

Supplementary Material

**Optimizing Proteolytic Digestion Conditions for the Analysis
of Serum Albumin Adducts of
2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, a
Potential Human Carcinogen Formed in Cooked Meat**

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Figure S-1. ESI/MS product ion spectra of (A) LQQC*PF (C-[SO₂]-PhIP sulfonamide [M+H]⁺ at *m/z* 989.5), (B) QQC*PF (C-[SO₂]-PhIP sulfonamide [M+H]⁺ at *m/z* 876.3), and (C) QC*PF (C-[SO₂]-PhIP sulfonamide [M+H]⁺ at *m/z* 748.2).

Figure S-2. Reconstructed ion chromatograms of Cys³⁴ peptide adducts recovered from *m*-CPBA oxidized HONH-PhIP-modified SA digests, following (A) Pronase E digestion at a protease:SA ratio of 1:2; (B) Pronase E digestion at a protease:SA ratio of 1:10; and (C) Pronase E/leucine aminopeptidase/prolidase digestion.

Figure S-3. UPLC-ESI/MS² chromatograms of (A) 24 fmol of LQQCPFEDHVK ([M+3H]³⁺ at *m/z* 488.6); (B) 24 fmol of LQQCPF ([M+H]⁺ at *m/z* 735.3); (C) 15 fmol of LQQC*PFEDHVK (C-[SO₂]-PhIP sulfonamide ([M+3H]³⁺ at *m/z* 533.7); and (D) 15 fmol of LQQC*PF (C-[SO₂]-PhIP sulfonamide ([M+H]⁺ at *m/z* 989.5).

Figure S-4. UPLC-ESI/MS² chromatograms of LQQC*PFEDHVK and LQQC*PF adducts recovered from trypsin/chymotrypsin digestions of 225 fmol IAA alkylated SA, 225 fmol *m*-CPBA oxidized SA and 450 fmol *m*-CPBA oxidized HONH-PhIP-modified SA digests, without or with denaturation of SA.

Figure S-5. Reconstructed ion chromatograms of LQQC*PFEDHVK (C-[SO₂]-PhIP sulfonamide and LQQC*PF (C-[SO₂]-PhIP) sulfonamide recovered from 1.5 pmol of *m*-CPBA oxidized HONH-PhIP-modified SA digests by (A) sequence grade trypsin and

chymotrypsin from Promega and (B) biochemical grade enzymes from Sigma.

Figure S-1.

