Supplemental Materials and Methods

Materials. A commercial digest of six bovine proteins (PN# PTD/00001/63 Michrom Bioresources, Inc., Auburn, CA) was used as a QC peptide standard in stability experiments. The six proteins making up the sample were beta lactoglobulin, lactoperoxidase, carbonic anhydrase, glutamate dehydrogenase, alpha casein and serum albumin. The standard digest was obtained as lyophilized powder and resuspended in 100 µl of HPLC grade H_2O w/ 3% acetonitrile and 0.1% formic acid at a concentration of 1 pmol/µl (as described in the manufacturer's instructions). The solution was then aliquoted into 1.7ml polypropylene tubes (Genesee Scientific, San Diego, CA) and stored at -80 ^{o}C before use. Three types of sample vials were used in the stability study including non-deactivated glass (P/N 186000384c, Waters, Milford, MA), deactivated glass (P/N 186000385DV, Waters, Milford, MA), and polypropylene vials (P/N 186002626, Waters, Milford, MA).

LC-MRM Analysis. For characterization of peptide stability, QC standard samples were analyzed by LC-MS using a nano-liquid chromatography system (NanoAcquity, Waters, Milford, MA) coupled to a triple quadrupole mass spectrometer (Xevo TQ, Waters, Milford, MA). The system was equipped with a nanoACQUITY UPLC BEH analytical column (Waters, C18, particle size 1.7 µm, pore size 130 Å, ID 100 µm, length 100 mm) and a nanoACQUITY UPLC Symmetry trap column (Waters, C18, particle size 5 µm, pore size 100Å, ID 180 µm, length 20 mm). Mobile phase A was 0.1% formic acid (v/v) in water, mobile phase B was 90% acetonitrile/0.1% formic acid (v/v) in water. Peptides were eluted using the following gradients. In the experiments to

evaluate storage stability, peptides were trapped for 6 min with 99% mobile phase A, followed by 7 min at 5% B, 4min gradient to 10%B, 22 min gradient to 30%B, 4 min gradient to 40%B, 1 min gradient to 90%B, 4 min hold at 90%B, 11 min re-equilibration at 5%B. In the experiments to evaluate freeze-thaw stability, peptides were trapped for 2 min with 99%A, followed by 3 min at 3%B, 2 min gradient to 10%B, 11 min gradient to 30%B, 10 min gradient to 40%B, 1 min gradient to 90%B, 7 min re-equilibration at 3%B. Instrument settings included a capillary voltage of 2.8 kV, a cone voltage of 35 V, an ion source temperature of 150 °C, a cone gas flow of 15 L/Hr, and a nanoflow gas flow of 0.20 bar. Autosampler temperature was maintained at 10 °C.

Data analysis. Skyline software [(1)] was used for building unscheduled MRM methods to monitor 50 peptides in the QC standard. 159 total transitions were monitored, corresponding to an average of 3 transitions for each peptide precursor ion. Collision energy (CE) was determined based on the linear equation CE=0.034*m/z + 3.314. The peptides were selected based on their detectability and ranged from 6 to 21 amino acids in length and 0.93-44.87 in relative hydrophobicity [(2)]. The total peak area (summed from all interference-free transitions for each precursor ion) was obtained using Skyline exports and was used for experiment calculations.

References

1. MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, Kern R, Tabb DL, Liebler DC, MacCoss MJ. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics. 2010;26(7):966-8. doi: 10.1093/bioinformatics/btq054. PubMed PMID: 20147306; PubMed Central PMCID: PMC2844992.

2. Krokhin OV. Sequence-specific retention calculator. Algorithm for peptide retention prediction in ion-pair RP-HPLC: application to 300- and 100-A pore size C18 sorbents. Analytical chemistry. 2006;78(22):7785-95. doi: 10.1021/ac060777w. PubMed PMID: 17105172.