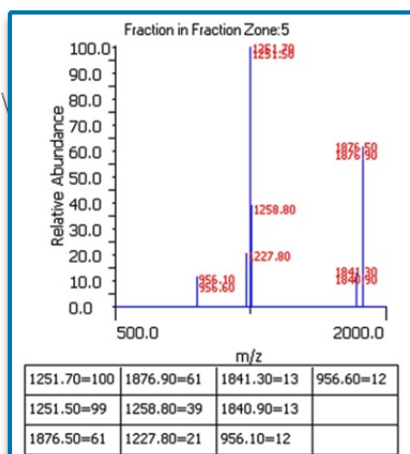
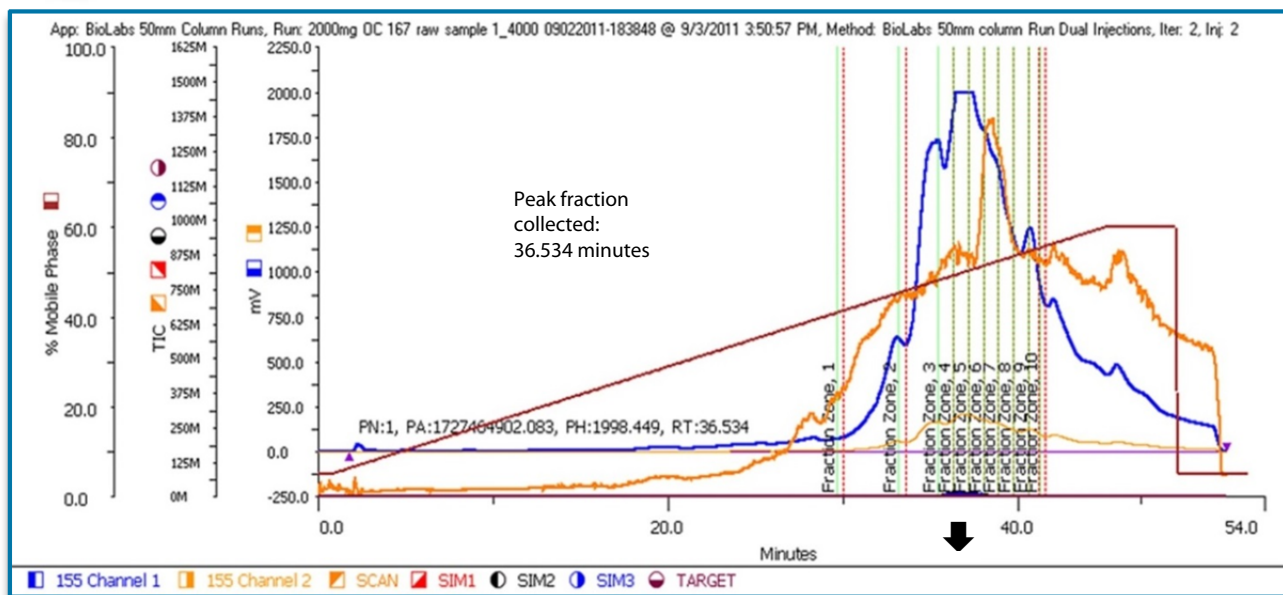




Preparative Purification of a Synthesized Peptide using an automated LC/MS purification system



The Gilson automated liquid handling GX-281 LC/MS Purification System was used to purify a synthesized peptide by monitoring three target masses. The C18 column was loaded with 2000 mg of synthesized peptide (average molecular weight of 3751.3 Da and the most abundant isotopic mass totaling 3749.0 Da). Using the conditional logic fraction collection, and UV and SIM settings in the TRILUTION® LC software, the purified protein was effectively collected at 36.534 minutes. The presence of one or more target masses was also confirmed in fraction zones 3–8 out of 1–10. Pictured to the left are two target masses contained within fraction zone 5.

Conditions

Sample: 2000 mg of synthesized peptide with residues in 30 mL water and 3 mL 25% ammonia in water

Average molecular weight: 3751.3 Da

HPLC column: Vydac (Grace) monomeric, 50 x 50 mm C18

Mobile phase:

- A: 0.1% trifluoroacetic acid in water
- B: 90% acetonitrile
10% of 0.1% trifluoroacetic acid in water

Make-up solvent: 0.1% formic acid in methanol

Wavelength: 215 and 280 nm

SIM (Single Ion Monitoring) channels:

- M4H + (938.8)
- M3H + (1251.3)
- M2H + (1876.5)

Other prominent ions in spectra :

- M3Na1H++++ (956.6)
- M1Na2H+++ (1258.8)

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