

# icIEF Analysis in a cGMP Environment

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*WCBP 12th Symposium, Technical Seminar*

January 30, 2008, Washington, DC USA

# Objectives

1. Imaged capillary isoelectric focusing methodology background
2. Comparison of IEC and icIEF cGMP methods
3. Case Studies: Identification and Purity/Identification
4. Conclusion

# Background

## IEC

Traditionally utilized for protein identification and purity testing in the biotechnology industry in a QC environment for stability and release material.

# icIEF Methodology

## New technology

Protein are analyzed based on their isoelectric points by imaged capillary isoelectric focusing (icIEF) with UV detection on the iCE280 analyzer by Convergent Bioscience.

## Currently in Biogen Idec QC

2 qualified methods for both purity and identity  
2 methods in validation for identity

New platform as the replacement of IEC method

# Great Interest for cGMP Environment

## IEC method:

Many system suitability and/or assay acceptance criteria failures due to retention time shifts, resulting more documentation.

### Criteria:

- RT of the peaks in the reference standard #1 and #2 must be is within one minute of corresponding peaks of each other.
- RT of the sample(s) is within one minute of the reference standard

## icIEF Method:

pI values of the sample are assigned by the calibrated internal pI markers, prevent the pI shift.

### Criteria:

- Both markers must be present
- pI of the main peak of the sample(s) is within 2% of the pI of the main peak of the reference standard

# Method Comparison

Advantages	
IEC	icIEF
<p><b>Easy to find experienced analysts</b></p> <p><b>HPLC in every lab</b></p>	<p><b>Specific</b></p> <p><b>Easy step-by-step system start-up within iCE280 Software</b></p> <p><b>pI markers calibrated, no pI shift</b></p> <p><b>Easy to use chemical test kits</b></p> <p><b>Possibility of data analysis using Empower Software without external modules</b></p> <p><b>Short method development time (typically 15 to 20 minutes per injection)</b></p> <p><b>New technology attracts analysts in QC environment</b></p> <p><b>Small amount of waste</b></p>

# Method Comparison

Disadvantages	
IEC	icIEF
<p><b>Long method development time / run time (typically 60 to 90 minutes per injection)</b></p> <p><b>Time consuming to condition and qualify a new column prior to use</b></p> <p><b>pH sensitive, causes retention time shifts</b></p> <p><b>Large amount of hazardous waste</b></p> <p><b>Requires preparing a large amount of buffers</b></p>	<p><b>Data analysis using EZChrom software</b></p> <p><b>Few vendors around for consumables</b></p> <p><b>Not many experienced analysts</b></p>

# Case Study #1 -Identification

## IEC method qualification

Specificity Study, two of the recombinant monoclonal antibodies had a very similar profile and eluted at approximately the same retention time.

