

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

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

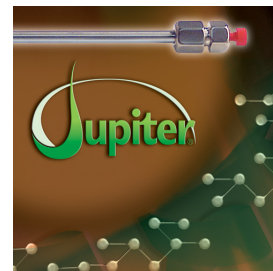
Flow Rate: 1 mL/min

Col. Temp.: 45 °C

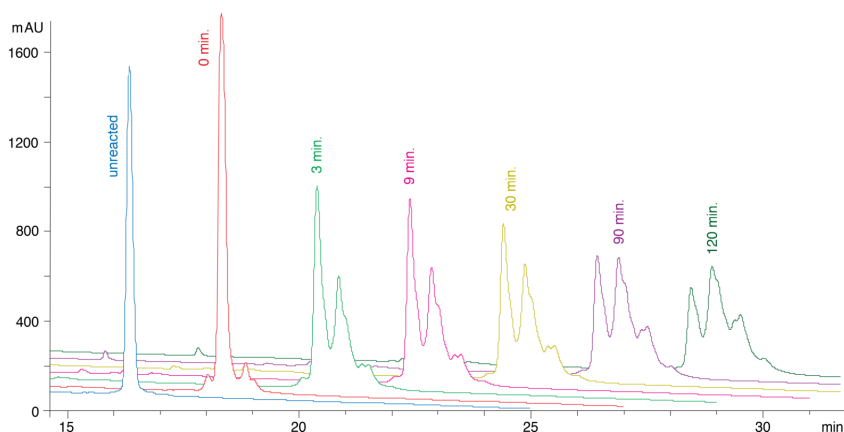
Detection: Refractive Index @ 0.0000000000 (ambient)

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

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



Products used in this application:



ANALYTES:

- 1 PEGylated Carbonic anhydrase

