



## Analytical Characterization of NIST MAb by Sepax SEC, HIC, RP and CEX

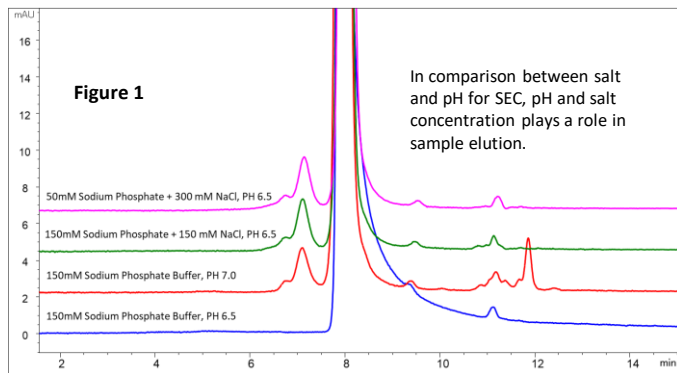
### Highlighted FACTS:

- Zenix SEC-300 provides aggregates, monomer and fragment separation based on sample size difference. In comparison between salt and pH for SEC, pH and salt concentration plays a role in sample elution.
- Using a simple native condition without IPA additives, Proteomix HIC is able to provide the variant separation based sample hydrophobicity. Peaks eluted prior to the main peak could possibly be the oxidation of the antibody or structural changes by aspartic acid isomerization. Further investigation is needed.
- Proteomix RP-1000 offers mAb fragment and structure variants analysis at 80 degree under denaturing condition.
- Using a simple salt gradient, Sepax Cation Exchange Chromatography (CEX) can successfully resolve acidic and basic charge variants of NIST reference mAb. Based on screening, Proteomix SCX provides slightly better separation than Proteomix WCX and Antibodix WCX with salt gradient.
- In comparison between salt and pH gradients, pH gradient provides a slightly better separation in acidic region and salt gradient provides better resolution in basic region.

### NIST MAb Separation on SEC

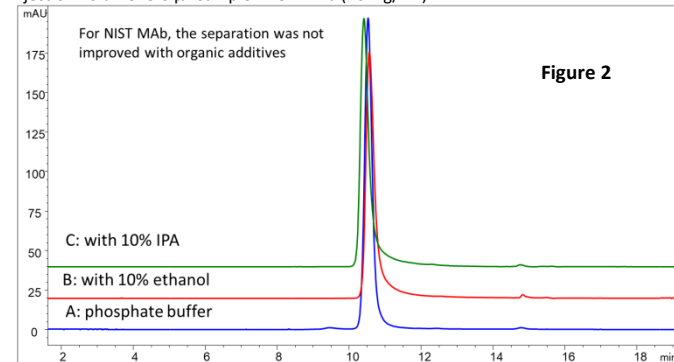
#### NIST MAb SEC Analysis on Zenix SEC- 300 – Mobile Phase Effect

Column: Zenix SEC-300, 3 $\mu$ m, 300 A 7.8 x 300 mm (PN:213300-7830)  
Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25 °C Injection volume: 3.0  $\mu$ L Sample: NIST MAb (10 mg/mL) (pI 9.18, in 12.5 mM histidine, pH 6.0)



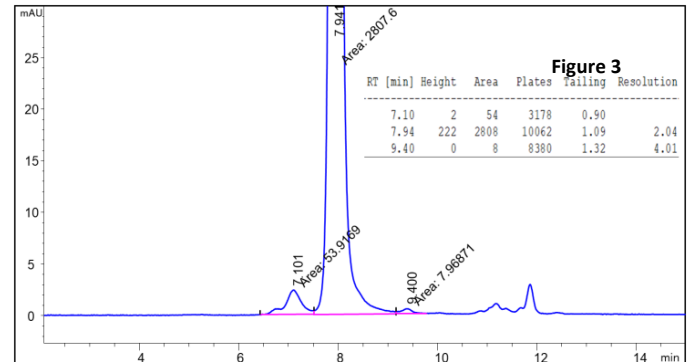
#### NIST MAb SEC Analysis on Zenix SEC- 300 – Organic Additives

