

# Your iCIEF Total Solution Provider



***CEInfinite* Multifunctional iCIEF System**

# CEInfinite System Application Note

## *Acidic Peaks Focused Fractionation Collection for Bevacizumab*

### 1. Instruments

The following system items and consumables should be used for performing this procedure.

- Instrument: C01 Preparative
- Autosampler: 840
- Syringe Pump: 2226 High Precise with 250 µl and 5ml Syringe
- Computer: Dell OptiPlex with Windows 7/10 Professional 64-bit
- Control Software: CEInsight version 3.0 or higher
- Cartridges
  - Preparative type for fraction collection: CP00307
  - Analytical type for reinjection: CP00201/CP00301

### 2. Method

Step 1: Verification of Bevacizumab Electropherogram with standard condition, and the Bevacizumab Electropherogram is obtained below:

#### Bevacizumab standard condition

- pI marker 6.14 and 9.33
- 0.35% Methyl Cellulose,
- 4% Pharmalyte 3-10
- 2M Urea
- Sample Concentration: 0.5mg/ml
- Focusing: 1 min at 1,000V, 1 min at 2,000V, 7 min at 3,000V
- Using AES FC coated cartridge CP00201

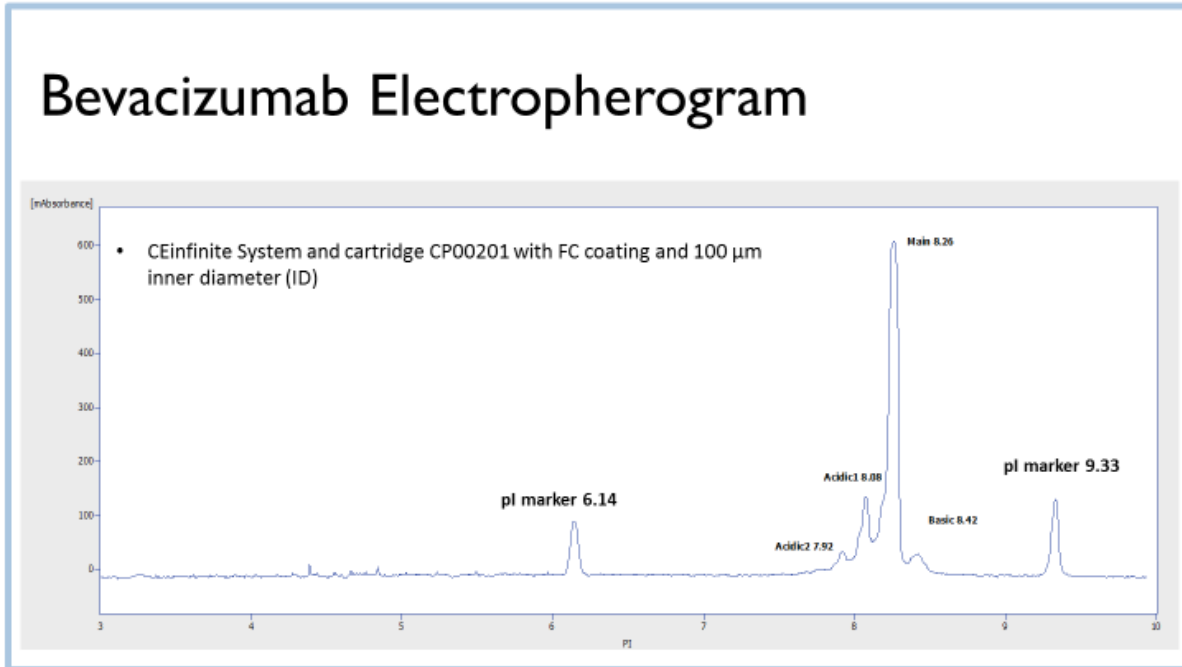


Fig.1a) Analytical conditions and method for Bevacizumab 2) E-gram of Bevacizumab

Step 2: Optimize condition for fractionation

## Bevacizumab AES optimized condition for fractionation

- pI marker 7.65 and 8.71
- 4% 7-9 HR AESlytes (Narrow range)
- 2 M Urea
- Sample Concentration: 0.5 mg/mL
- Focusing: 1 min at 1,000 V, 1 min at 2,000 V, 9min at 3,000 V
- Using AES AD coated cartridge CP00301

Fig.2 Method and conditions for preparation of bevacizumab

Optimized Bevacizumab Egram with AESlyte Narrow range carrier ampholyte and omitted the MC:

