

Sepax HIC

HIC hydrophobic interaction separation

Polymer based HIC

Proteomix HIC Butyl-NP5, 4.6x35mm,
P/N: 431NP5-4603

Proteomix HIC Phenyl-NP5, 4.6x35mm,
P/N: 433NP5-4603

Proteomix HIC Ethyl-NP5, 4.6x35mm,
P/N: 432NP5-4603

Proteomix HIC Propyl-NP5, 4.6x35mm,
P/N: 434NP5-4603

**Proteomix HIC Kit, includes all 4 above
phases, P/N: HICKIT-4603**

Silica based HIC

BioHIC 100A, 3um, 4.6x100mm,
P/N: 313100-4610

BioHIC 300A, 3um, 4.6x100mm,
P/N: 313300-4610



Herceptin and its ADCs separation on Proteomix[®] HIC

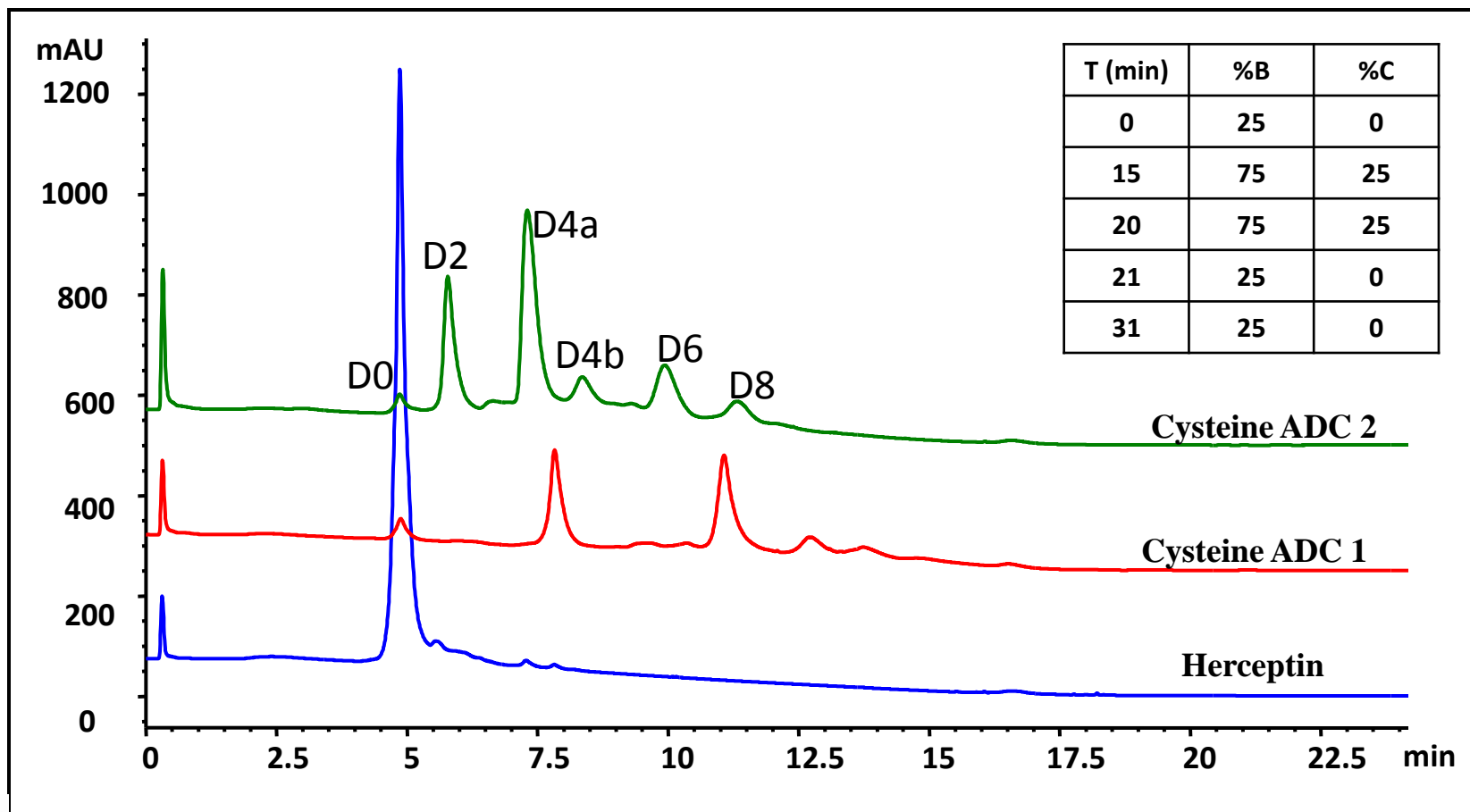
Column: Proteomix[®] HIC Butyl-NP5 (5 μ m, 4.6 x 35 mm)

