

Supplementary Material

Qualitative and quantitative evaluation of derivatization reagents for different types of protein-bound carbonyl groups.

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Supporting Information

Fig S1 – Chemical structures of carbonylated peptides used in the current study. **Fig S2** – Chemical structures of derivatization reagents DNPH, BHZ and ARP and typical products with their increment masses. **Fig S3, S4, and S5** - MALDI-TOF mass spectra of aldehyde- and ketone- and lactam-containing peptides derivatized with DNPH, BHZ and ARP. **Fig S6, S7** - ESI-LTQ-tandem mass spectra of ketone-containing peptide derivatized with DNPH, BHZ and ARP and aldehyde-containing peptide derivatized with BHZ, DNPH and reduced with NaCNBH₃. **Fig S8** - Recovery rates of aldehyde- and ketone-containing peptides after incubating them with and without BSA. **Fig S9** - MALDI-TOF mass spectra of a mixture of the ketone- and aldehyde-containing peptides after reduction, alkylation and trypsin digestion.

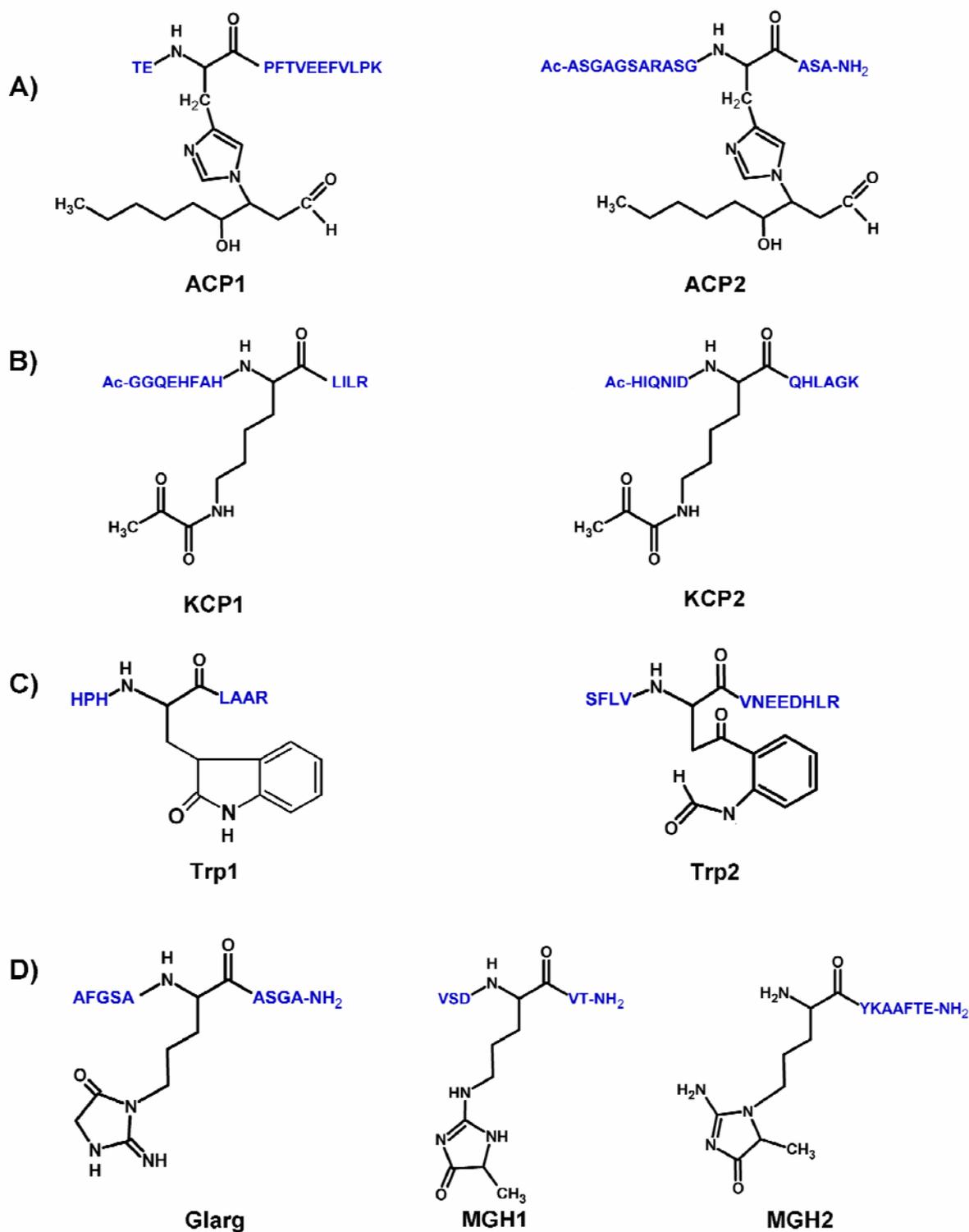


Figure S1: Chemical structures of carbonylated peptides used in this study. ACP1, ACP2- hydroxynonenal adduct on histidine residues. KCP1, KCP2- pyruvic acid coupled to the ϵ -amino group of lysine residue. Trp1, Trp2- carbonyl containing tryptophan oxidation products oxindolylalanine and N-formylkynurenine. Glarg- glyoxal-derived oxoimidazolidine, MGH1, MGH2- methylglyoxal-derived hydro-imidazolone.

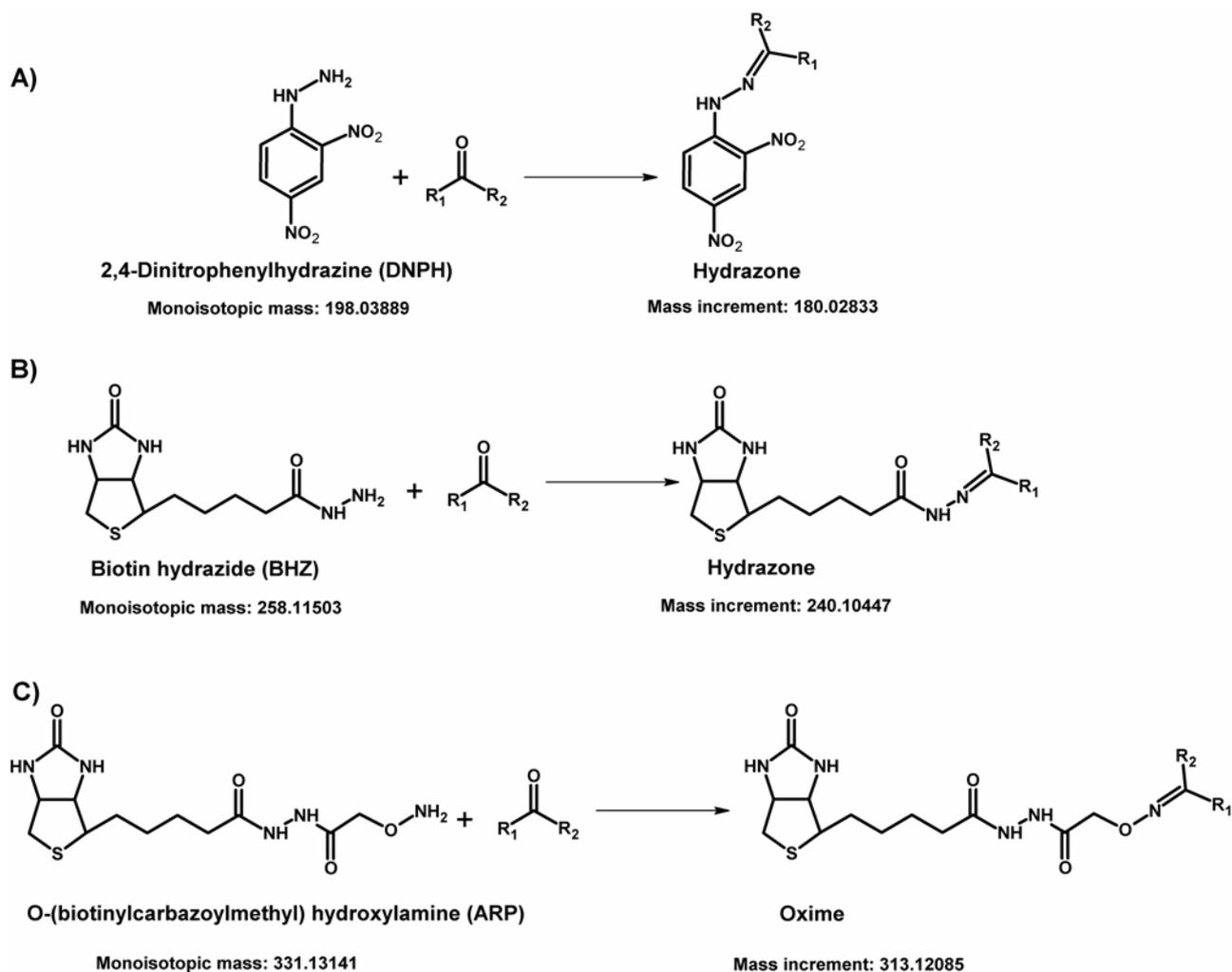


Figure S2: Chemical structures of all derivatization reagents used in this study and the expected products with their mass increments. DNPH and BHZ, the hydrazine and hydrazide based reagents, react with the carbonyl groups yielding the corresponding hydrazones. ARP, a hydroxyl amine derivative, reacts to the corresponding oximes.

