

# Advantages of Performing icIEF Analysis in cGMP Environment

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## ABSTRACT

The imaged capillary isoelectric focusing (icIEF) with UV detection is an upcoming new technology used to analyze the protein based on the isoelectric points. It can be a desirable tool in biotechnology industry in cGMP laboratories that support contract product fill sites requiring fast turnaround for protein identification. This poster will discuss the use and benefits of ICE280 analyzer over conventional IEC in identity determination for two recombinant monoclonal antibodies at Biogen Idec, Inc. Some of the advantages of this method compared to conventional IEC method include better specificity and identity, as well as shorter preparation and analysis time.

## INTRODUCTION

Ion exchange chromatography (IEC) is traditionally utilized for protein identification and purity testing in biotechnology industry in Quality Control environment for stability and release material. Multiple product line contract fill sites and manufacturing need product identification testing performed by Quality Control laboratories for material intended for use in animals and humans. Potency assay and Cation Exchange Chromatography (CEX) are typically used for product identification to ensure that there is no cross contamination from other products. Fast testing turnaround time is critical to support these processes moving forward with the product.

During the routine Weak Cation Exchange method qualification for one of the recombinant monoclonal antibodies at Biogen Idec, Inc., it was noticed that another recombinant monoclonal antibody had a very similar profile and eluted at approximately the same retention time. This initiated an investigation since there was no specificity in the method used for identification of these two products.

## DESCRIPTION OF METHODS

### IEC method

Weak Cation Exchange (CEX) is an analytical method used for determining the charge distribution of protein. The positively charged protein is bound to the negatively charged resin of the weak cation exchange column and in return exchanged into the mobile phase with increasing concentration of the sodium cations. The chromatographic results give a distribution of the charged species (acidic, main and basic) which is commonly used for identity and stability indications.

Investigation included method development for the existing CEX method with the goal to obtain different profiles for the two products. The optimized CEX method used different mobile phases and flow rate. In the end, the same retention time and similar profile were seen as in the previous CEX method thus not successfully meeting the criteria for the specificity for product identification.

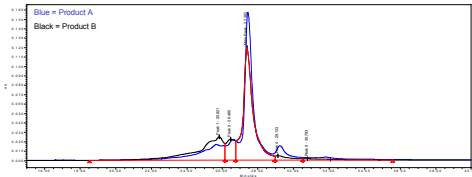


Figure 1. CEX chromatogram overlay of Product A and B.

### icIEF method

During the CEX method development Quality Control Chemistry laboratory had acquired an upcoming new technology used to analyze the protein based on the isoelectric points: imaged capillary isoelectric focusing (icIEF) with UV detection, ICE280 analyzer by Convergent Bioscience. It is being used in the QC Chemistry laboratory for two new clinical products as a replacement of conventional CEX method. Initially the icIEF method was an interest to QC Chemistry Laboratory because in routine testing the traditional CEX method produces many system suitability and/or assay failures due to retention time shifts. These failures are caused by the retention time shift of the sample in comparison to the reference material.

The icIEF method using ICE280 instrument (Convergent Bioscience, Ltd., Toronto, Canada) is a tool that utilizes a column cartridge containing a short separation silica capillary column with its outside polyimide coating removed for the whole-column image detection at  $\lambda = 280$  nm. Protein sample is mixed with selected ampholytes, pI markers and methyl cellulose prior to introduction into the separation capillary column. The cartridge uses porous hollow-fiber membranes to isolate protein samples within the column from two external electrolytes (catholyte and anolyte). Upon applying of the electric field, protein sample is focused along pharmalyte-created pH gradient and its profile detected by a charge coupled device (CCD) sensor. The icIEF method is a great interest for cGMP environment because sample pI values are assigned by the calibrated internal pI markers that would not cause the pI shift from sample to sample.

After unsuccessful further method development results for the CEX method, these two recombinant monoclonal antibodies with overlaying profiles were analyzed using icIEF instrument. The ICE280 method was able to provide different profiles for these two products and separate them based on their pI. As a result, icIEF method was further developed and transferred to use as an identity test to QC Chemistry department from Analytical Development.

