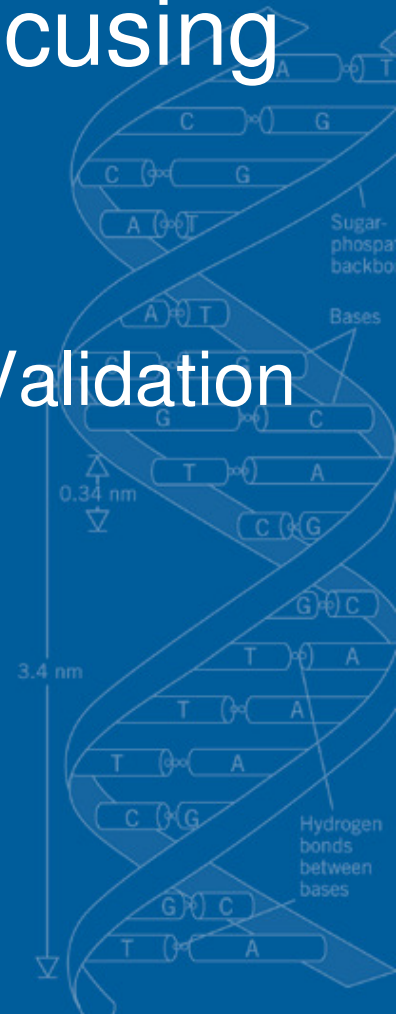


Imaged Capillary Isoelectric Focusing (ICIEF)

From Method Selection, Development, Validation
to Transfer

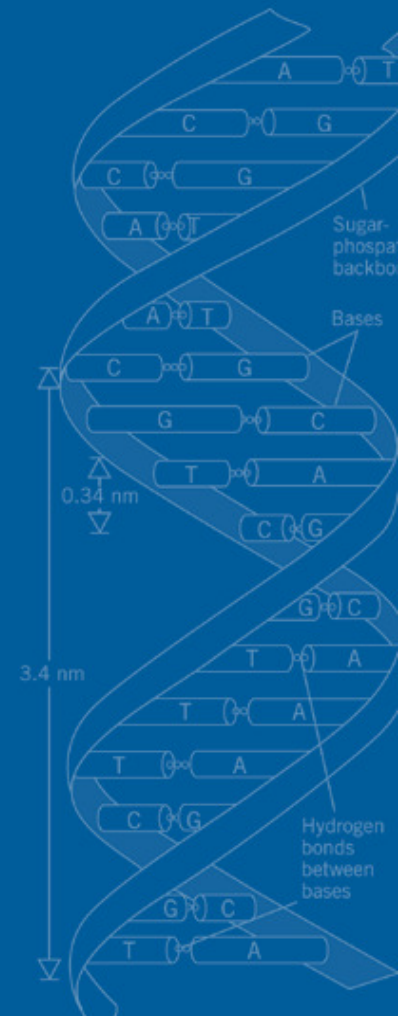
Zara Safarian, PhD
January 13, 2009
San Francisco