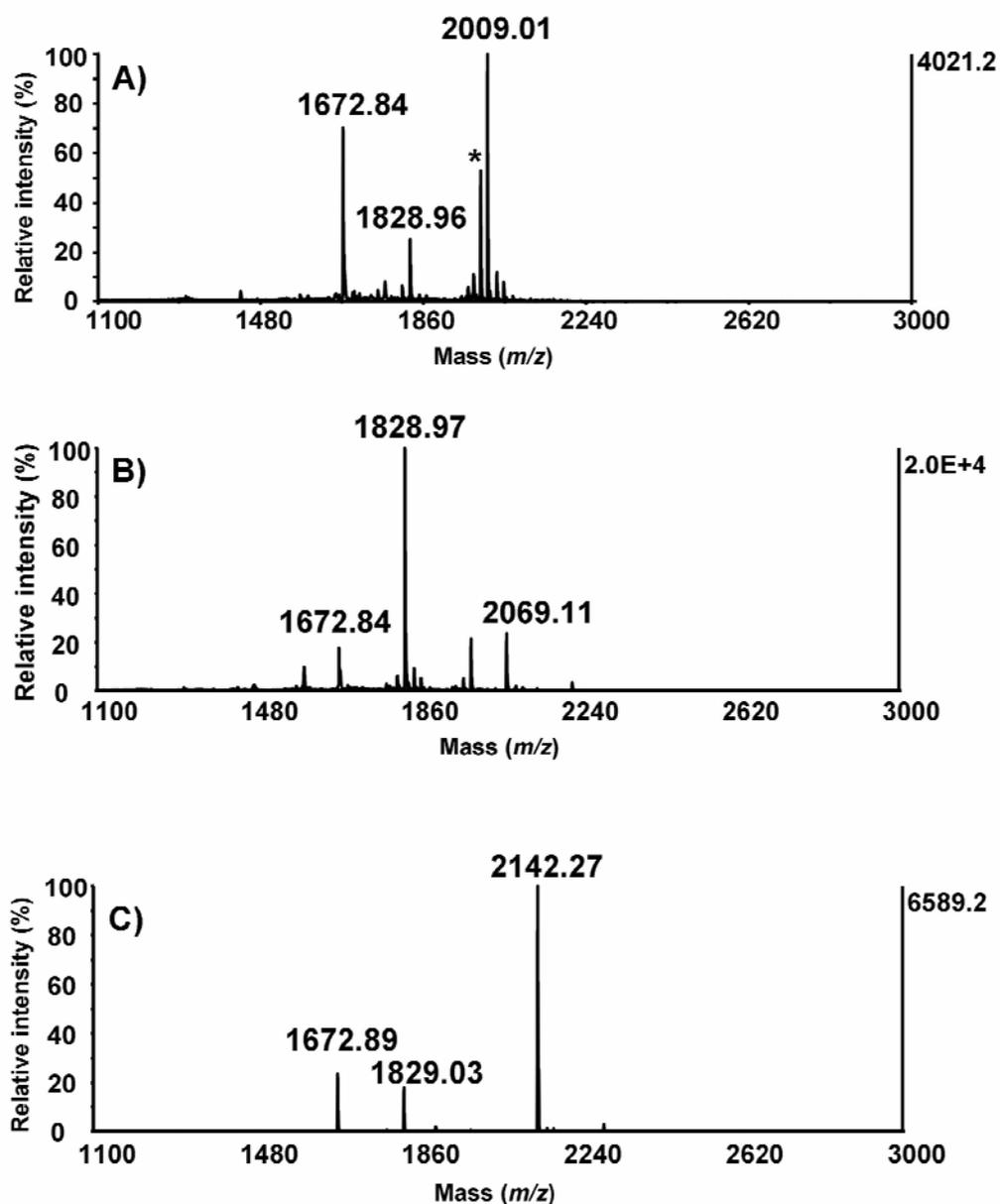


Figure S3: MALDI-TOF mass spectra of the aldehyde-containing peptide TEHPFTVEEFVLPK (ACPI, m/z 1828.97) derivatized with DNPH (A, m/z 2009.01), BHZ (B, m/z 2069.11), and ARP (C, m/z 2142.27). The peak at m/z 1672.84 corresponds to the original peptide without HNE. * indicates the peak at -16 Da, probably due to the loss of oxygen atom from one of the nitro groups in DNPH.