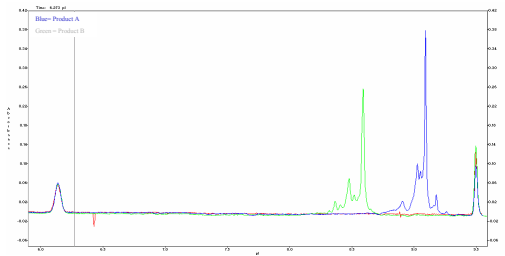


Figure 2. icIEF electropherogram overlay of Product A and B.

## COMPARISON OF METHODS

	IEC	icIEF
Reagent preparation	Time consuming in making mobile phases, large volume of chemicals	Chemical test kits with minimal time for preparation
Instrument analysis	Long run time, typically 60 to 90 minutes per injection	Short run time, typically 15 to 20 minutes per injection
Result	pH sensitive, which causes retention time shifts	pI markers calibrated, no pI shift

### Advantages of IEC Method:

- Easy to find experienced analysts
- HPLC in every lab

### Disadvantages of IEC Method:

- Long method development time
- Time consuming to qualify a new column prior to use
- Slow to make big volume of buffers
- pH adjustment is hard, narrow ranges
- Large amount of waste
- Long run time

### Advantages of icIEF Method:

- Easy to use chemical test kits
- Small amount of waste
- Possibility of data analysis using Empower software without external modules
- Short method development time
- New technology attracts analysts in QC environment
- Easy step-by-step system start-up within ICE280 software
- Specific

### Disadvantages of icIEF Method:

- Data analysis using EZChrom software
- Few vendors around for consumables
- Not many experienced analysts

## MATERIALS

Recombinant monoclonal antibodies (mAb) were produced by Biogen Idec, Inc.

### IEC

The positively charged protein is bound to the negatively charged resin of the weak cation exchange (WCX) column (Dionex, Sunnyvale, CA). It binds to the carboxyl groups when it is injected into the column. A gradient of the increasing salt concentration (Phosphate, Acetonitrile and salt buffer, ACS grade with variety of vendors) is used to exchange the proteins into the liquid phase and elute the protein isoforms from the column on an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) with Empower software data analysis (Waters, Medford, MA).

### icIEF

Protein sample is mixed with selected ampholyte (GE Healthcare, Sweden), pI markers and methylcellulose (Convergent Bioscience, Ltd., Toronto, Canada) prior to introduction into the separation capillary column (Convergent Bioscience). The cartridges use porous hollow fiber membranes to isolate protein samples within the column from two external electrolytes, catholyte and anolyte (Chemical Test Kit, Convergent Bioscience). Using the ICE280 software (Convergent Bioscience), the pI values for the acidic, main and basic peaks are determined. The EZChrom (Convergent Bioscience) or Empower (Waters) software is used to produce a chromatographic result.

## SYSTEM SUITABILITY / ASSAY ACCEPTANCE CRITERIA

### IEC

- Retention time of the sample(s) is within one minute of the reference standard
- Sample(s) should be visually comparable to reference standard

### icIEF

- The pI of the main peak of the sample(s) is within 2% of the pI of the main peak of the reference standard
- Sample(s) should be visually comparable to reference standard

## CONCLUSION

The imaged capillary isoelectric focusing (icIEF) with UV detection as a new technology is a suitable and desirable tool for use in biotechnology industry in cGMP laboratories that support contract product fill sites requiring fast turnaround for protein identification. icIEF has appeared to be a more specific method for identification for two recombinant monoclonal antibodies at Biogen Idec Inc. ICE280 analyzer is easy to use, and it has shorter method development, sample preparation and analysis times than conventional IEC method. In comparison to the conventional IEC method, icIEF method decreases the system suitability and/or assay failure rate since sample pI values are calibrated and assigned by the internal pI markers. In Quality Control environment the new technology attracts talented staff and keeps them motivated by learning new skills as well as keeps the laboratory up to date.

## ACKNOWLEDGEMENT

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