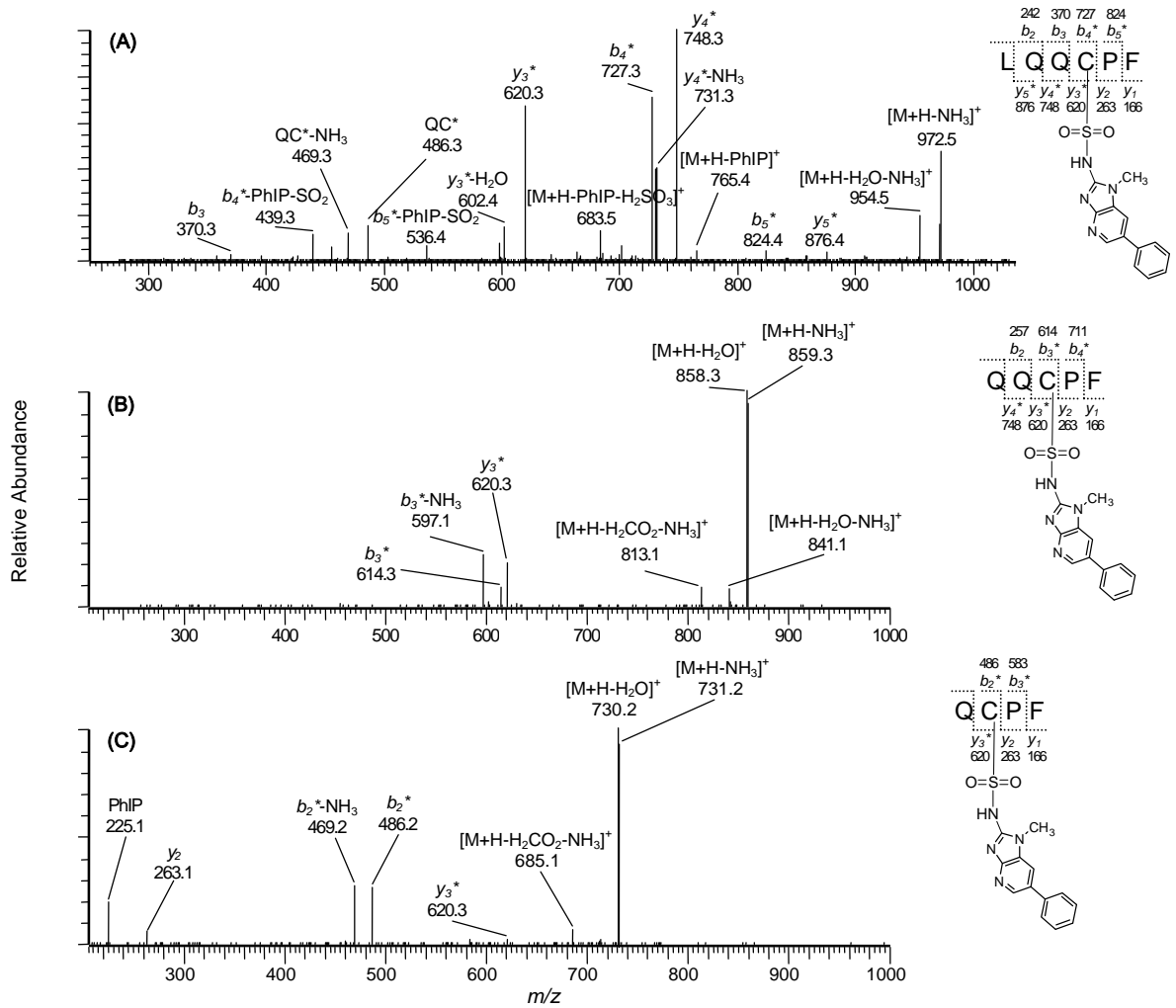


Figure S-2.