Column: Zenix SEC-300, 3 $\mu$ m, 300 A 7.8 x 300 mm (PN:213300-7830)  
Mobile phase: A. 150mM Sodium Phosphate Buffer, PH 7.0  
Flow rate: 0.75 mL/min; Detector: UV 280 nm; Column temperature: 25 °C  
Injection volume: 3.0  $\mu$ L Sample: NIST mAb (10 mg/mL)



### NIST MAb SEC Analysis on Zenix SEC- 300 – Zoom In

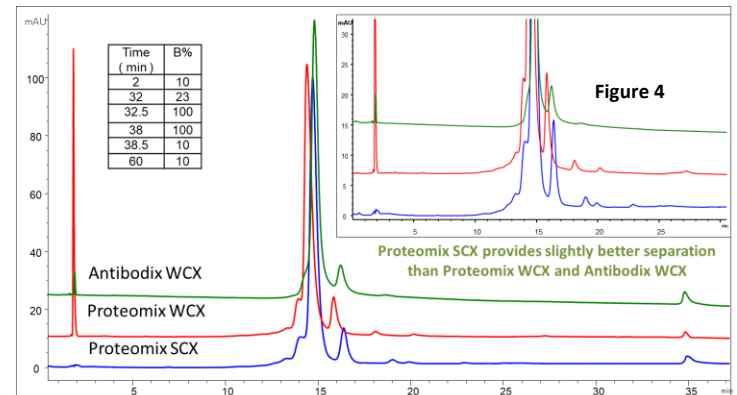
Column: Zenix SEC-300, 3 $\mu$ m, 300 A 7.8 x 300 mm (PN:213300-7830)  
Mobile phase: 150mM Sodium Phosphate Buffer, PH 7.0  
Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25 °C  
Injection volume: 3.0  $\mu$ L Sample: NIST MAb (10 mg/mL) (pI 9.18, in 12.5 mM histidine, pH 6.0)



### NIST MAb Separation on CEX

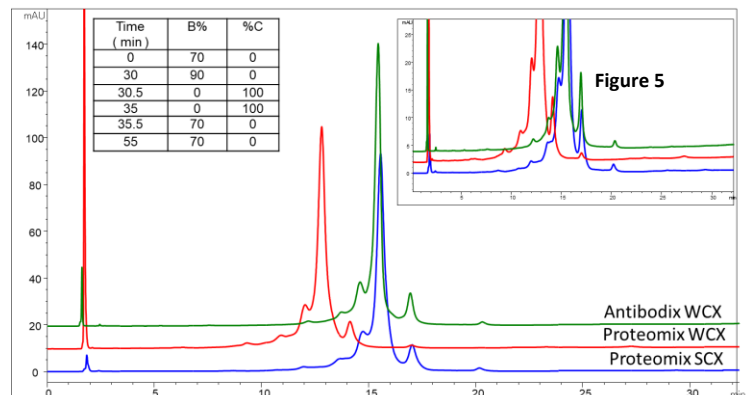
#### Three CEX Phases Screening – Salt Gradient

Column: Proteomix SCX, WCX and Antibodix WCX NP5  
(5  $\mu$ m, 4.6 x 250 mm PEEK) PN:CEXKIP-4625  
Mobile phase: A: 10 mM phosphate, pH 7.0, B: A+ 0.5 M NaCl;  
Flow rate: 1 mL/min, Detector: UV 280 nm, Column temperature: 25 °C,  
Samples: NIST MAb; Injection volume: 60  $\mu$ g (pI 9.18, in 12.5 mM histidine, pH 6.0)



#### Three CEX Phases Screening – pH Gradient

Column: Proteomix SCX, WCX & Antibodix WCX (5  $\mu$ m, 4.6 x 250 mm PEEK) (PN:CEXKIP-4625)  
Mobile phase A: 16 mM MES, 10 mM MOPS, 12 mM TAPS, 10 mM CAPSO, pH 5.6  
B: 10 mM MES, 12 mM MOPS, 14 mM TAPS, 16 mM CAPSO, pH 10.2 C: B+ 150 mM NaCl Flow rate: 1 mL/min, Detector: UV 280 nm, Column temperature: 25 °C,  
Samples: NIST mAb; Injection volume: 60  $\mu$ g (pI 9.18, in 12.5 mM histidine, pH 6.0)