Mobile phase: A: 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0,

B: 0.025 M sodium phosphate pH 7.0, C : 100% IPA

Flow rate: 0.8 mL/min, Detector: UV 214 nm, Column temperature: 25 $^{\circ}$ C

Sample: Herceptin/ADC1/ADC2, 1 mg/mL in 25 mM sodium phosphate, Injection: 10 μ L,



Cysteine ADC DAR analysis on Proteomix[®] HIC

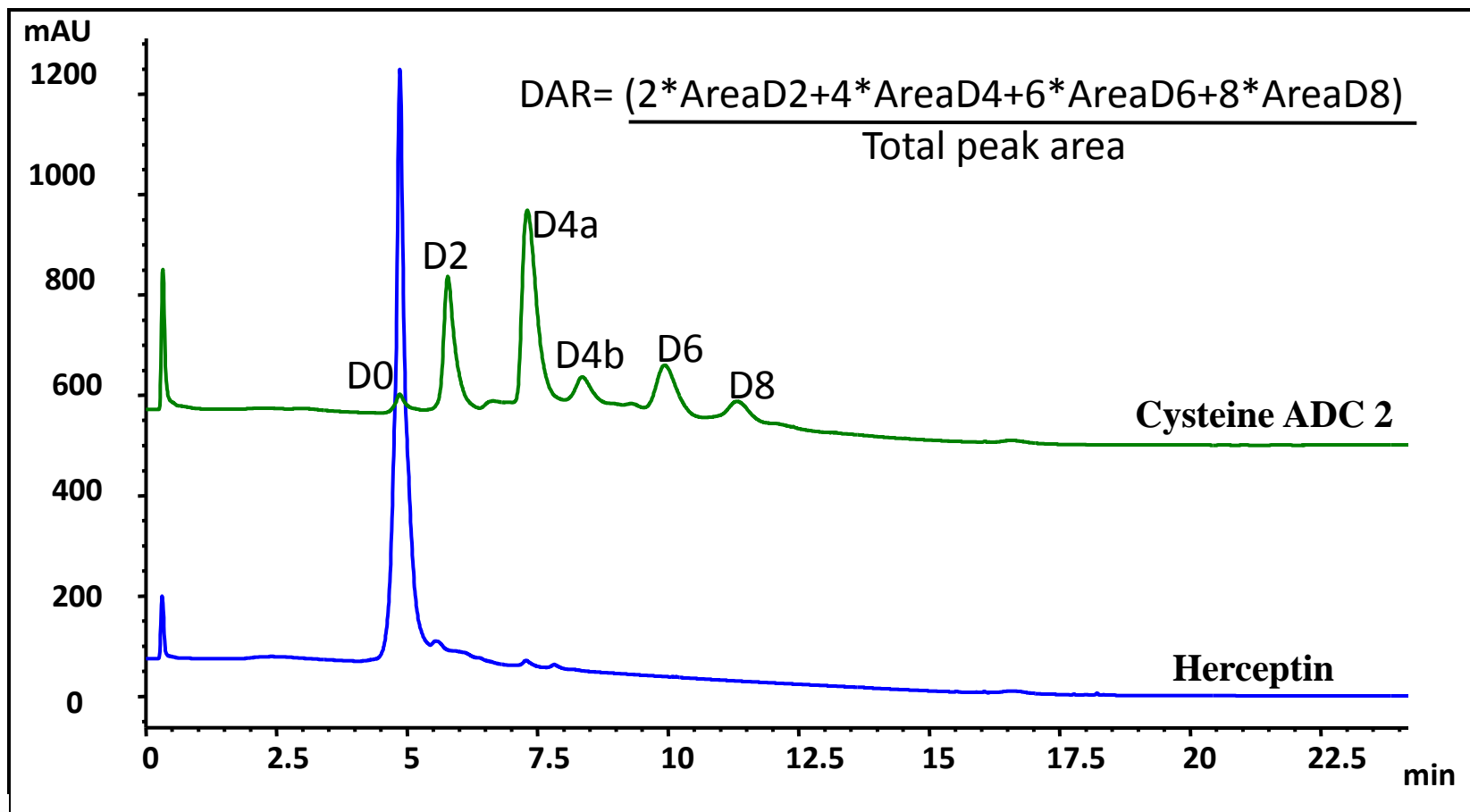
Column: Proteomix[®] HIC Butyl-NP5 (5 μm, 4.6 x 35 mm)

Mobile phase: A: 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0,

B: 0.025 M sodium phosphate pH 7.0, C : 100% IPA

Flow rate: 0.8 mL/min, Detector: UV 214 nm, Column temperature: 25 °C

Sample: Herceptin ADC2, 1 mg/mL in 25 mM sodium phosphate, Injection: 10 μL.