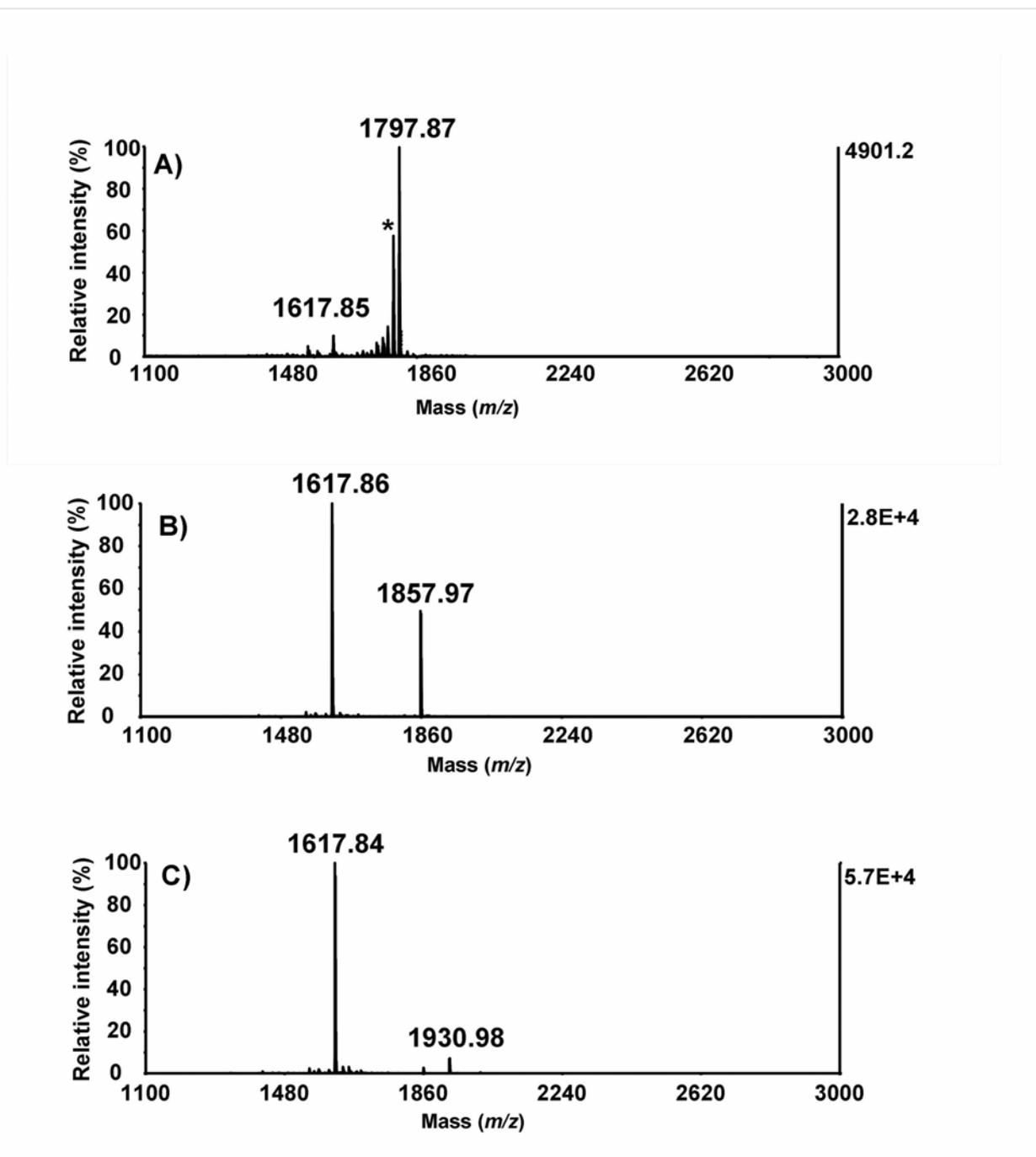


Figure S4: MALDI-TOF mass spectra of the ketone-containing peptide Ac-GGQE $\underline{HFAHKLILR}$ (KCP1, m/z 1617.85) derivatized with DNPH (A, m/z 1797.87), BHZ (B, m/z 1857.97), and ARP (C, m/z 1930.98). * indicates the peak at -16 Da, probably due to the loss of oxygen atom from one of the nitro groups in DNPH.

