

HPLC Application

ID No.: 17514

PEGylated alpha-Chymotrypsinogen A on Jupiter® 300 C18 3µm, reaction at different time-points

Column: Jupiter® 3 µm C18 300 Å, LC Column 150 x 4.6 mm, Ea

Dimensions: 150 x 4.6 mm ID

Order No: 00F-4263-E0

Elution Type: Gradient

Eluent A: 0.1% TFA, 2% ACN in Water

Eluent B: 90% ACN, 0.085% TFA in Water

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	80	20
	2	25	35	65

Flow Rate: 1 mL/min

Col. Temp.: 45 °C

Detection: UV-Vis Abs.-Variable Wave.(UV) @ 214 nm (25 °C)

Analyst Note: Application Focus: Using larger (20KDa) PEGylation reagents and seeing better performance with Jupiter 300 3u C18.

In other studies proteins were modified with relatively small polyethylene glycol moieties (PEG) which may not be applicable to all pharmaceutical applications. In this application chymotrypsinogen was PEGylated using two different 20 KDa PEGylation reagents that are commonly used in

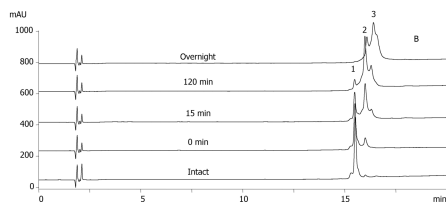
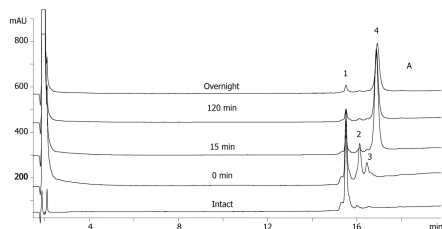
polymerization, RP chromatography can also separate PEGylated species based on site of PEG attachment. Time course results for App ID 17514 shows multiple PEG/protein conjugates with unique retention by RP-HPLC dictated by both the site and degree of modification. Although other



Products used in this application:



17514



ANALYTES:

- 1 PEGylated alpha-Chymotrypsinogen A

