

093041

I first dissolved the samples in 5 uL of 50/50 ACN/0.1%TFA, waited 10 min, vortexed, then added 45 uL of 50 mM ammonium bicarbonate solution as a buffer. I added 0.25 ug of trypsin to A, 0.5 ug to B, then digested in a microwave digester (~10 min at 50 degrees). After digestion, I added 2 uL of neat TFA to each before separating on a C18 column (Magic C18, 5u, 200A, 0.15 x 150 mm).

The separation was done on a Michrom Paradigm (5-65%B in 35 min @ 1.0 uL/min; A=5%ACN, B=95% ACN). About 40 spots were collected onto an ABI 4700 sample plate with a ProBot. After separation, the samples were automatically mixed with matrix (alpha-CN) via a tee just before they were deposited on the sample plate. I ran the MS of the fractions by hand, then did MS/MS and de novo interpretation of the MS/MS spectra that were good enough to do. Calibrations were all default, except MS spectra were calibrated internally as needed.