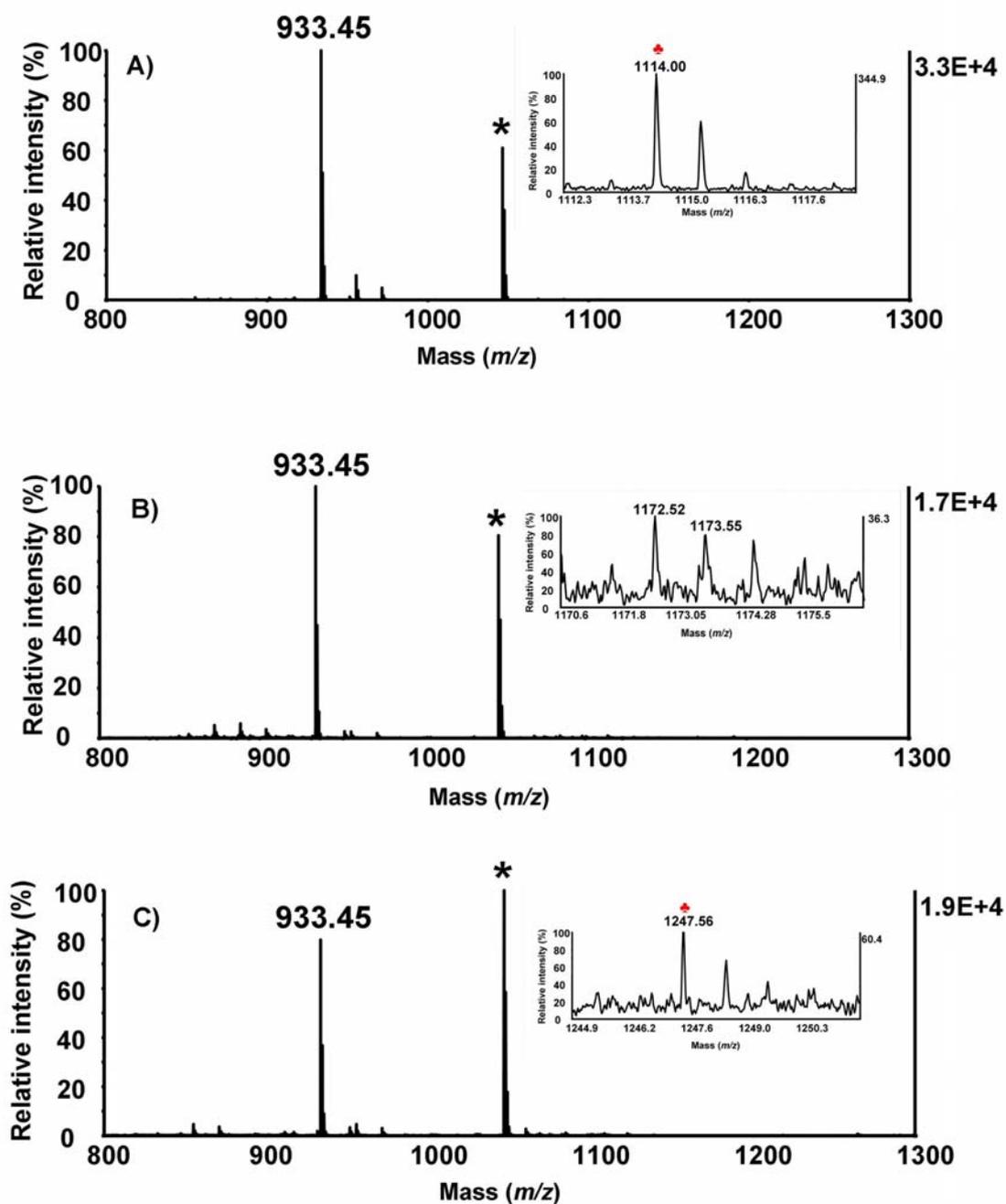


Figure S5: MALDI-TOF mass spectra of the lactam-containing peptide AFGSARASGA-NH₂ (Glarg, m/z 933.46) derivatized with DNPH (A, m/z 1114.0), BHZ (B, m/z 1173.5) and ARP (C, m/z 1247.5). Inserts show the MALDI-TOF mass spectra recorded after incubating peptide with the corresponding reagents at 37°C, over night, ♣ indicates the corresponding derivatized product. * indicates internal standard, angiotensin.