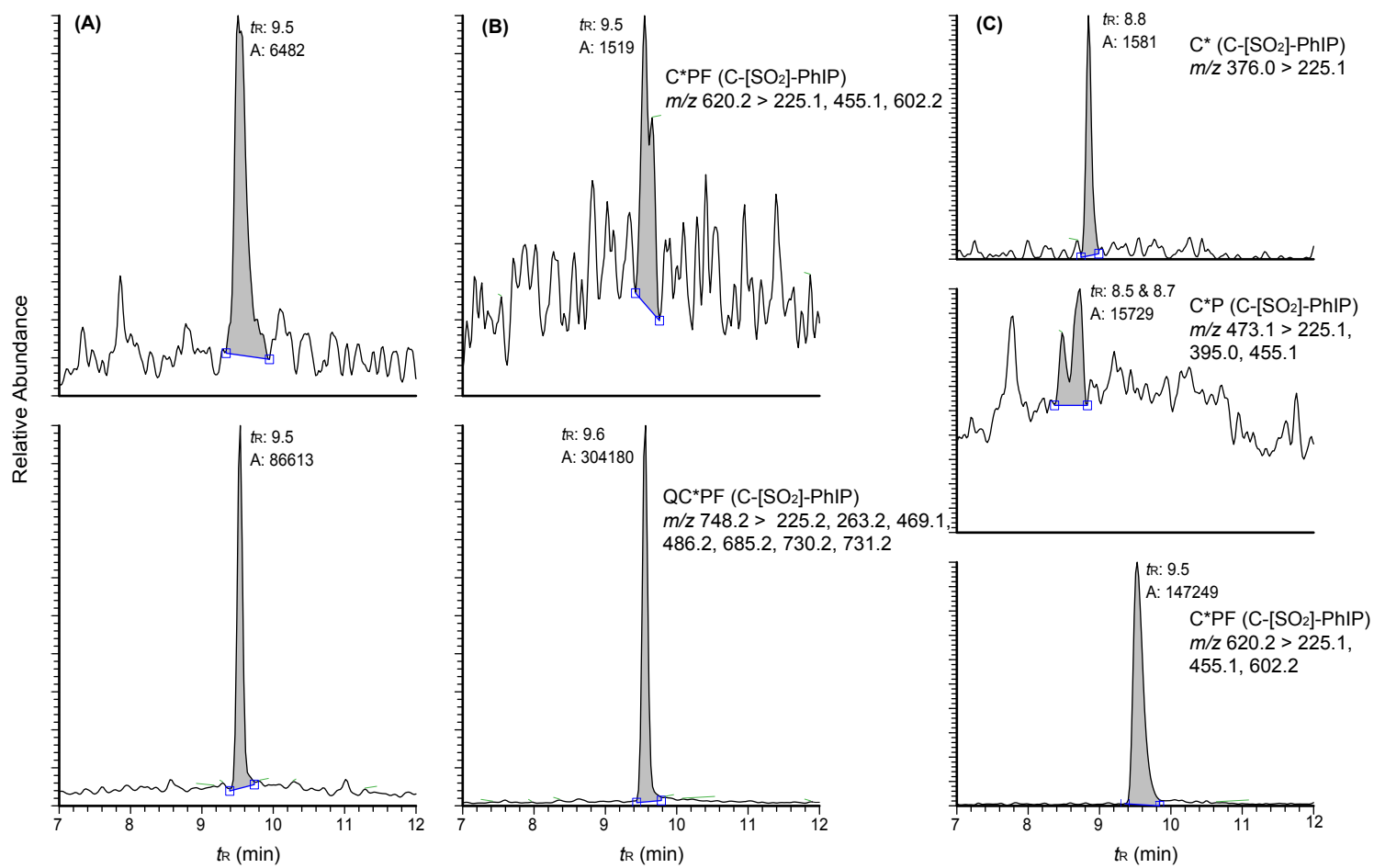


Figure S-3.

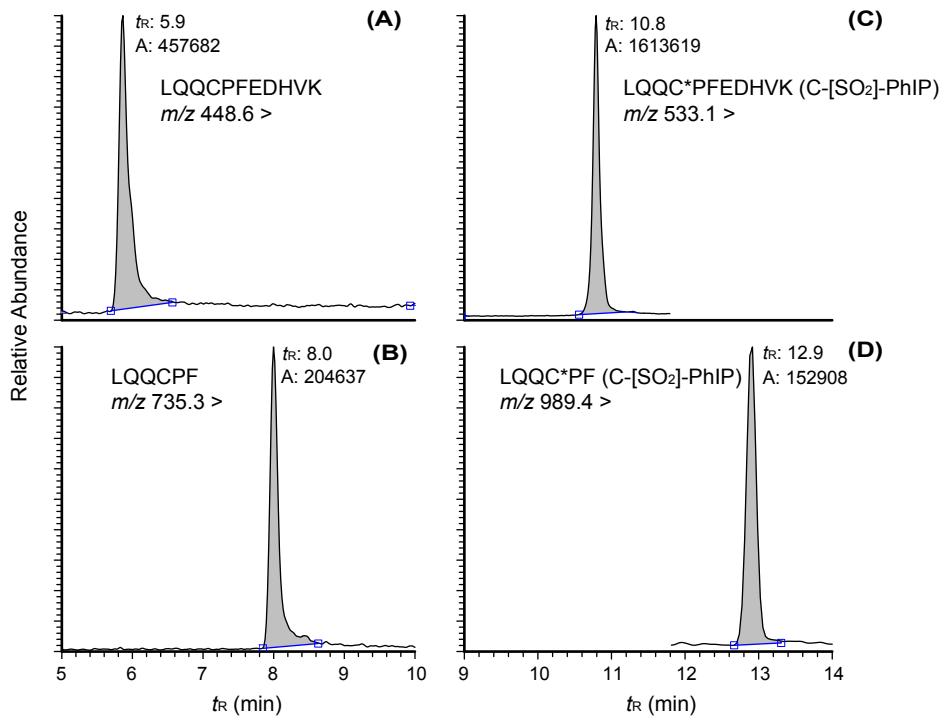


Figure S-4.