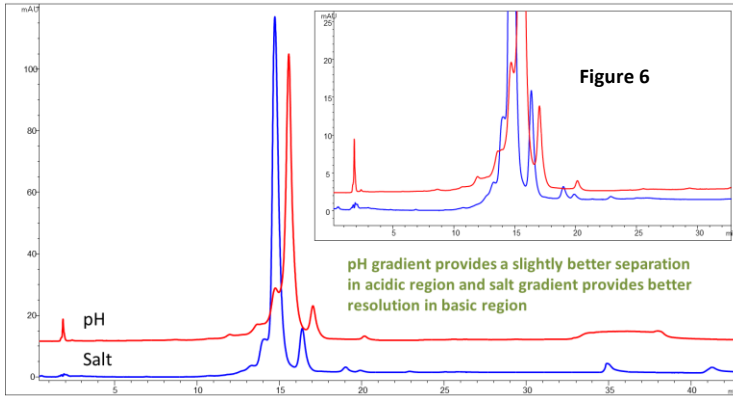




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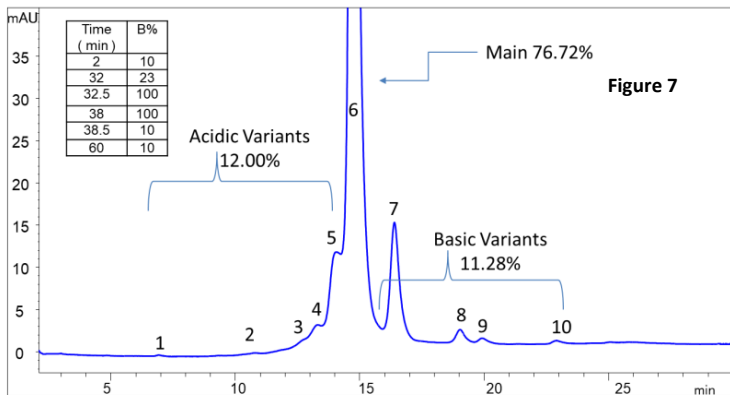
### Proteomix SCX - Salt vs. pH Gradient

Column: Proteomix SCX-NP5, 5  $\mu$ m, 4.6 x 250 mm PEEK (PN:401NP5P-4625)  
Flow rate: 1 mL/min, Detector: UV 280 nm, Column temperature: 25  $^{\circ}$ C,  
Samples: NIST MAb; Injection volume: 60  $\mu$ g (pI 9.18, in 12.5 mM histidine, pH 6.0)



### Proteomix SCX - Salt Gradient Zoom In

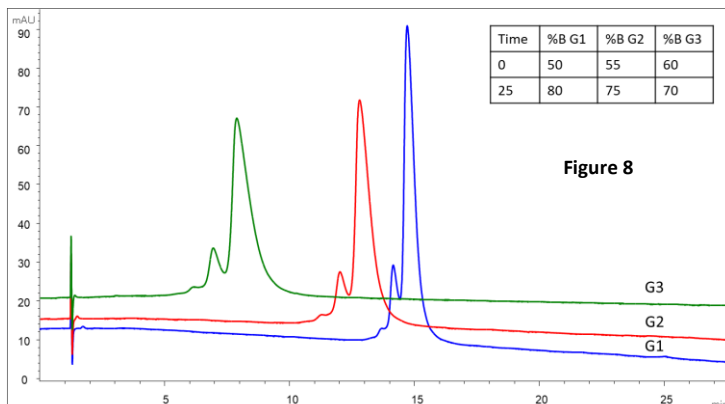
Column: Proteomix SCX-NP5, 5  $\mu$ m, 4.6 x 250 mm PEEK (PN:401NP5P-4625)  
Mobile phase: A: 10 mM phosphate, pH 7.0, B: A+ 0.5 M NaCl;  
Flow rate: 1 mL/min, Detector: UV 280 nm, Column temperature: 30  $^{\circ}$ C,  
Samples: NIST MAb; Injection volume: 60  $\mu$ g (pI 9.18, in 12.5 mM histidine, pH 6.0)