Genentech
IN BUSINESS FOR LIFE



Goal

To determine charge-based purity
of the drug product



Objective

Select and Develop method: - Validatable

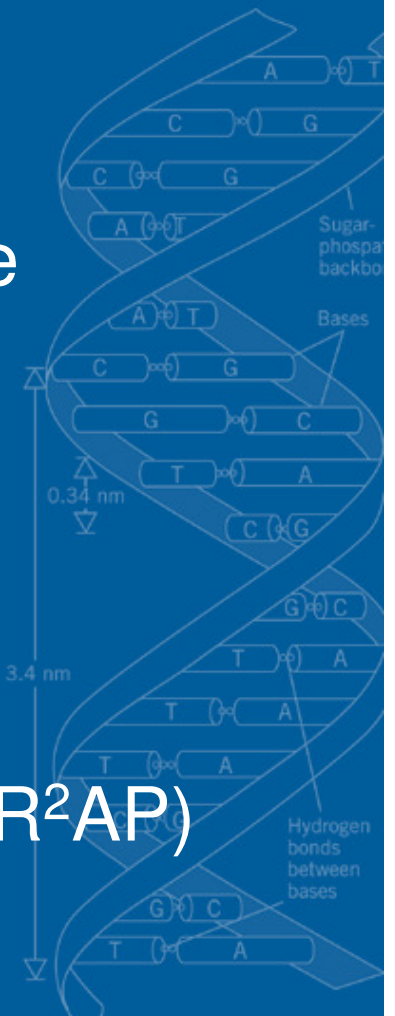
- Transferable

- Robust

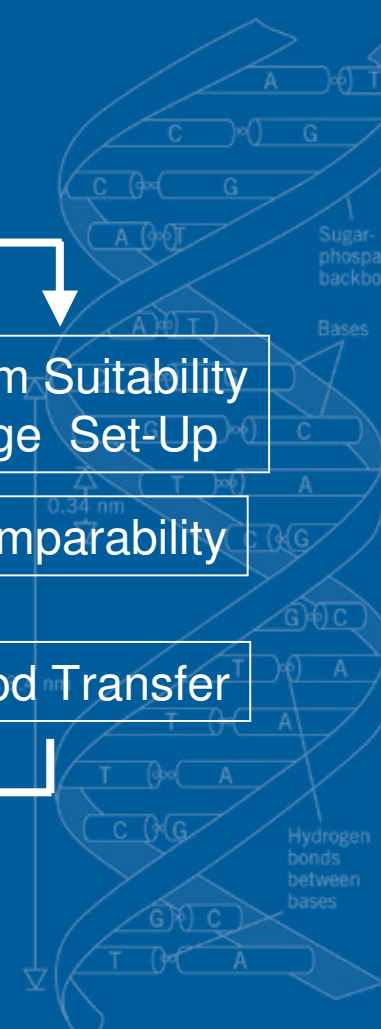
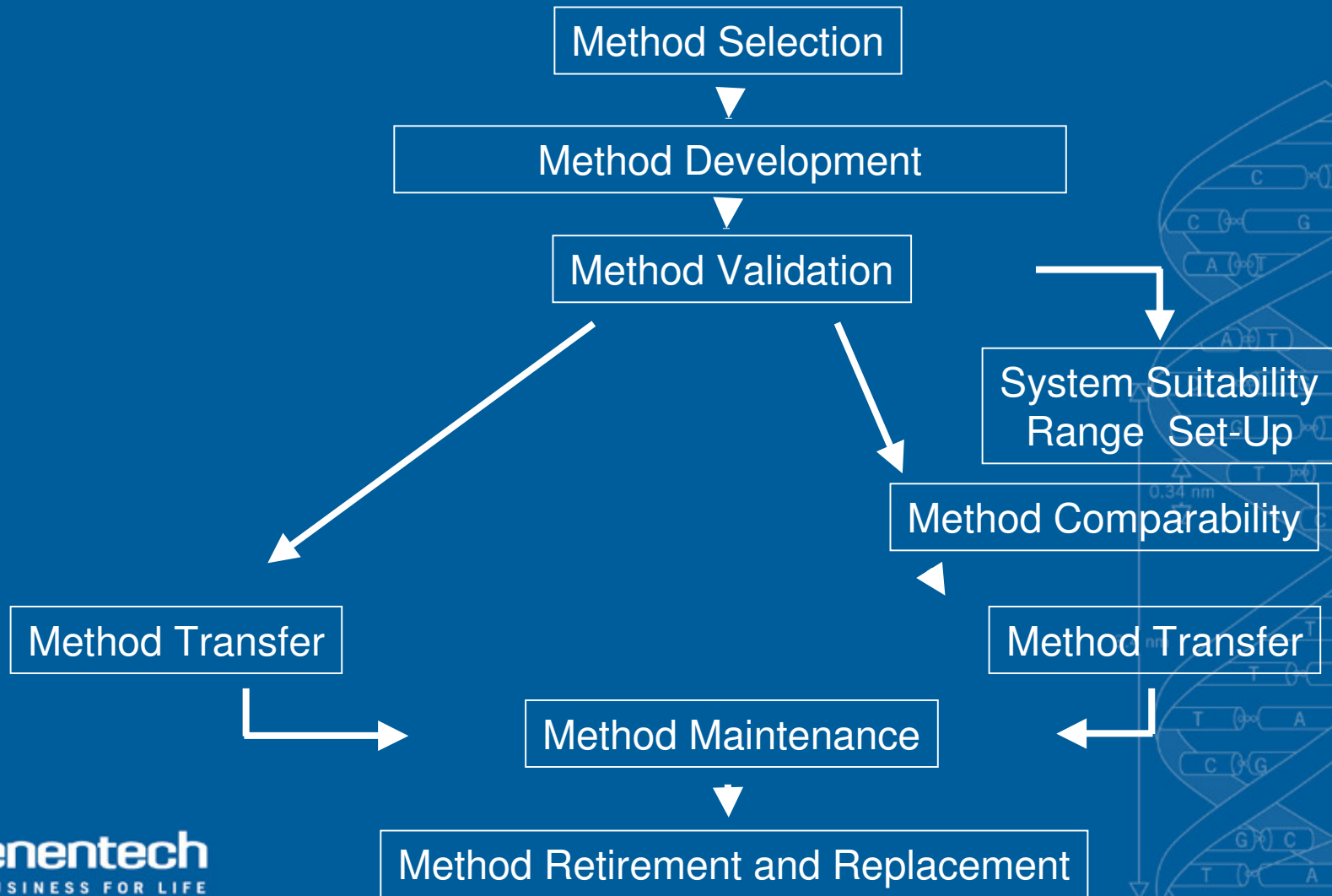
- Reliable

- Accurate

- Precise (VTR²AP)



QC Methods Lifecycle Approach

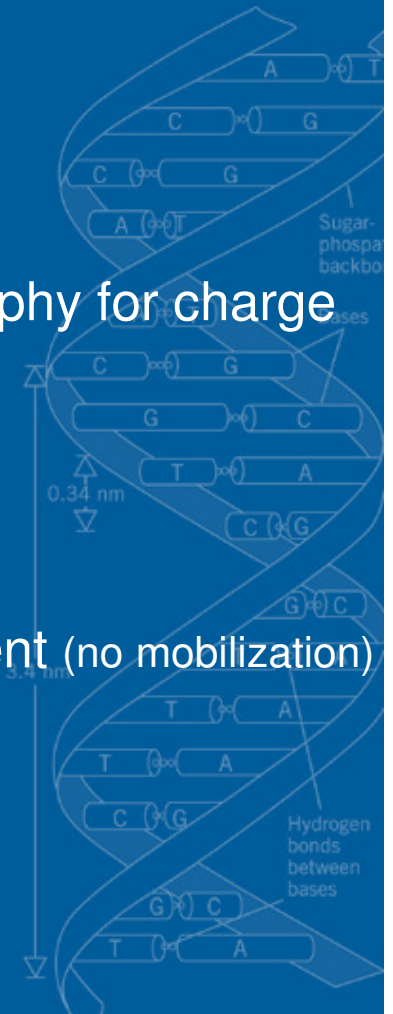


Method Selection - ICIEF

1. Applicable for charge-based purity determination
2. Comparable with standard ion-exchange chromatography for charge heterogeneity analysis

Advantages:

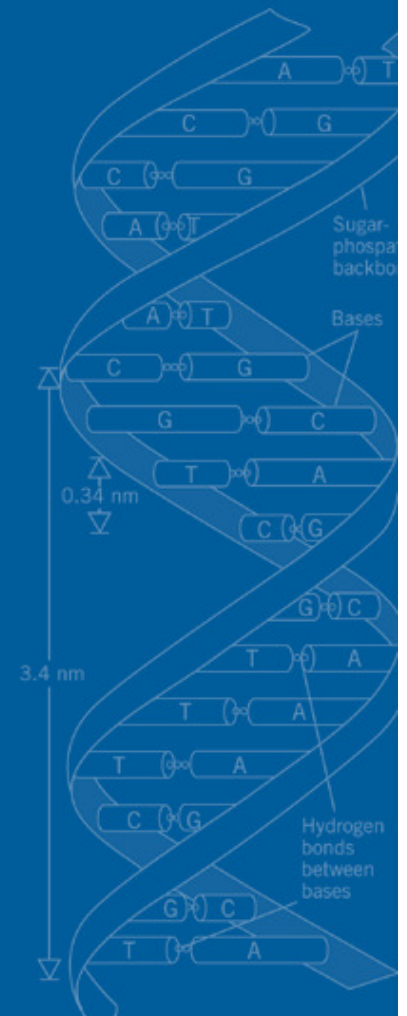
- Generic for mAbs
- Operational simplicity
- Short run time
- Fast Method development (no mobilization)
- Easy analyst training
- Easy transferable



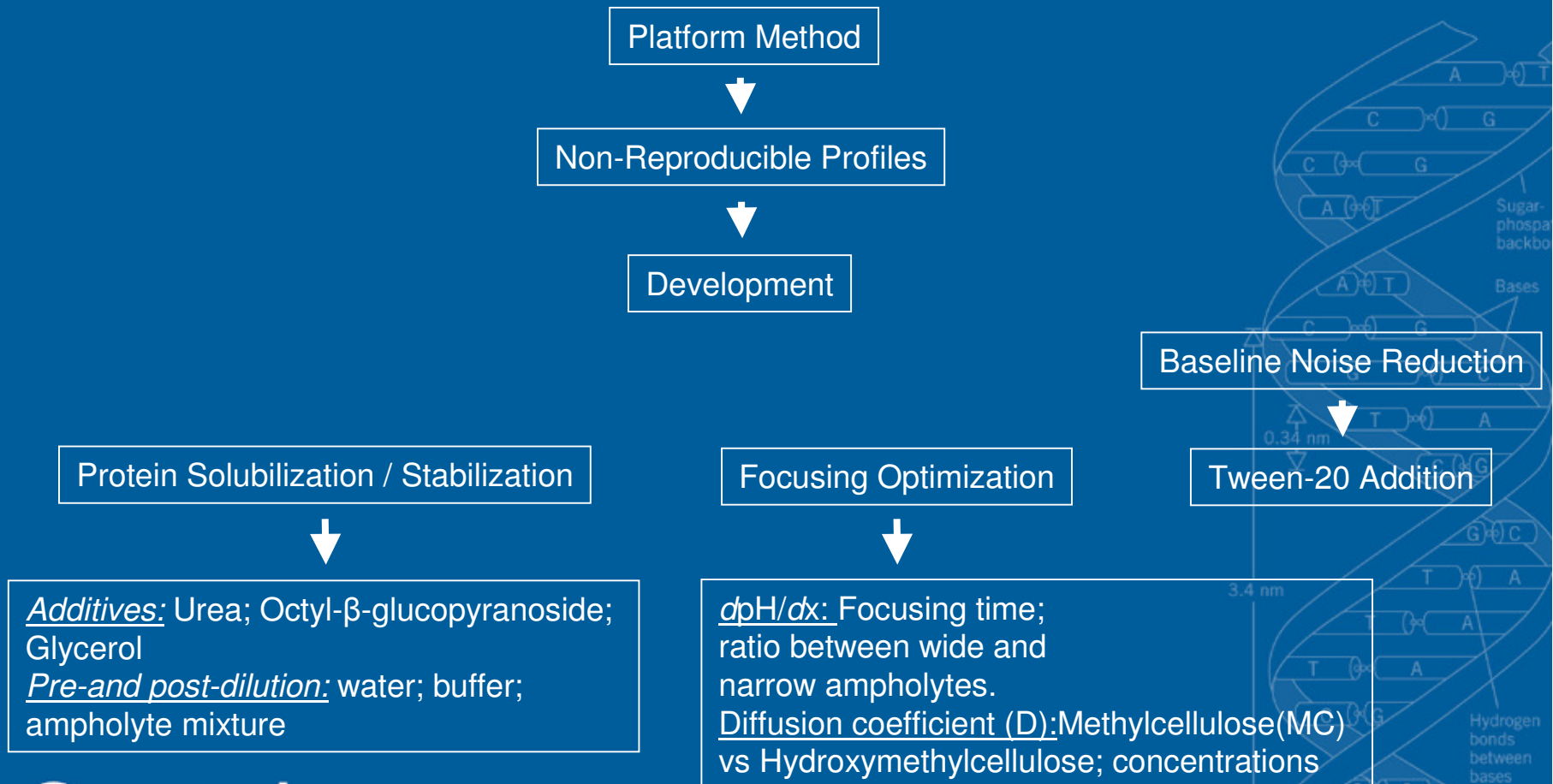
Method Development – Initial MD: Targets

Profiles of the peaks should be:

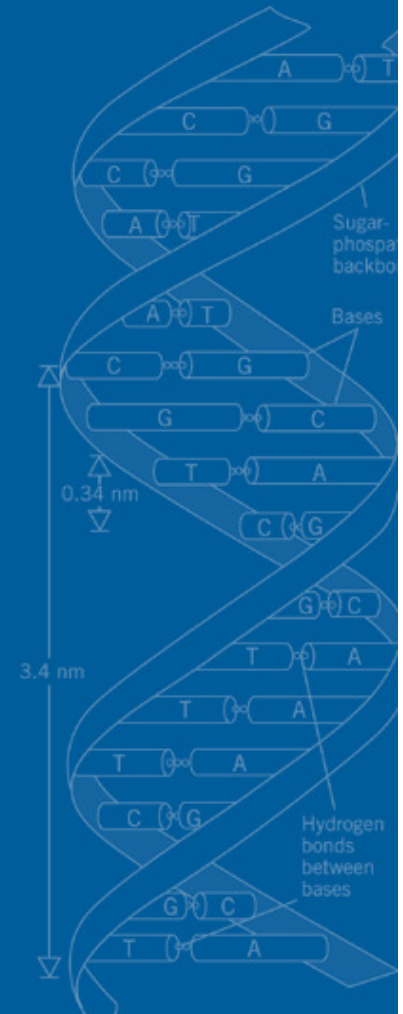
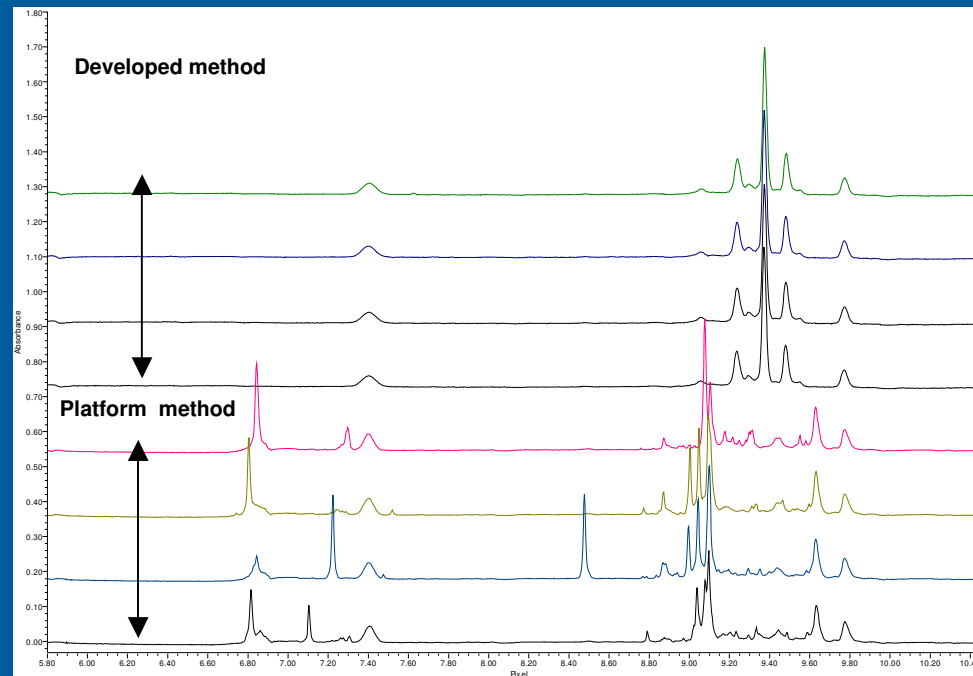
- Reproducible
- Quantitative
- Comparable with IEC