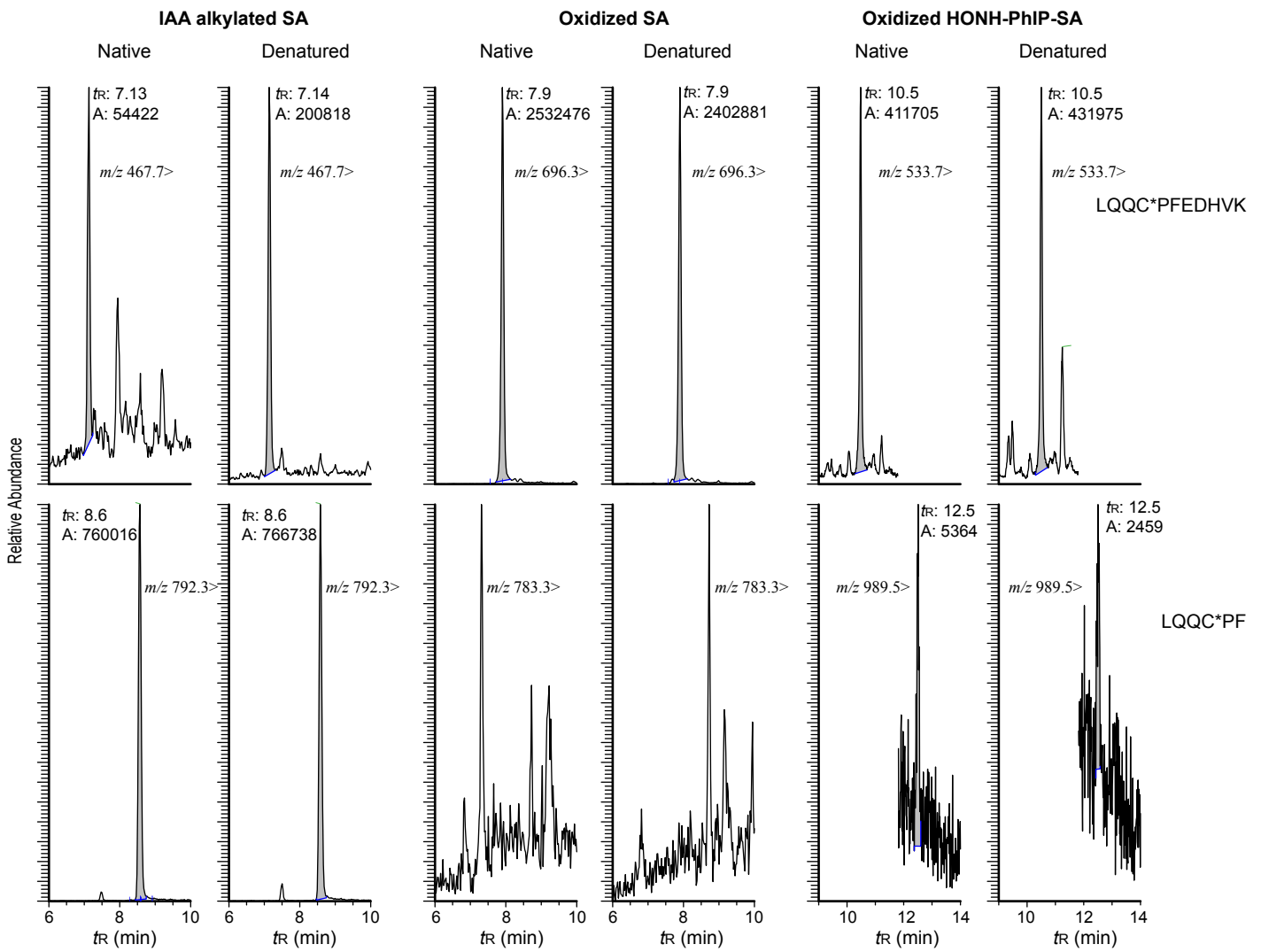


Figure S-5.