### NIST MAb Separation on HIC

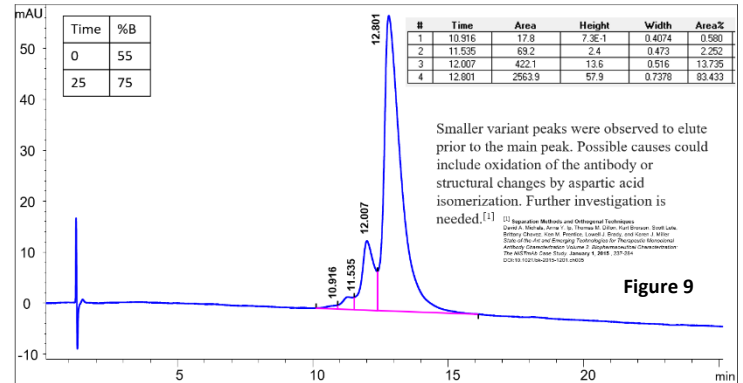
#### NIST MAb on Proteomix HIC Butyl-NP5 – Gradient Optimization

Column: Proteomix HIC Butyl-NP5, 5 $\mu$ m, NP 4.6 x 100 mm (PN:431NP5-4610)  
Mobile phase: A: 2 M Ammonium Sulfate, in 100 mM Sodium Phosphate pH 7.0,  
B: 100 mM Sodium Phosphate pH 7.0  
Flow rate: 0.5 mL/min; Detector: UV 280 nm; Column temperature: 30 $^{\circ}$ C  
Sample: NIST MAb 10 mg/mL, Injection volume: 2  $\mu$ L



### NIST MAb on Proteomix HIC Butyl-NP5 – Gradient 2 Zoom In

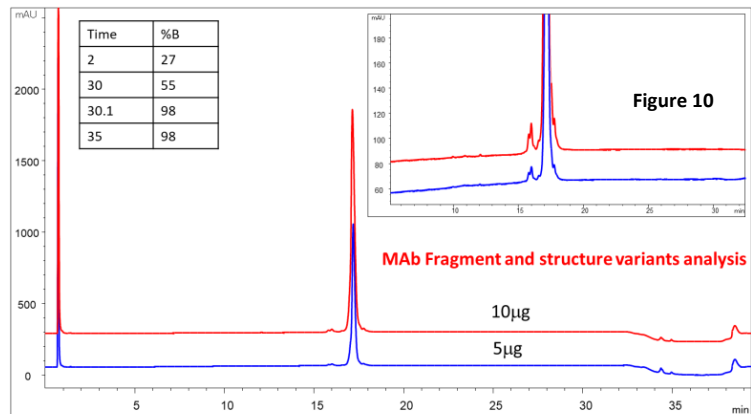
Column: Proteomix HIC Butyl-NP5, 5 $\mu$ m, NP 4.6 x 100 mm (PN:431NP5-4610)  
Mobile phase: A: 2 M Ammonium Sulfate, in 100 mM Sodium Phosphate pH 7.0,  
B: 100 mM Sodium Phosphate pH 7.0  
Flow rate: 0.5 mL/min; Detector: UV 280 nm; Column temperature: 30 $^{\circ}$ C  
Sample: NIST MAb 10 mg/mL (pI 9.18, in 12.5 mM histidine, pH 6.0), Injection volume: 2  $\mu$ L



### NIST MAb Separation on RP

#### NIST MAb on Proteomix RP-1000 2.1 x 100 mm

Column: Proteomix RP-1000, 5  $\mu$ m, 1000  $\text{Å}$ , 2.1 x 100mm (PN:465950-2110)  
Mobile phase A: Water + 0.1% TFA, ACN + 0.1% TFA  
Flow rate: 1.0 mL/min; Detector: UV 214nm; Column temperature: 80 $^{\circ}$ C  
Sample: NIST MAb 10 mg/mL, Injection volume: 0.5, 1  $\mu$ L



### NIST MAb on Proteomix RP-1000 2.1 x 100 mm Zoom In