## Initiated an investigation

Method not specific for these two products

Original formulation of the proteins are the same

Initial sample concentrations are the same

# IEC Chromatograms

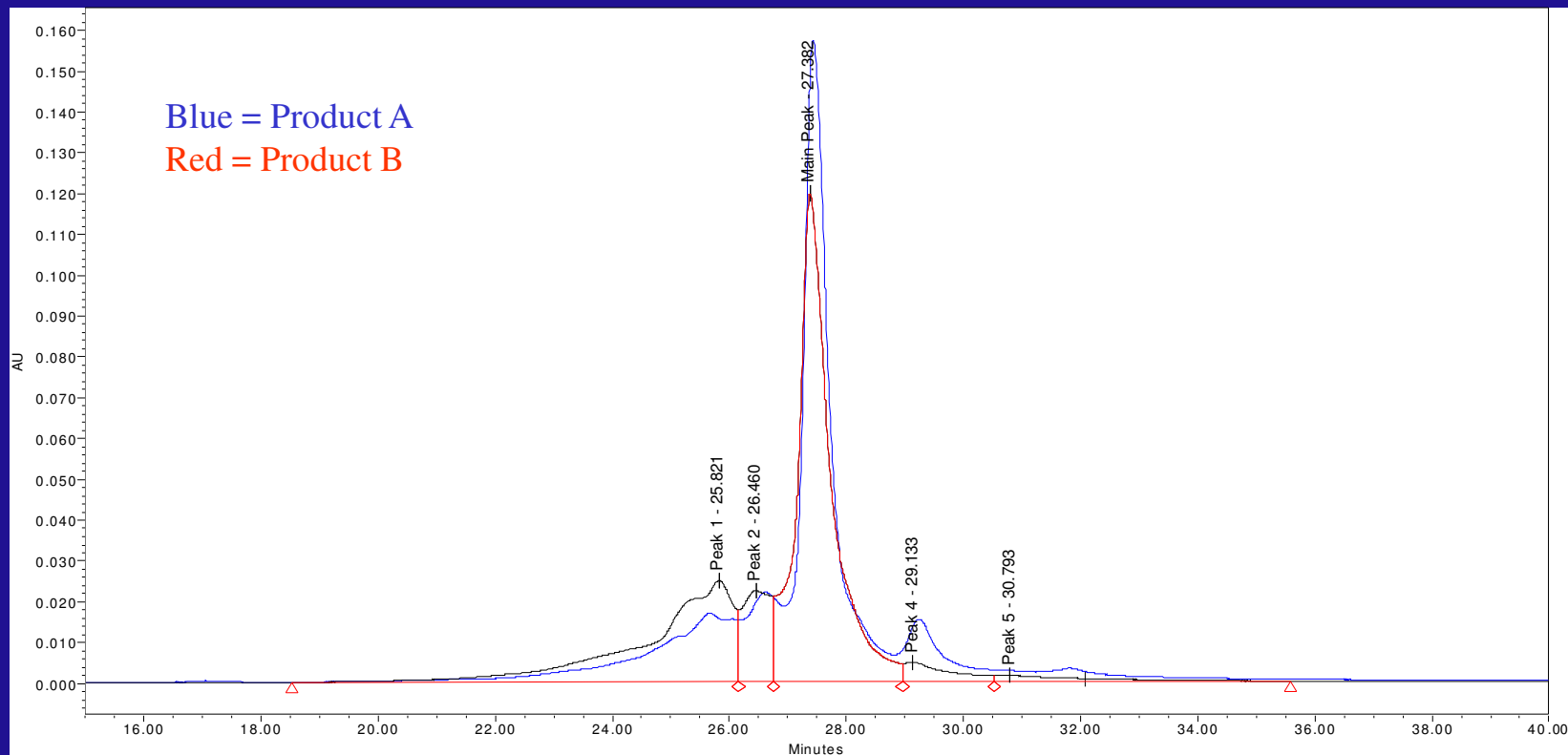


Figure 1. IEC chromatogram overlay of Product A and B.

# Case Study #1 -Identification

## Initial Investigation of IEC Method

Method optimization:

Different mobile phases, flow rate, and gradients

Result:

Not successful, method not product specific for product identification.

# Case Study #1 -Identification

## icIEF method

Same molecules were analyzed using icIEF method.

### Result:

Provided different profiles for these two products.  
Separated based on their pI.

Developed by Analytical Development in collaboration with QC Chemistry to use as an identity test.  
Validation is in progress in QC Chemistry.

