

Quantitative UV HPLC Analysis of Trifluoroacetic Acid in Peptide/Protein on Amaze HA Column

Equipment

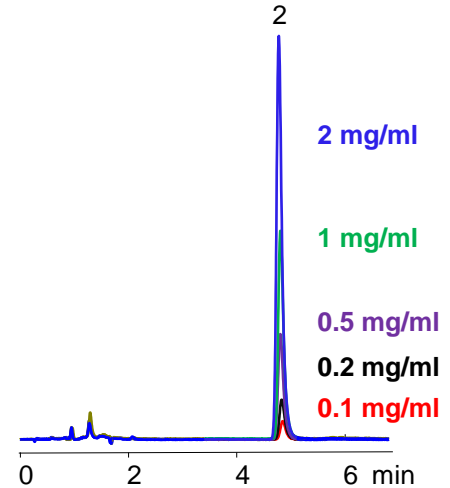
Agilent 1100 HPLC system with DAD
 Analytical Balance
 HPLC Column: Amaze HA, 3.0x100 mm, 3 um, 100A

Materials:

Peptide/protein NS3278
 Trifluoroacetic acid (Aldrich, catalog #74564)
 ACN (Tedia, catalog #AS1127)
 Distilled water (Aldrich, catalog #270733)
 Sulfuric acid (Aldrich, catalog #258105)

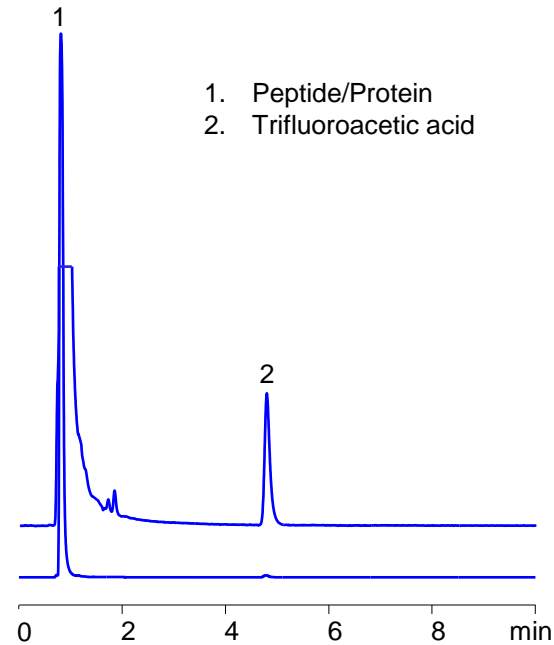
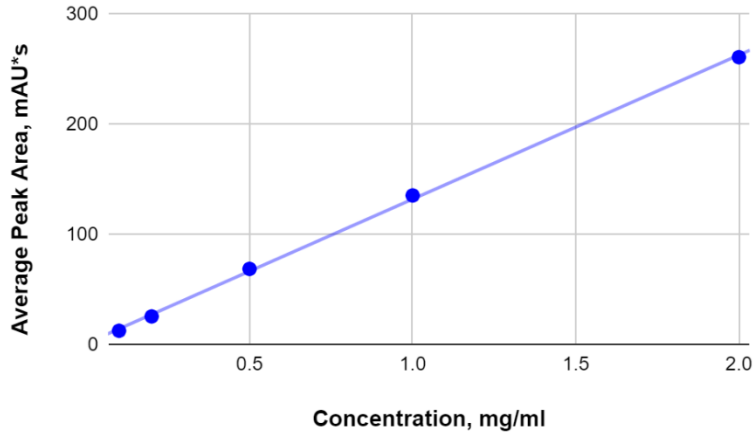
Method Description

HPLC Q-Pump set up: **A:** DI water, **B:** Acetonitrile, **C:** 1% H₂SO₄
 Mobile phase: 60% ACN with 0.1% H₂SO₄
 Mobile phase composition: : **A:** 30%, **B:** 60%, **C:** 10%



TFA Calibration and Sample Analysis

Calibration Plot for Trifluoroacetic Acid



| Concentration, mg/ml | Average Peak Area, mAU*s | Concentration of TFA, mg/ml |
|------------------------|--------------------------|-----------------------------|
| 0.1 | 12.9 | - |
| 0.2 | 25.9 | - |
| 0.5 | 69 | - |
| 1 | 135.6 | - |
| 2 | 261 | - |
| Peptide/Protein | 41.8 | 0.31 |

Column: Amaze HA
Dimensions: 3.0 x 100 mm, 3 um, 100A
Mobile phase: 60% ACN with 0.1% H₂SO₄
Flow: 0.5 ml/min
Detection: 215 nm
Sample: Standards and sample