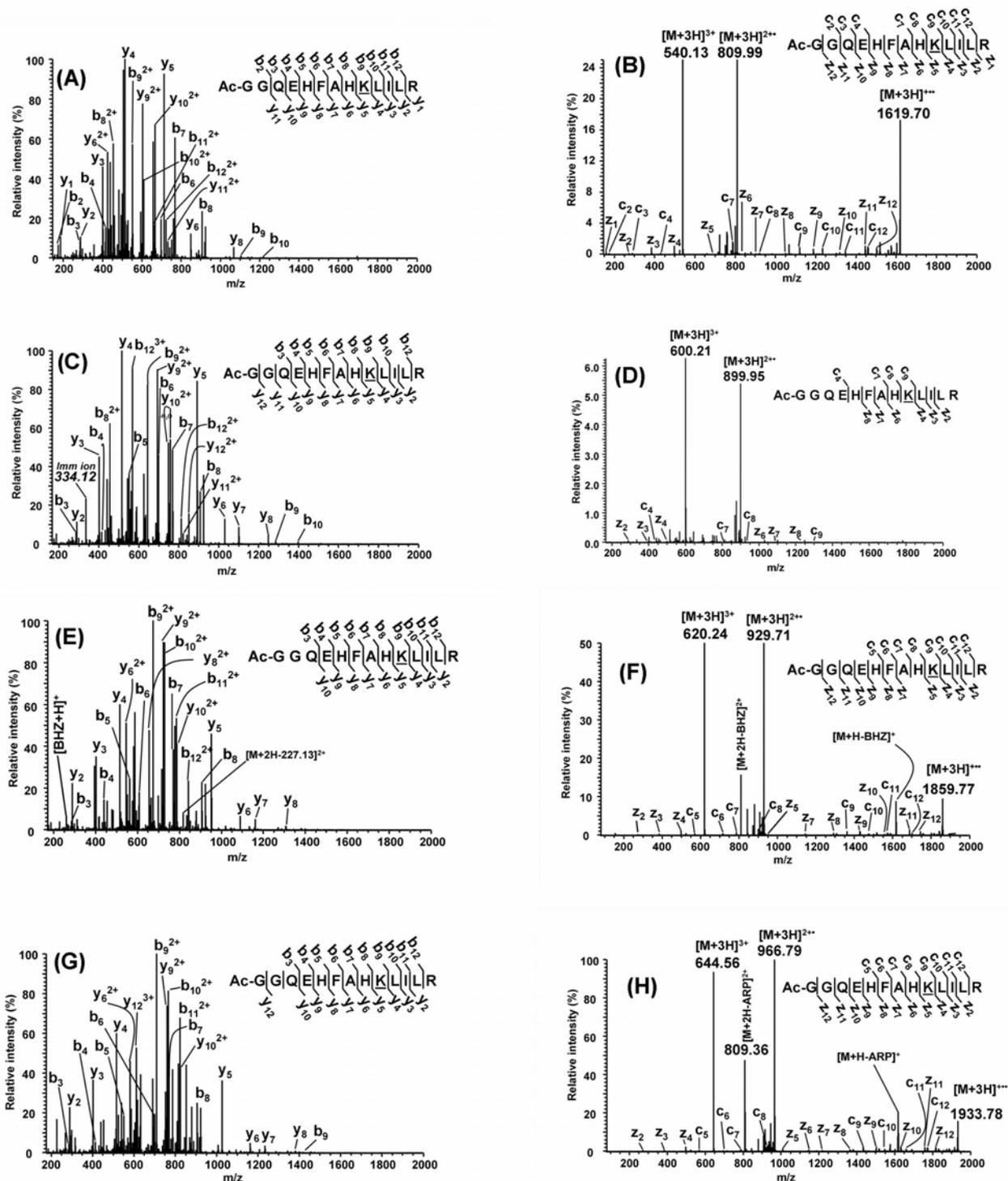


Figure S6: ESI-LTQ-tandem mass spectra of the triply protonated ketone-containing peptide Ac-GGQE \underline{H} FAHKLILR (KCP1, m/z 539.95³⁺; A, B) and its DNPH (m/z 599.96³⁺; C, D), BHZ (m/z 619.00³⁺; E, F) and ARP (m/z 644.66³⁺; G, H) derivatives. Left and right rows show the CID- and ETD-spectra respectively.