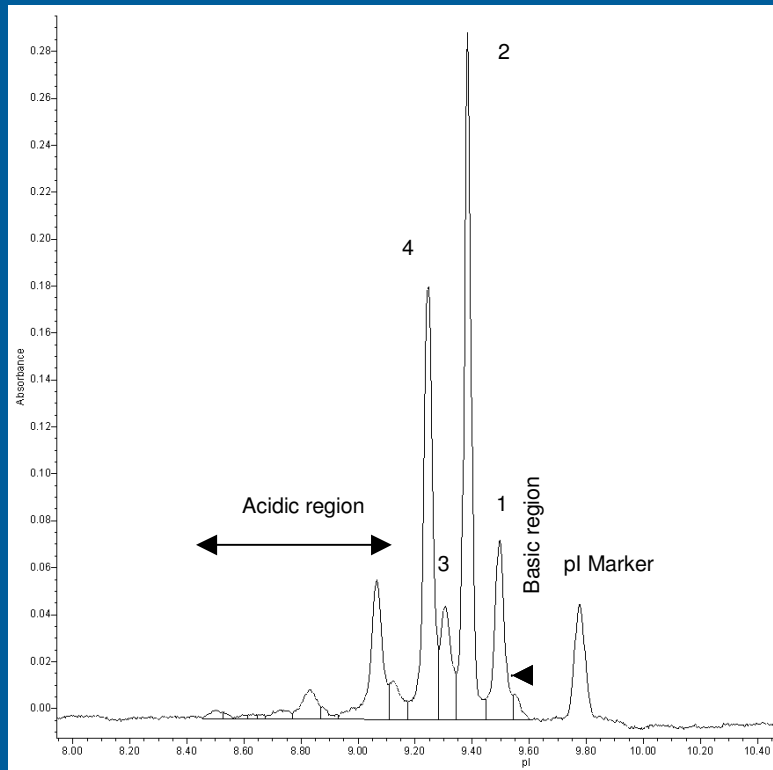
Initial MD: Approach



Initial MD: Reproducible profiles of the peaks

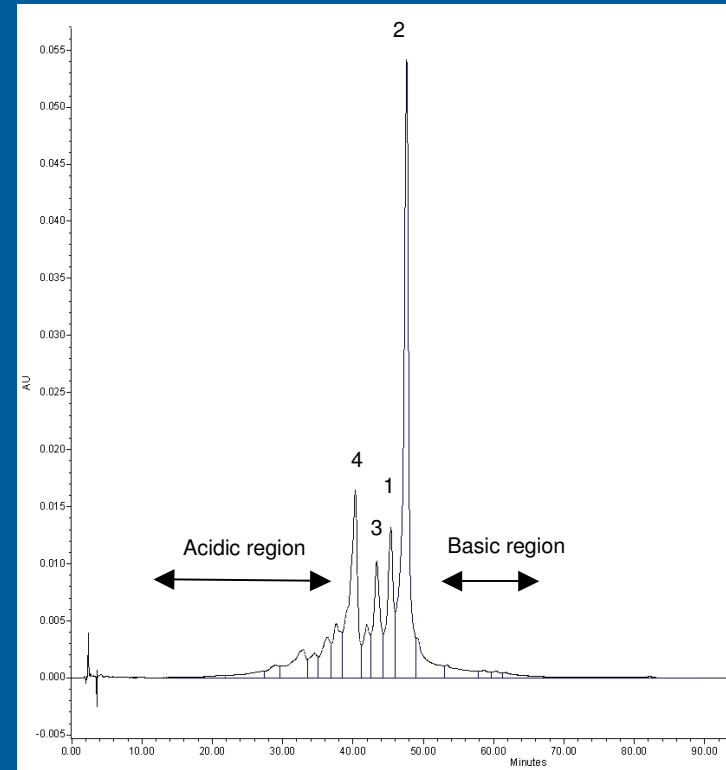


Initial MD: Quantitative and comparable profiles



ICIEF Area % Of Total Peak Area

Main Peaks	Acidic region	Peak1	Peak2	Peak3	Peak4	Basic region
78.9	20.0	11.8	33.1	7.9	26.1	1.1



IEC Area % Of Total Peak Area

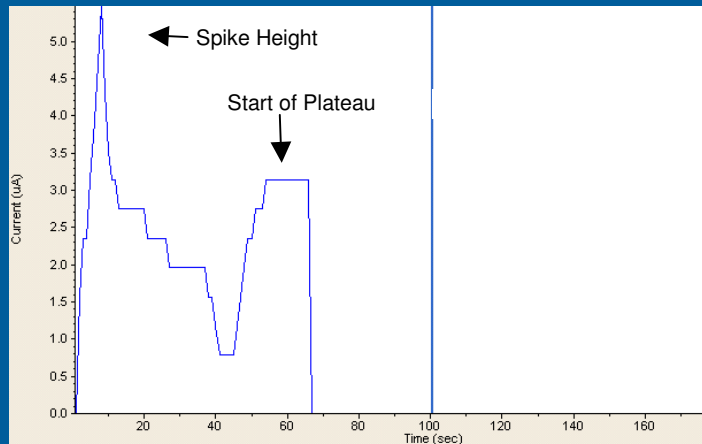
Main Peaks	Acidic region	Peak1	Peak2	Peak3	Peak4	Basic region
71.3	17.9	9.5	34.6	11.5	15.6	10.8

Method Development

Predefined System Suitability: Instrument Related

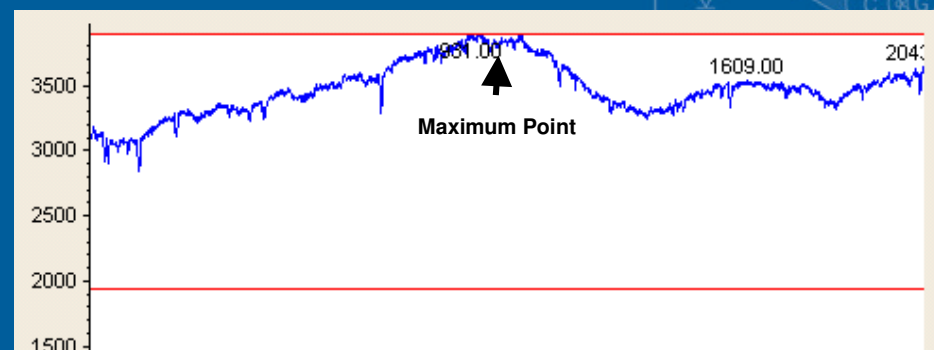
To check plugged capillary:

Typical Current vs. Time Plot
 Start of the plateau < 150 sec
 Spike height > Sample related μA



To check whole column UV detector

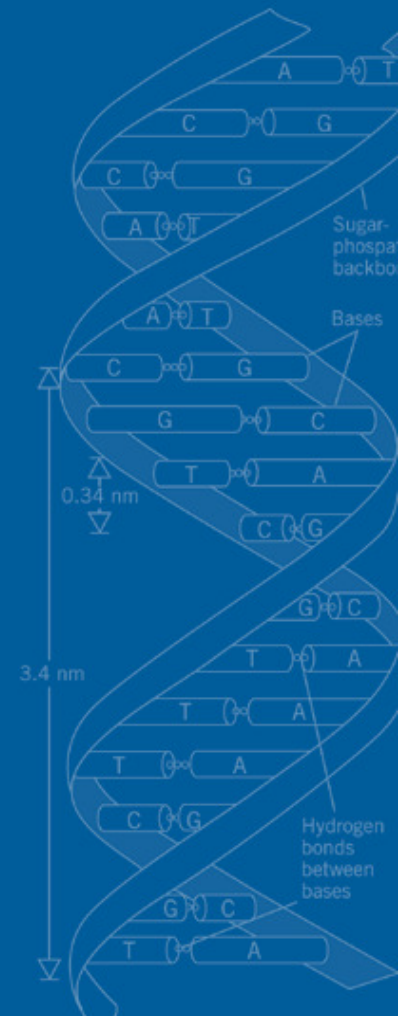
Typical light intensity profile
 Intensity of max. point of the cartridge light profile is between 3500-3600 a.u.



