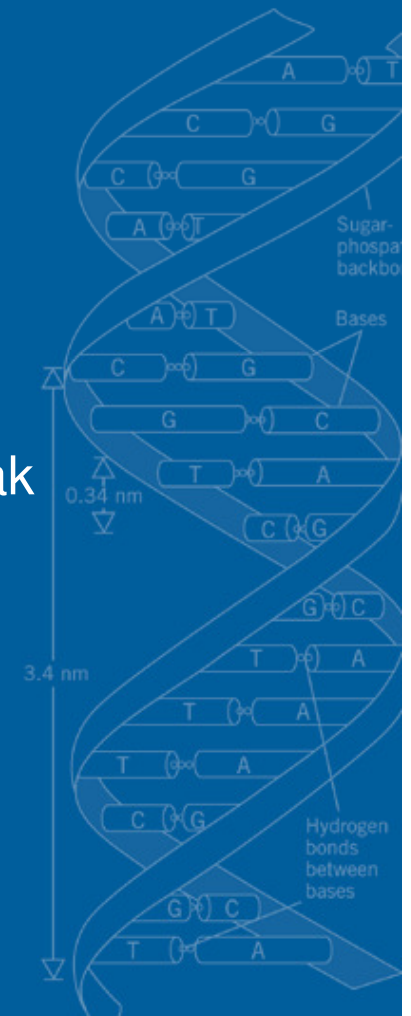
- Typical light intensity profile
- Intensity of maximum point of the cartridge light profile is between 3500-3600 a.u.
- Updated exposure time is within ± 3 mseconds from default