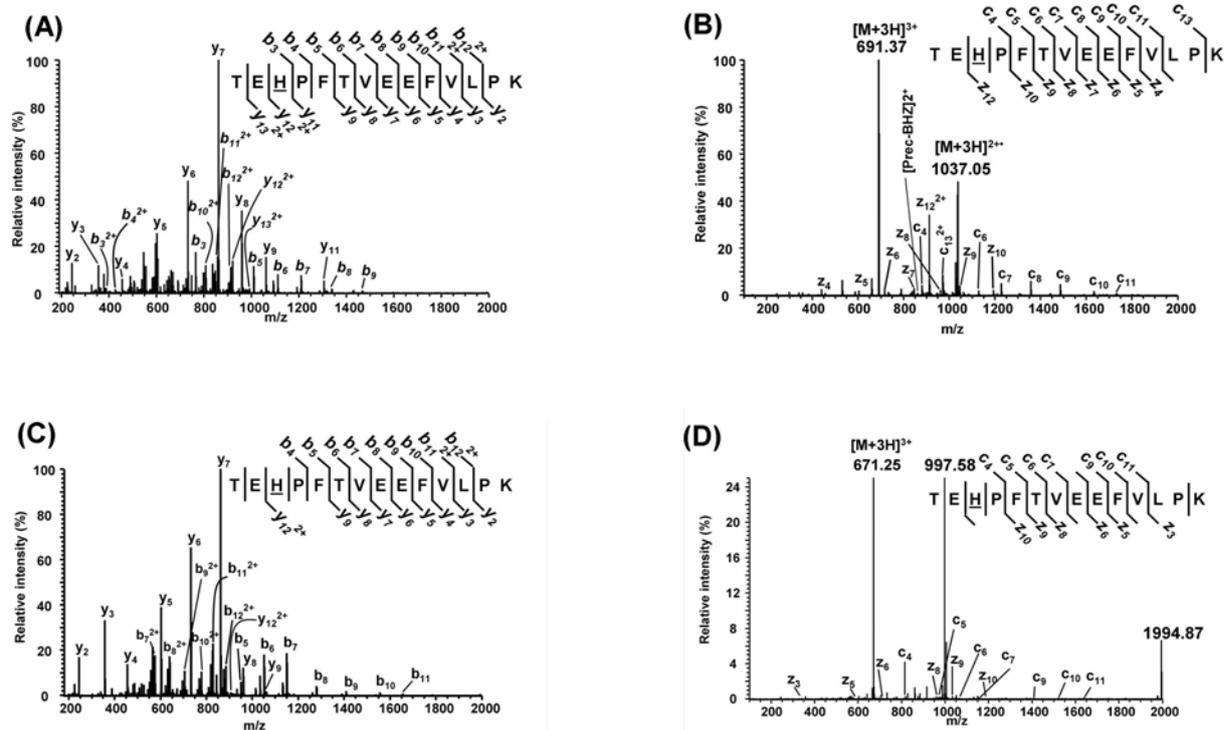


Figure S7: ESI-LTQ-tandem mass spectra of triply protonated aldehyde-containing peptide TEHPFTVEEFVLPK (ACP1) derivatized with BHZ and reduced with NaCNBH₃ (m/z 691.03³⁺; A, B) or derivatized with DNPH and reduced with NaCNBH₃ (m/z 671.01³⁺; C, D). Left and right rows show the CID- and ETD-spectra respectively.

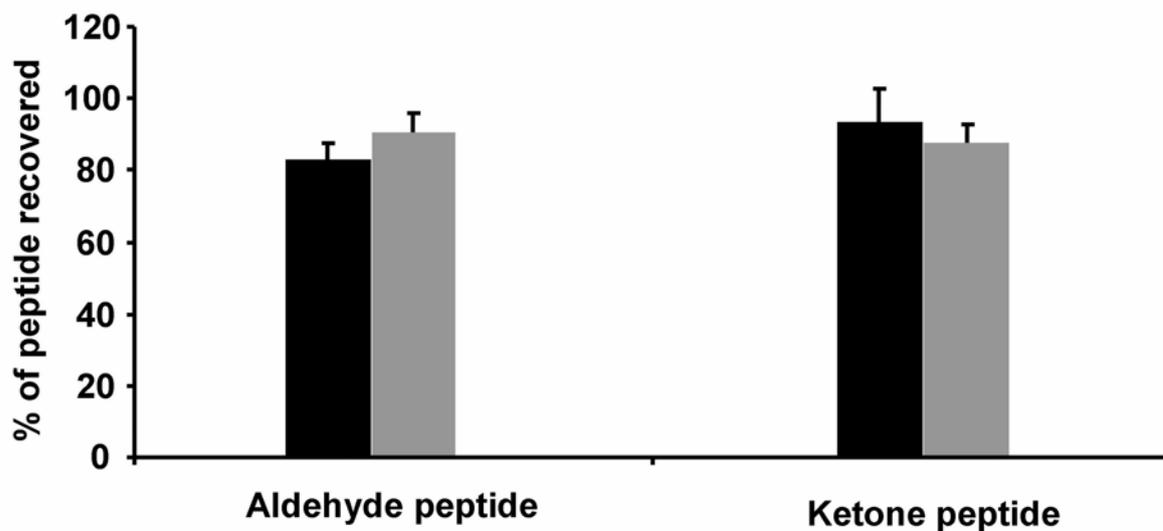


Figure S8: Recovery rates of aldehyde (ACP1)- and ketone (KCP2)-containing peptides after incubating them with (black bars) and without BSA (grey bars) for 24 hours. Afterwards TCA was added to precipitate BSA and the supernatants were analyzed by RP-HPLC. Recovery rates were calculated by the peak areas.