Method Development

Predefined System Suitability: Sample Related

1. System Suitability Sample – Reference material
2. Bracketing reference injections
3. Less than 2% RSD for the relative percent peak area of the main peak and up to 10 % RSD for acidic and basic regions



Method Development

Pre-validation (Approach)

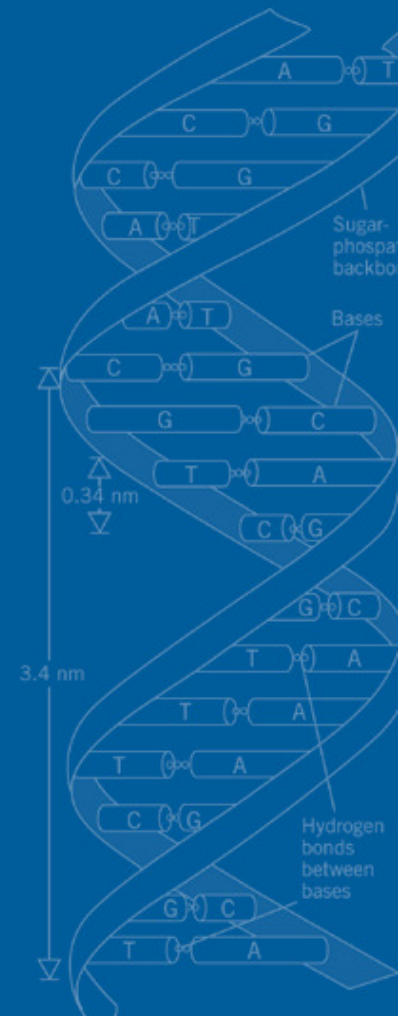
1. Perform extensive testing to define method with meaningful system suitability
2. Establish critical method parameters
3. Compare reproducibility of quantitation under different conditions with normal conditions (reference material at target)
4. Show that method is clear and precise prior to validation



Method Development

Pre-validation (Studies)

1. Precision: intra- and inter-day, sample preparation and injections repeatability
2. Accuracy: Sample recovery and Protein Loading
3. Linearity
4. LOQ
5. Stability: sample, methylcellulose, electrolytes
6. Electrolytes depletion
7. Cartridge-to-Cartridge
8. Instrument-to-Instrument
9. Stressed samples analysis
10. Alternative instrument: Beckman PA800



Method Development

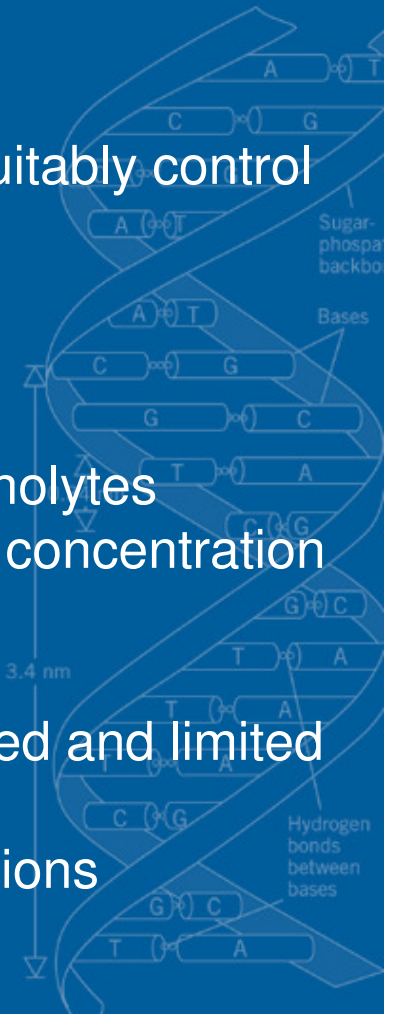
Method Optimization/Robustness evaluation

To establish ranges around critical method parameters to suitably control the analytical conditions.

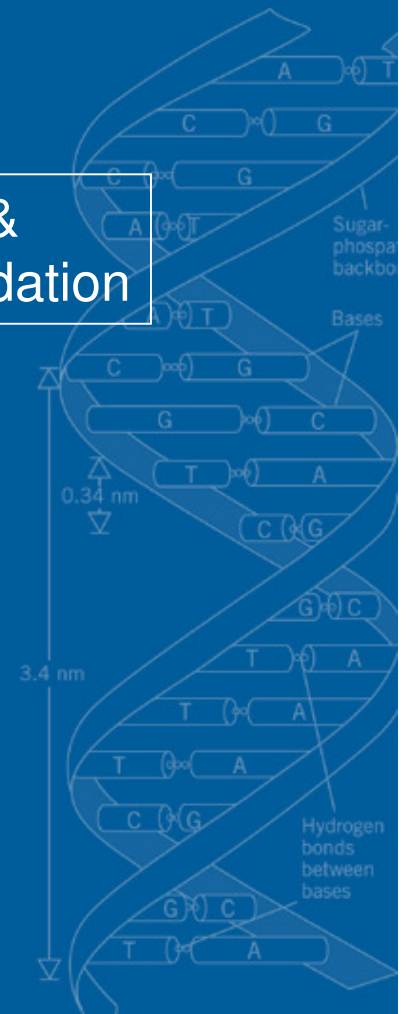
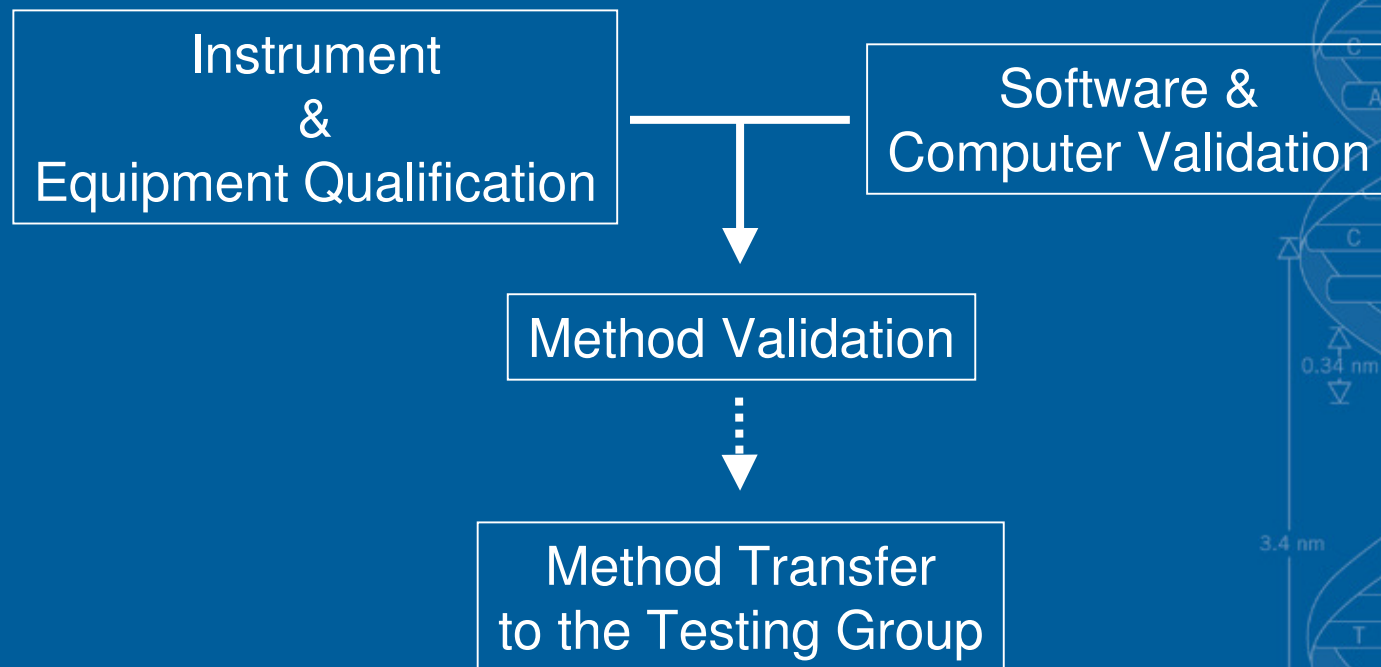
Studies: Variations in MC/HPMC concentrations
Ampholyte concentrations
Ratio of wide range and narrow range ampholytes
Additives (urea, Octyl- β -glucopyranoside, glycerol) concentration
Focusing time
Tween-20

