



Column Name: FLARE WP C18

Column Dimensions: 2.1 x 100 mm

HPLC System: Acquity I-Class UPLC system

Injection Volume: 0.5 μ l

Detection: FLD (280 - 360 nm)

Flow Rate: 0.15 ml/min

Mobile Phase: A: 0.5% TFA in H₂O, B: 0.5% TFA in ACN

Gradient: Time (mins)	%A	%B
0.00	75	25
10.00	67	33
10.20	75	25
12.00	75	25 end

Temperature: 90°C

Analyte: Intact Rituximab (MW= 144 kDa)

Sample Preparation:

- Intact mAb aqueous solutions were prepared to a concentration of 2 mg/ml.
- Reduction of mAbs were performed by addition of 0.05 mg of dithiotreitol (DTT) to a 100 μ l solution of mAb stock solution. The solution was incubated at 40°C for 60 minutes.
- Digestion was done by addition of 50 μ l of papain (1.0 mg/ml) to the reduced sample. The solution was incubated at 40°C for 5 hours.

Sample Characterization:

-Rituximab: The reduction and digestion processes yielded three main fragments of ca. 25 KDa, namely the native LC, the single-chain Fc (sFc) and the Fab portion of the HC (Fd)