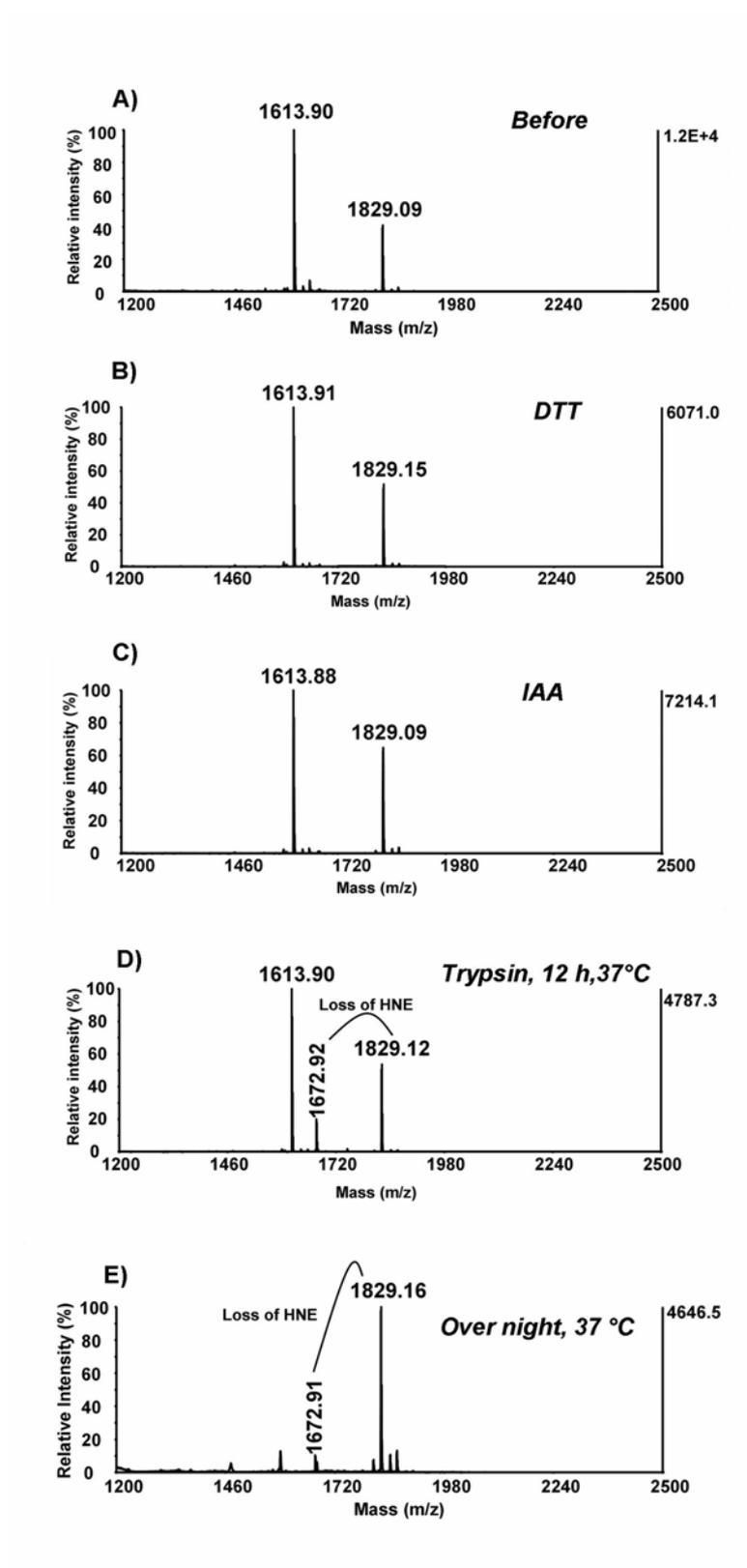


Figure S9: MALDI-TOF mass spectra of a mixture of the ketone- (KCP2, m/z 1613.88) and aldehyde-containing peptides (ACP1, m/z 1829.12, A) after DTT reduction (B), iodoacetamide alkylation (C) and overnight incubation with trypsin (D) as well as the aqueous peptide mixture incubated overnight at 37°C (E)