

Sample A was analyzed with online LC-MS using a 1 mm polymer column with a 0.08 mL/min flow rate. The sample ran through a split flow on an Advion Triversa with 0.3 microliters/min being introduced to the LTQ-FT mass spectrometer, and the remaining solvent fraction collected in 96 well plate for later offline analysis. For FT detection, we employed a strategy of ion trap marching from 800-1200 m/z with 60 m/z isolation windows. MS/MS was data dependent. Fragmentation was induced in the ion trap with CID at 41 V of collision energy, 0.5 activation Q, and 30 ms activation time. The fragments were detected in the FT. One ion trap full scan was performed to detect any proteins outside the FT isolation scans. In this approach no protein was detected in the online analysis. An attempt to lyophilize the plate and resuspend in a 49/ 49/ 1 percent methanol/ water/ formic acid yielded no additional proteins.

Based off the results from Sample A, Sample B was loaded on a 8 cm, 75 micron c4 nano column and attached to a Triversa LC-coupler. While this prevents sample collection and therefore limits us to a set number of scans per protein's elution time, the increase in sensitivity was deemed necessary. The detection and fragmentation parameters were the same as above. With this setup 2 proteins, 11.1 and 12.5 kDa, were detected in the FT and the ion trap, while 1 protein, 24 kDa, was detected in the ion trap only. The 24 kDa protein had a charge state distribution above 1200 m/z and therefore was outside our FT windows and not automatically targeted for fragmentation. Of the two proteins that were targeted, fragmentation was sparse yielding no more than 8 fragments per protein.