An On-Line, High-Pressure Digestion System for Protein Characterization by Hydrogen Deuterium Exchange

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Supplementary material

EXPERIMENTAL SECTION

Digestion Apparatus. The apparatus was plumbed with silica tubing (O.D. 360 µm, I.D. 150 µm), and a 20 µL sample loop was used for protein injection. For digestion, a constant flow of solvent A (99.9% H_2O, 0.1% formic acid) was kept through the system. An 8 min gradient from 0-100% solvent B (99.9% acetonitrile, 0.1% formic acid) with a flow rate of 40 µL/min was run prior to MS analysis.

Back Exchange Correction. To account for back exchange during analysis, the deuterium content was calculated from:

$$D_{\mathrm{H}\rightarrow\mathrm{D}} = \frac{m - m_{0\%}}{m_{100\%} - m_{0\%}} \times N$$

where, m, $m_{0\%}$, and $m_{100\%}$ are the centroids for each peptide peak after a specified HDX period, zero-time control, and the 100% control. The 100% exchange control was the result for 48 hr exchange. The average back exchange for both the high and low pressure digestion was ~20%.