Sepax Proteomix[®] HIC Butyl-NP5 for Herceptin-cysteine ADC separation Three resin lot to lot consistency testing

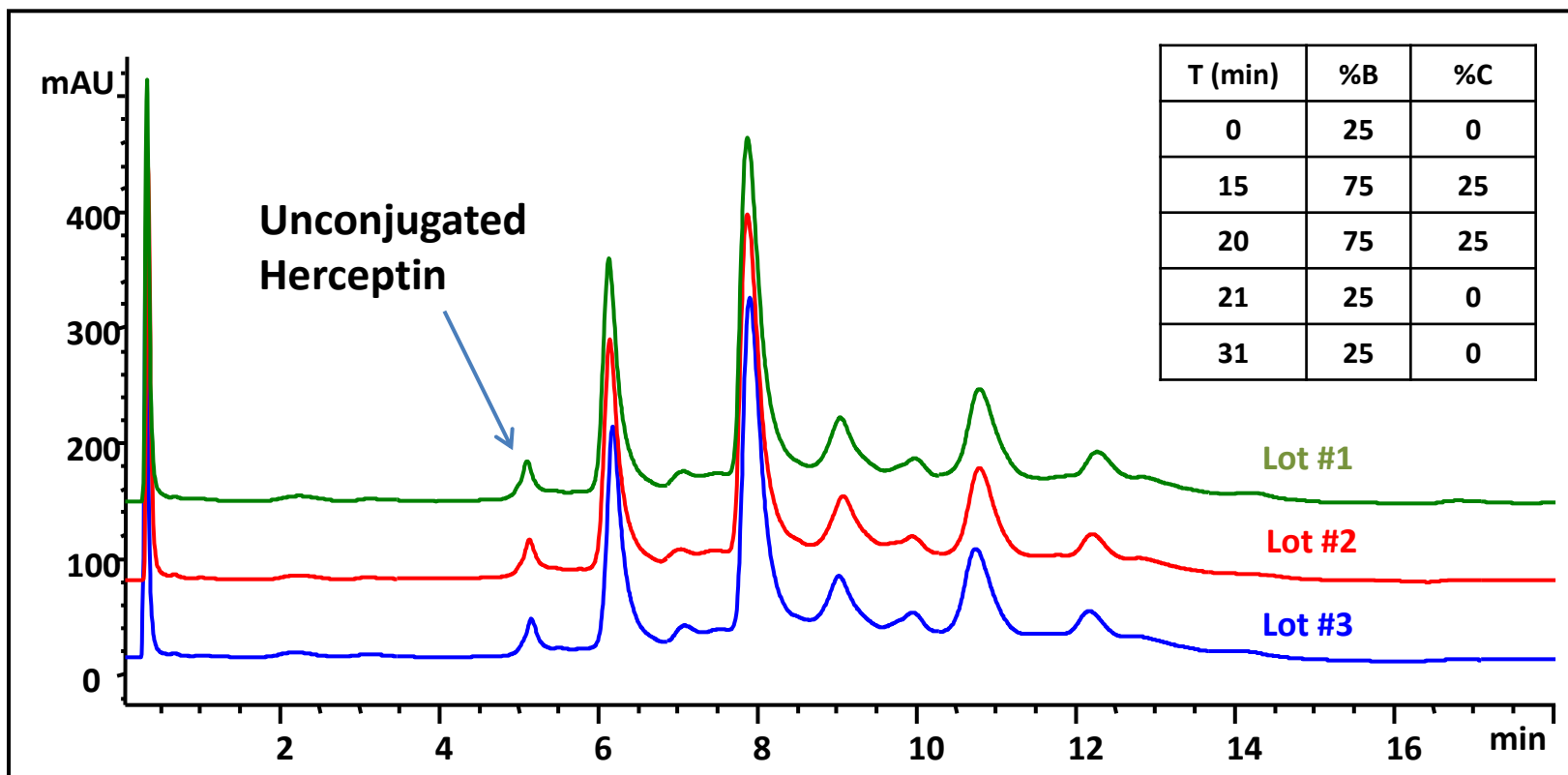
Column: Proteomix[®] HIC Butyl-NP5 (5 μ m, 4.6 x 35 mm);

Flow rate: 0.8 mL/min, Detector: UV 214 nm, Column temperature: 25 $^{\circ}$ C

Mobile phase: A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0,

B: 0.025 M sodium phosphate pH 7.0, C : 100% IPA ;

Sample: ADC, 1mg/mL in 1M ammonium sulfate; Injection: 10 μ L



Herceptin-cysteine ADC separation Sepax proteomix[®] HIC Butyl-NP5 vs. Vendor Y butyl-NPR