# icIEF Chromatogram

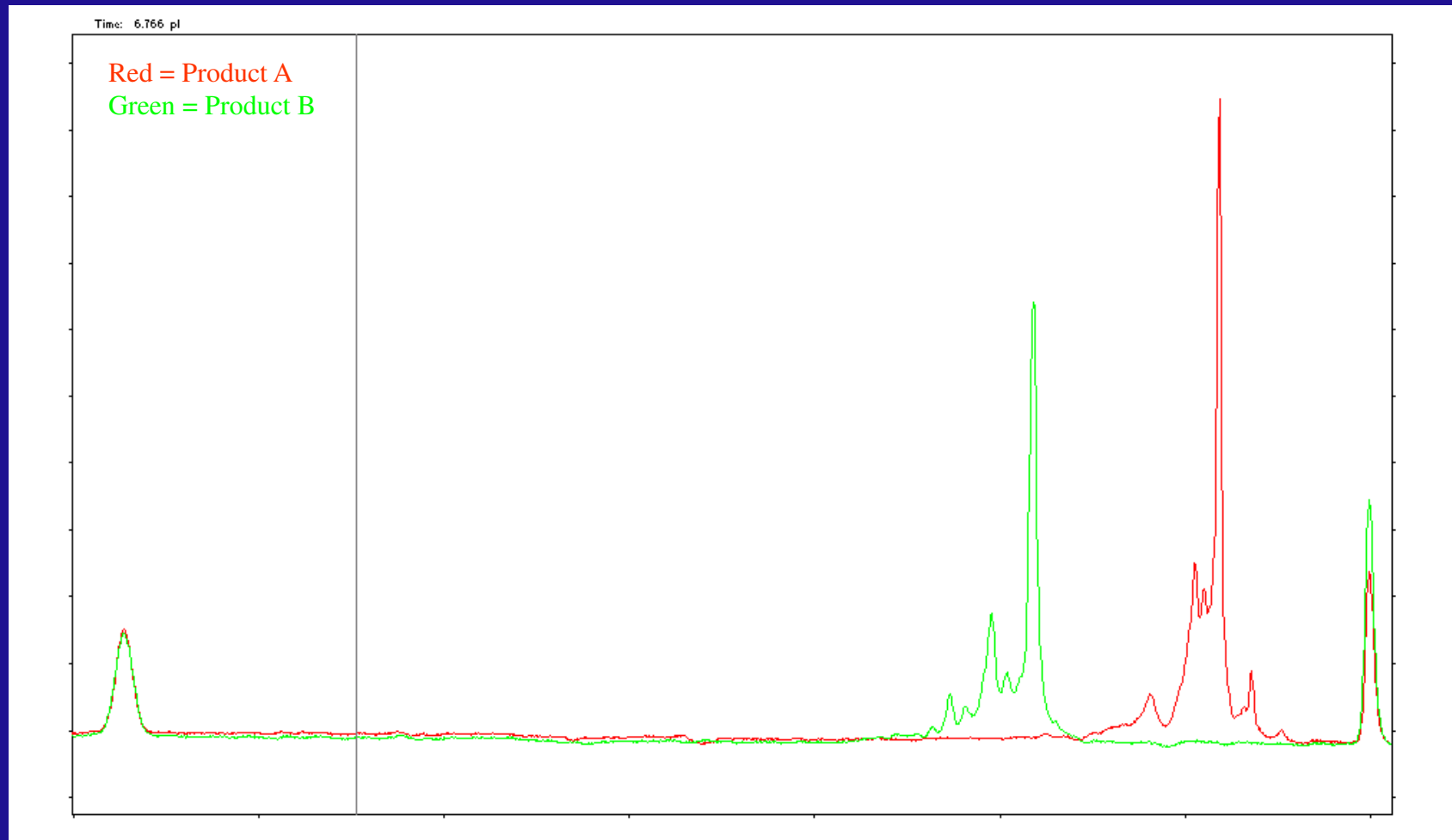


Figure 2. icIEF Chromatogram overlay of Product A and B.

# Challenges during Qualification

## Pharmalytes

Overall, icIEF has a better resolution than IEC.

For this particular basic protein, purity analysis did not work since no higher basic range ampholytes were available.

However, icIEF method is specific for identification, and replaced the IEC method for identification.

