HPLC Application

ID No.: **16198**

Jupiter® 300 C4 - PEGylation Reaction Time Course (Carbonic Anhydrase)

Products used in this application:

Column: Jupiter® 5 µm C4 300 Å, LC Column 150 x 4.6 mm, Ea
Dimensions: 150 x 4.6 mm ID
Order No: 00F-4167-E0
Elution Type: Gradient
Eluent A: 0.1% TFA in Water
Eluent B: 90% ACN 0.09% TFA in Water

Gradient Profile:

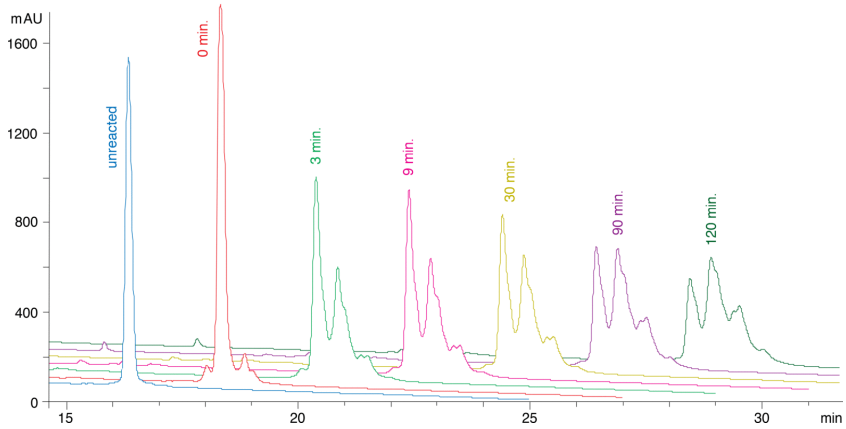
Step No.	Time (min)	Pct A	Pct B
1	0	80	20
2	25	45	55

Flow Rate: 1 mL/min
Col. Temp.: 45 °C
Detection: UV-Vis Abs.-Variable Wave.(UV) @ 214 nm (ambient)

Analyst Note: Application Focus: To demonstrate the utility of using reversed phase chromatography with Jupiter 300 media for purifying PEGylated proteins.

The addition of PEG groups to a protein complicates both the characterization and purification of such PEG/protein conjugates away from the "non-PEGylated" protein species. Protein PEGylation was performed using Methyl-PEO12-NHS Ester. A protein solution in PBS (pH 7.4) was reacted In general, the PEGylation reaction concurrently occurs rapidly at several different protein sites in a fixed ratio. As the reaction continues, more heavily PEGylated (and later eluting) forms were observed. In every protein tested there was always more than one PEGylated protein peak

16198



ANALYTES:

1 PEGylated Carbonic anhydrase

For more information contact your Phenomenex Representative at info@phenomenex.com

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