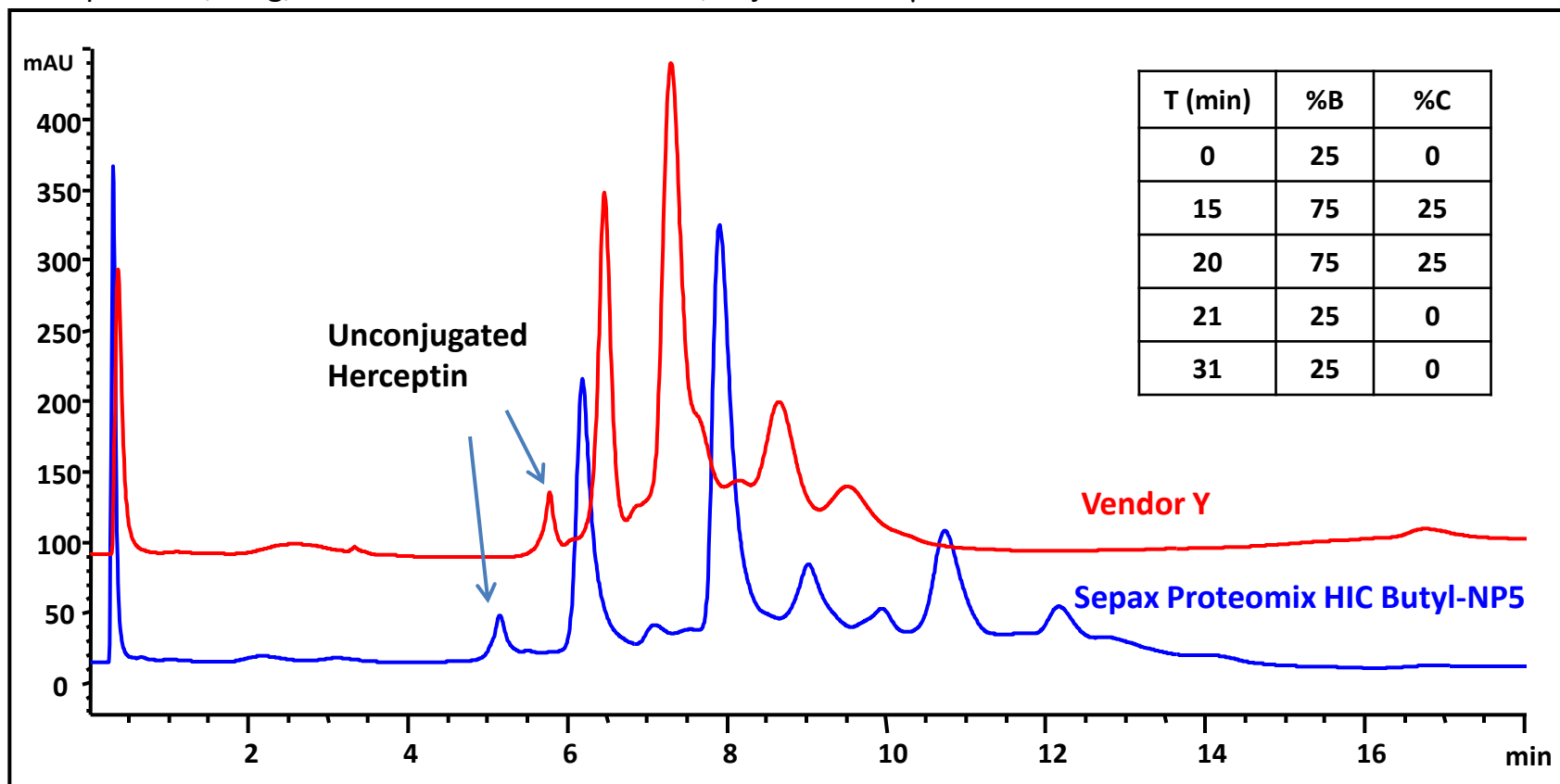
Column: **Proteomix[®] HIC Butyl-NP5 (5 mm, 4.6 x 35 mm)**

Flow rate: 0.8 mL/min, Detector: UV 214 nm, Column temperature: 25 °C

Mobile phase: A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0,

B: 0.025 M sodium phosphate pH 7.0, C : 100% IPA ;

Sample: ADC, 1mg/mL in 1M ammonium sulfate; Injection: 10 μ L



Protein Mixture on Different Proteomix[®] HIC Phases

Column: Sepax HIC Phenyl, Butyl, Propyl and Ethyl phases, NP, 5um, 4.6 x 35 mm,

Sample: Ovalbumin 1.0 mg/mL, Chymotrypsinogen 0.5 mg/mL,

Mobile phase: A: 2M ammonium sulfate in 0.1M sodium phosphate, pH 7.0,

B: 0.1M phosphate pH 7.0,

Flow rate: 0.4 mL/min, Detector: UV 214 nm, Column temperature: 25 °C, Injection 4 µL.