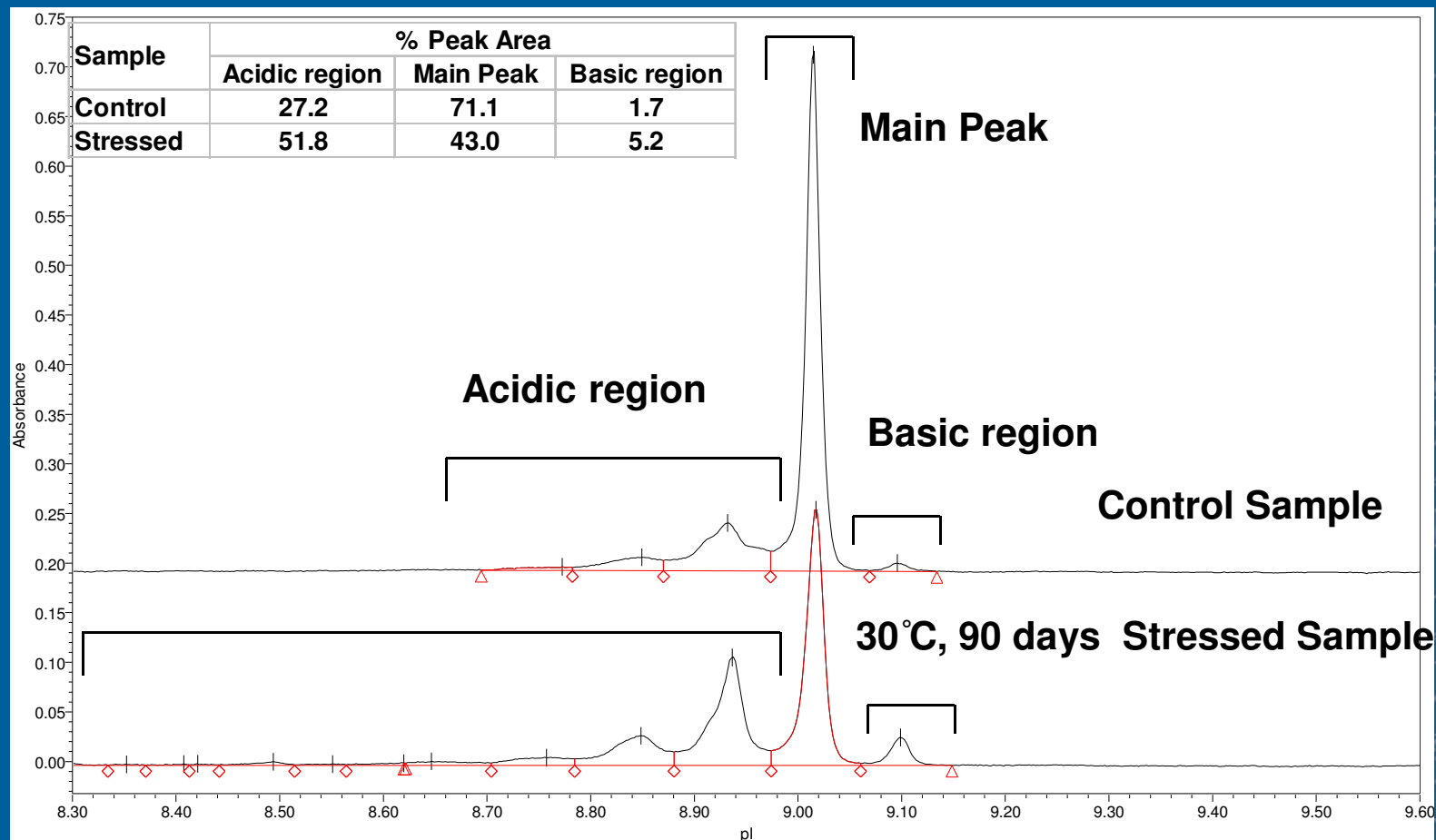
SYSTEM SUITABILITY – Sample related

3. Consistency of the separation profile

- Sample sequence with bracketing reference material injections
- Quantitative acceptance criteria: $\pm 3SD$ range on % peak area for acidic region, basic region and main peak



STABILITY – INDICATING PROPERTIES



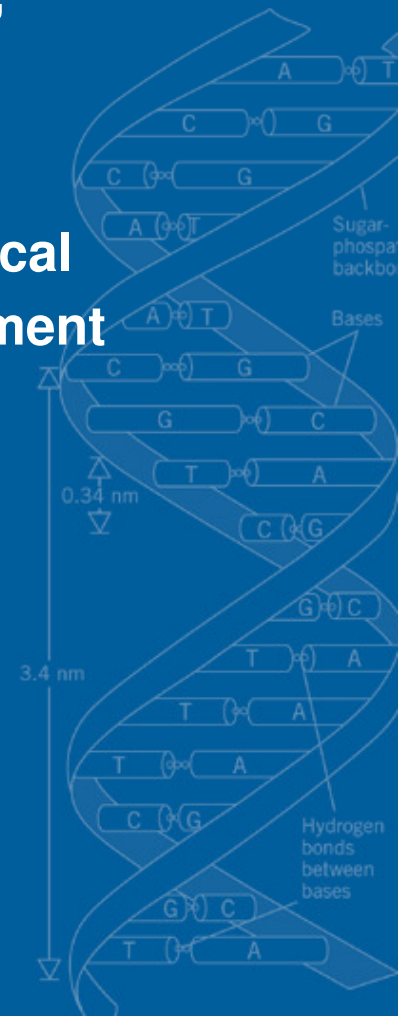
CONCLUSIONS (1)

1. Replaced product-specific IEC method with generic ICIEF for routine lot release and stability testing of early stage clinical products
2. Gained efficiency: 0.3 FTEs/Phase 1 project
3. Imaged CIEF method was successfully implemented in Quality Control
4. All electronic data archiving paths were validated in compliance with 21 CFR part 11 regulation



CONCLUSIONS (2)

5. Demonstrated that iCIEF method is robust, precise, accurate, specific and stability-indicating
6. Close collaboration and team work between analytical development laboratory, quality control and instrument manufacturer is critical.



ACKNOWLEDGMENTS

1. Genentech

Quality Control Analytical Technologies:

Koman Joe, Chantal Felten, Dieter Schmalzing, Amir Malek and others.

Analytical Development:

Oscar Salas-Solano, Will McElroy, David Michels

Quality Control Equipment & Software Validation:

Lyle Covino, Dave Watson

2. Convergent Team:

Ed Chase, Jiaqi Wu, Tiemin Huang, Ravi Mandke