Outcome: Critical method parameters identified, defined and limited

Test Procedure - draft for validation with final method conditions



Method Validation & Method Transfer



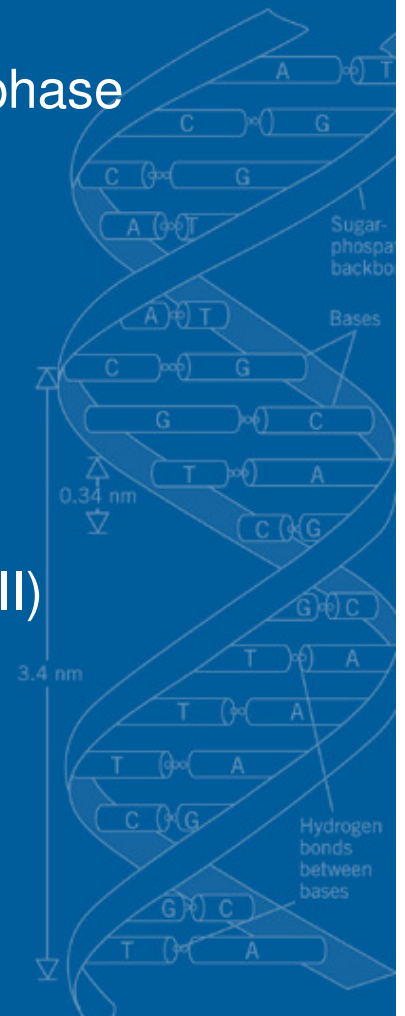
Method Validation Approach

Validation is performed to ensure compliance with ICH Q2(R1)

The level of validation is based upon the intended use, the phase of product development and the stage of production

Validation characteristics to be evaluated for Phase I/II/III:

- Specificity
- Precision: Repeatability and Intermediate
- Robustness (can be part of validation for Phase III)



Setting System Suitability Ranges (1)

Quantitative Acceptance Criteria:

$\pm 3SD$ range on % peak area for acidic region, basic region and main peak

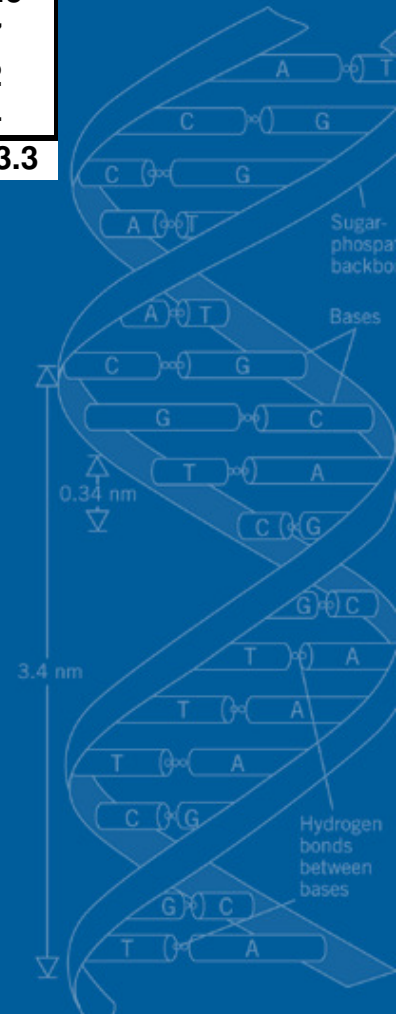
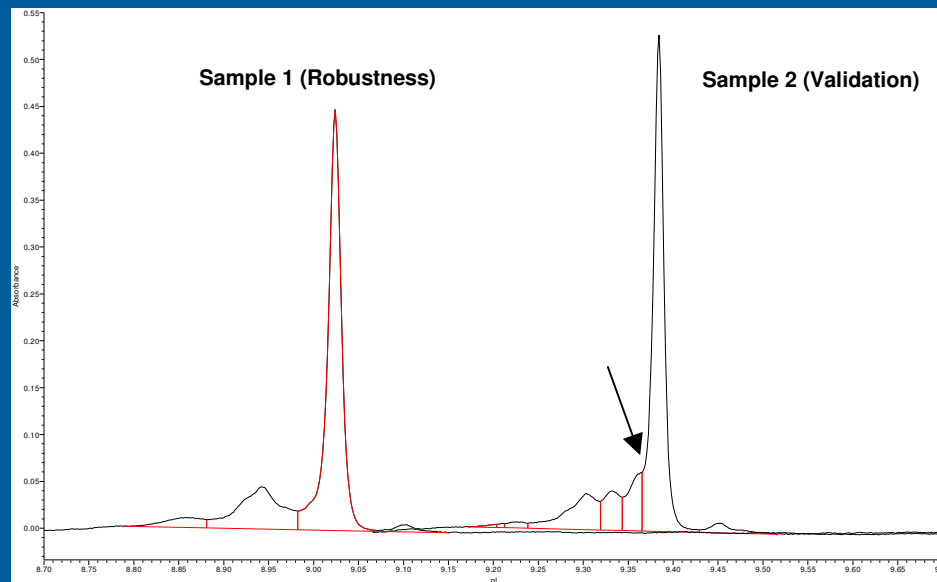
	Acidic Region	Main Peak	Basic Region
Mean	27.5	65.3	7.2
Std. Dev.	0.5	0.6	0.1
% RSD	2.0	0.9	1.9
Mean $\pm 3SD$	25.9-29.1	63.5-67.1	6.8-7.6