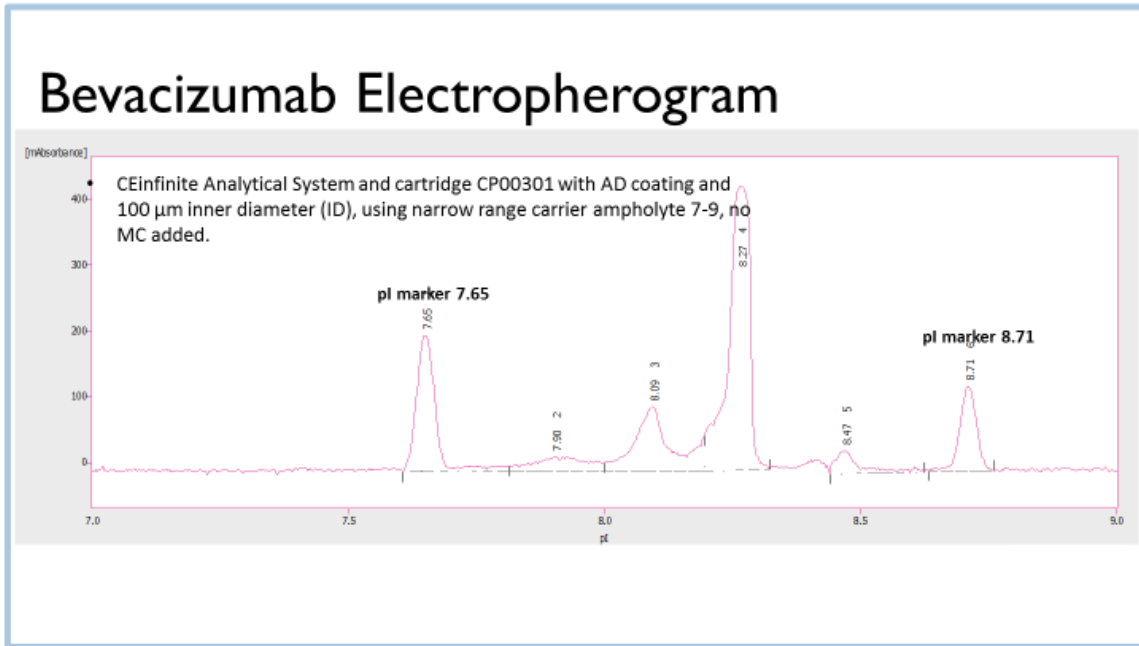


Fig.3 Bevacizumab e-gram with narrow range AESlyte

### Step 3: Fractionation

Fractionation collection condition is shown in below figure,

## Fraction Collection conditions

- 4% 7-9 HR AESlytes (Narrow range)
- 2 M Urea
- Sample Concentration: 0.5 mg/mL
- Focusing: 1 min at 1,000V, 1 min at 2,000V, 10min at 3,000V
- Using AES AD coated cartridge with 320um ID CP00307
- Focusing: 12 minutes
- Mobilization: 35 minutes, 0.2 uL/min with 10nM Acetic Acid
- Makeup liquid: 2ul/min

Fig.4 Bevacizumab preparative method and conditions with narrow range AESlyte

10mM Acetic acid was used as mobilization fluid. Collections were performed while focusing on acidic peaks. Fractionation results are shown below, >80% purity of acidic peaks were collected for Bevacizumab:

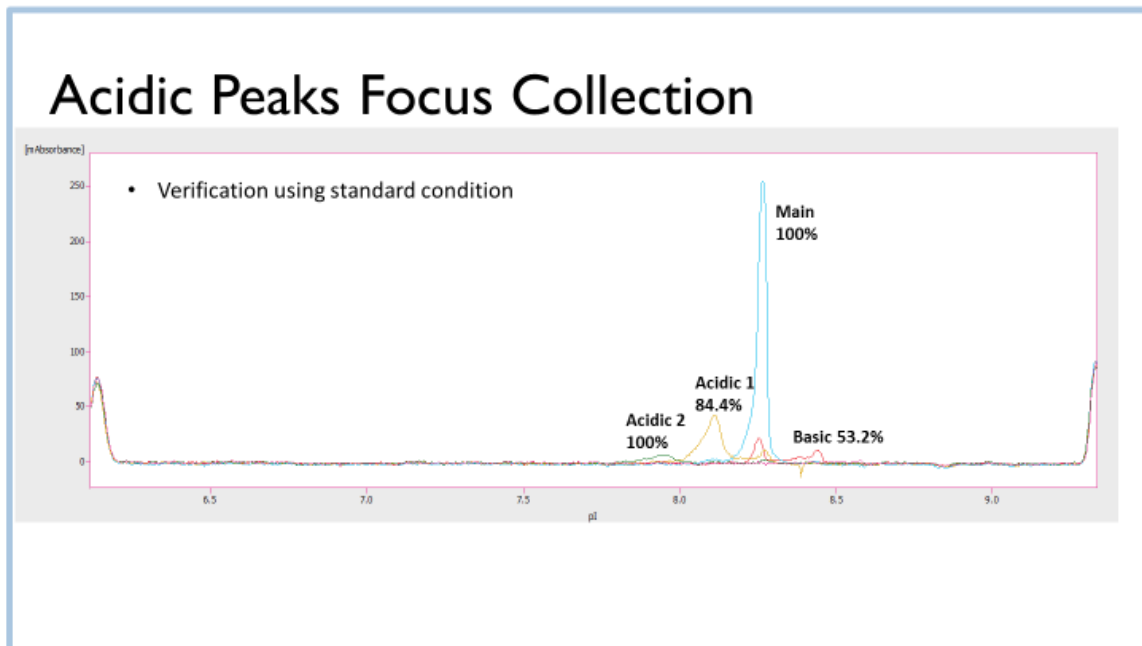


Fig.5 Bevacizumab fractions collected from CEInfinite preparative system

## System Specifications:

SYSTEM	Automated Preparative
Detection mode	Whole column iCIEF
Detector noise	Less than 0.001 AU, 280 nm
Operation mode	<b>Automatic</b>
Sample capacity	84+3 vial tray, 96 well plate
Sample tray temperature	4 - 10 °C
Detection dynamic range	250 (0.004 – 1.0 AU, 280nm)
Resolution	0.03 pI (3-10 ampholyte)
Fractionation capability	<b>&lt;0.1 pI difference</b>
Minimum load volume	35ul
pI CV	<1 %
Typical run time (one run)	<1 Hour
Fractionation efficiency	Up to 99% Purity
Fractionation amount	<b>~ 8 µg isomer/run</b> <b>~100 µg collections/day.</b>
High voltage range	0 – 3000 volts (continuously adjustable)
Exposure time	0.02 – 99.9 ms
Separation pH range	2.1-12
Electrical requirement	100/240 VAC, 50 - 60 Hz
Weight	40kg



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