# Challenges during Validation

## pI markers

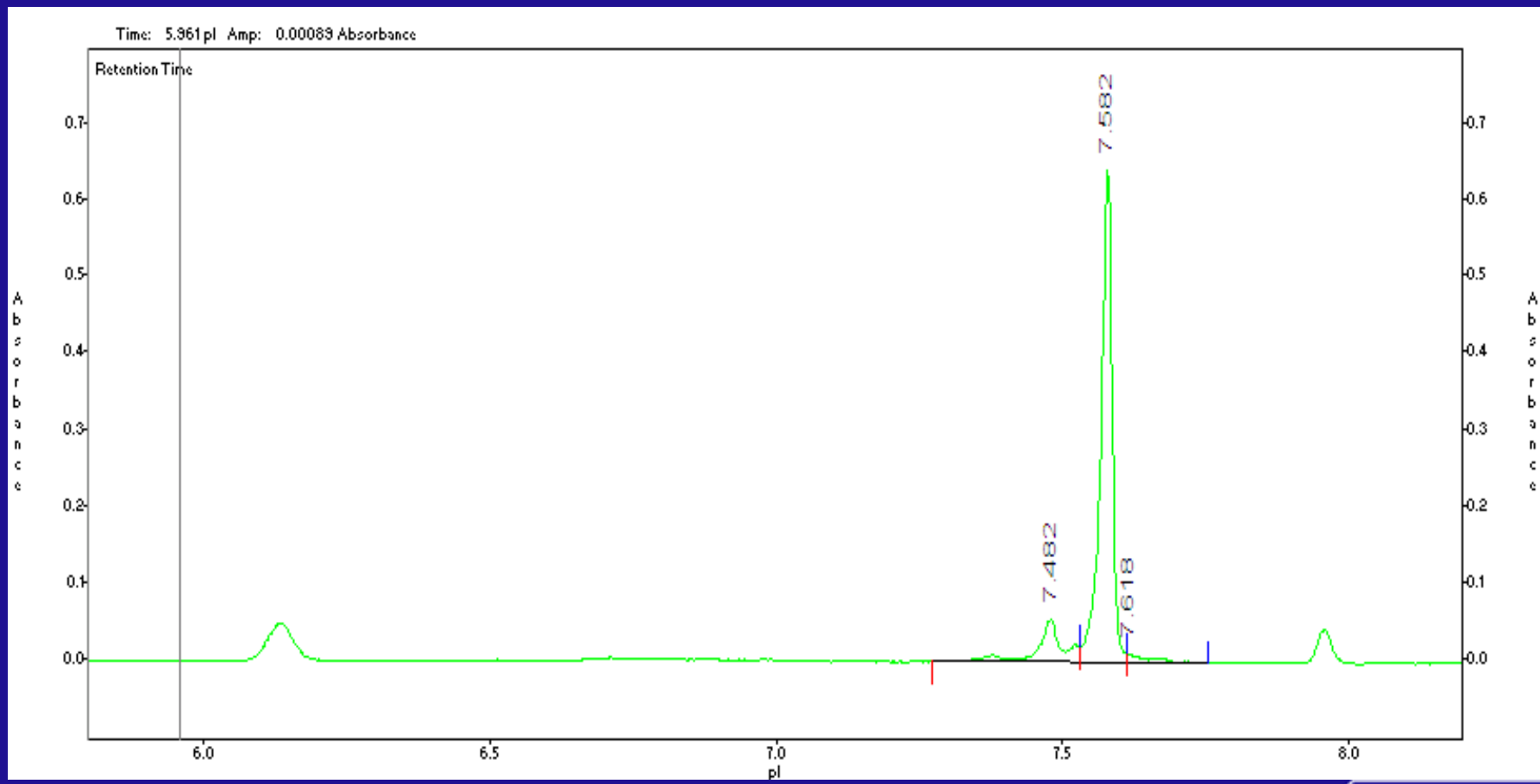
Communication from the vendors

Different pI marker values will result in slight difference in the assigned pI of the molecule in question.

pI marker changes require re-qualification, re-validation, revision of the SOP and re-training in cGMP labs

# Case Study #2 –Purity and Identification

Currently two qualified icIEF methods in use for both purity and identity QC for release and stability testing.



biogen idec

# Case Study #2 –Purity and Identification

Example of trend chart

Date	pI			% Peak Area		
	Acid	Main	Basic	Acid	Main	Basic
23-May-07	7.47	7.57	7.61	15.62	81.72	2.67
4-Jun-07	7.48	7.58	7.61	14.68	81.99	3.32
14-Jun-07	7.48	7.58	7.62	15.71	81.51	2.78
27-Jul-07	7.48	7.58	7.61	16.59	80.66	2.75
24-Oct-07	7.47	7.57	7.61	15.88	81.43	2.70
12-Nov-07	7.47	7.57	7.61	16.93	80.10	2.97
<b>Avg</b>	<b>7.48</b>	<b>7.58</b>	<b>7.61</b>	<b>15.90</b>	<b>81.24</b>	<b>2.87</b>
<b>SD</b>	<b>0.01</b>	<b>0.01</b>	<b>0.00</b>	<b>0.79</b>	<b>0.71</b>	<b>0.25</b>
<b>RSD</b>	<b>0.07</b>	<b>0.07</b>	<b>0.05</b>	<b>4.98</b>	<b>0.88</b>	<b>8.60</b>

# Conclusion

## icIEF

- Suitable and desirable tool to use in biotechnology industry in cGMP laboratories for purity and identity testing
- Support contract product fill sites requiring fast turnaround for protein ID
- More specific method for identification for two recombinant monoclonal antibodies at Biogen Idec
- Currently 2 qualified methods for both purity and identity at Biogen Idec for release and stability testing
- 2 methods in validation for identity

# Conclusion

## iCE280 analyzer

- Easy to use
- Short method development, sample preparation and analysis times
- Decreases system suitability and/or assay failure rate, generating less documentation
- New technology attracts talented staff and keeps the laboratory up to date

# Acknowledgment

Analytical Development:

Damian Houde, Zoran Sosic, Tyler Carlage

QC Chemistry, Assay Technology:

Päivi Albaiti, Elena Belitsky, Catherine Bilodeau,  
Joseph Molon