The system suitability range is based on robustness and validation studies of reference material

N=55

Setting System Suitability Ranges (2)

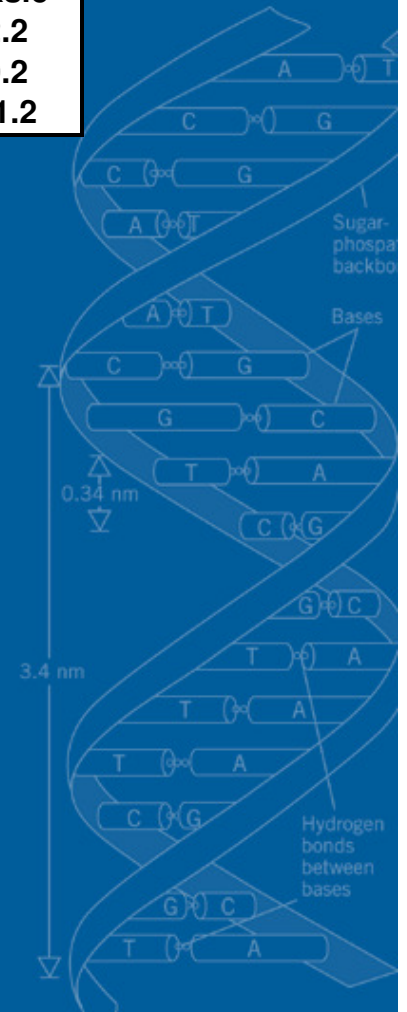
Studies	Robustness (N = 229)			Validation (N = 51)		
	Acidic	Main	Basic	Acidic	Main	Basic
Mean	26.8	71.1	2.1	30.4	66.9	2.7
SD	0.7	0.8	0.3	1.1	1.1	0.2
% RSD	2.6	1.1	12.8	3.8	1.7	7.4
	Mean \pm 3SD			27.0 - 33.8	63.5 - 70.3	2.1 - 3.3



Setting System Suitability Ranges (3)

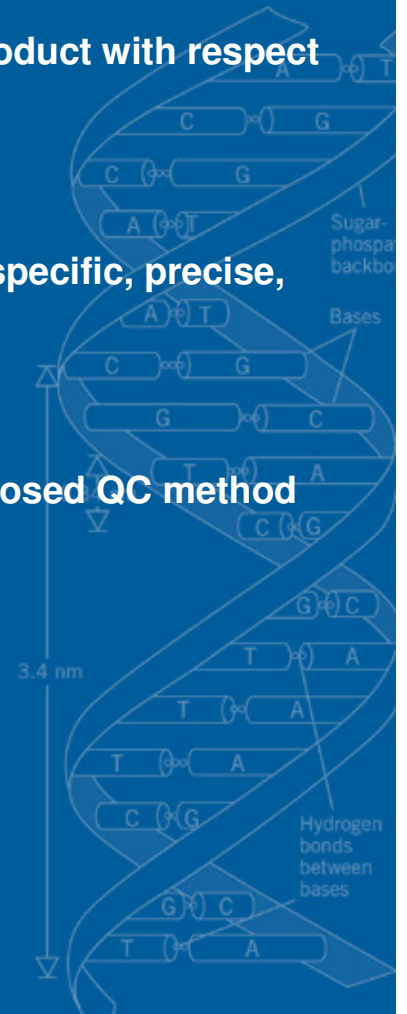
Studies	Robustness (N = 22)			Precision (Validation) (N = 48)		
	Acidic	Main	Basic	Acidic	Main	Basic
% Peak Area						
Mean	38.7	59.1	2.2	38.6	59.3	2.2
SD	0.6	0.62	0.2	0.5	0.57	0.2
% RSD	1.5	1.1	7.3	1.2	1.0	11.2

% Peak Area	Acidic region	Main Peak	Basic region
Mean±3SD	37 - 41	57 - 61	1 - 3



Conclusions

1. Analytical Method Selection and Development Processes demonstrated that ICIEF is quantitative method, and appropriate for determining purity of the drug product with respect to charge heterogeneity.
2. Analytical Method Validation Process demonstrated that ICIEF method is specific, precise, and suitably robust.
3. 14 molecules were successfully validated in Genentech based on the proposed QC method lifecycle approach.



Acknowledgment

1. Genentech

Protein Analytical Chemistry Department

2. Convergent Team:

Ed Chase, Jiaqi Wu, Tiemin Huang, Ravi Mandke and Alice Lam

