Development of a Capillary LC Method for Co-Elution of Isomeric Peptide Oxidation Products

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Overview:

- Developing a ZIC-HILIC chromatography method for quicker analysis of FPOP samples
- Co-elution of isomeric oxidized peptides
- Separation of different peptides

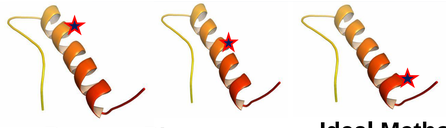
Introduction:

In hydroxyl radical protein footprinting (HRPF), accurate relative quantification of oxidative modifications at the amino acid level by ETD is hampered by the separation of the peptides by reverse phase chromatography. This requires custom MS/MS methods to equally sample all isomeric oxidation products. We are developing a chromatography method to ideally co-elute peptide modification isomers while separating different peptides. Use of large ID SEC columns can ideally co-elute peptide oxidation isomers while providing some resolution for different peptides, but give poor lower limits of quantitation. Hybrid trap-capillary SEC yielded ideal co-elution of oxidation isomers, but had issues with limits of quantification. Zwitterionic hydrophilic interaction chromatography (ZIC-HILIC) showed a similar ability to ideally co-elute oxidation isomers with better sensitivity.

Methods:

We performed the hybrid trap-capillary SEC method by placing in-line a C18 trap column immediately prior to a 0.3*300 mm custom-packed Sepax Zenix-C SEC-80 column. ZIC-HILIC used a 0.3*150 mm Milipore ZIC-HILIC column. Both methods were tested using unoxidized bovine serum albumin (BSA), oxidized [Glu]-Fibrinopeptide B (GluB), myoglobin and three synthetic oxidation isomeric peptides (R(Hyp)MFAIWK, RPMYAIWK, and RPMFSIWK). GluB and myoglobin samples were mixed to a final concentration of 10µM peptide, 1mM adenine as a radical dosimeter, 17mM glutamine to balance radical scavenging, 100mM hydrogen peroxide, and 20mM sodium phosphate buffer at pH 7.0. GluB and myoglobin samples were oxidized by FPOP and quenched immediately with catalase and methionine amide. MS analysis was conducted on Orbitrap Fusion mass spectrometer.

Background



Oxidation

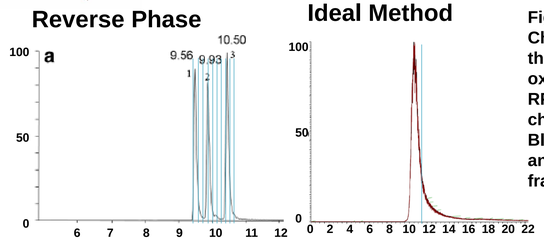


Figure 1. Chromatogram of three isomeric oxidized peptides via RP and ZIC-HILIC chromatography. Blue line represents an ETD fragmentation scan.

Results:

Hybrid Capillary Trap-SEC

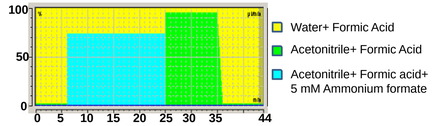


Figure 2. The optimal gradient used for hybrid capillary-trap SEC to obtain the co-elution of isomers and separation different peptides.

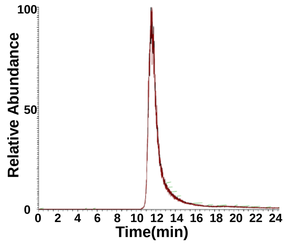


Figure 3. Selected Product Ion Chromatograms of oxidized RPMFAIWK demonstrating co-elution of isomers obtained via hybrid trap-capillary SEC. Black traces are the unoxidized c2 ion, specific for the two isomers containing oxygen on F or A, and red traces are the oxidized c2 ion, specific for oxidation of the proline.

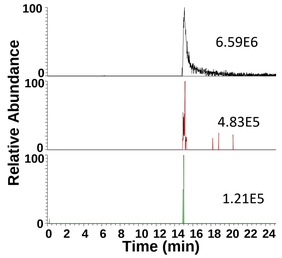


Figure 4. Selected product ion chromatogram of unoxidized, singly, and doubly oxidized of GYSLGNWVCAAK, a tryptic digested lysozyme peptide. Demonstrating the low intensity of oxidized peptides hampering the general applicability of the method.

ZIC-HILIC

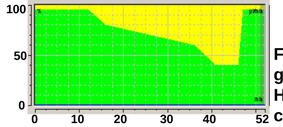
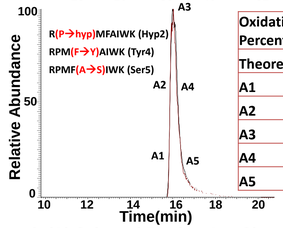


Figure 5. The optimal gradient used for ZIC-HILIC to obtain the co-elution of isomers.



Oxidation Percentage	Hyp2	Tyr4	Ser5
Theoretical	33.33	33.33	33.33
A1	29.81	31.9	38.27
A2	35.59	31.16	33.24
A3	36.53	33.69	29.76
A4	37.16	39.14	23.69
A5	34.04	39.34	26.61

Figure 6. (A) Selected Product Ion Chromatograms of oxidized RPMFAIWK demonstrating co-elution of isomers obtained via ZIC-HILIC, (B) measured oxidation ratio of peptide isomers calculated using ETD spectra at 5 different retention times. Peptides were mixed in 1:1:1 molar ratio. Black traces are the unoxidized c2 ion, specific for the two isomers containing oxygen on F or A, and red traces are the oxidized c2 ion, specific for oxidation of the proline.

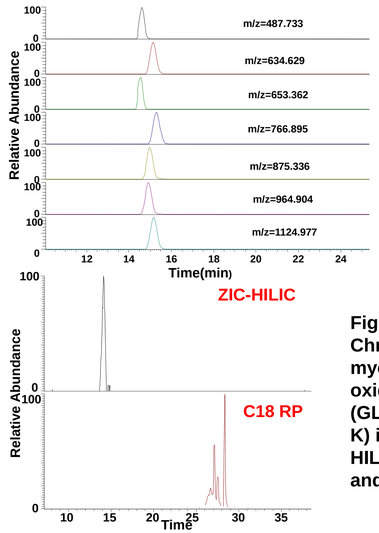


Figure 7. Partial separation of different BSA peptides via ZIC-HILIC.

Figure8. Chromatography for myoglobin singly oxidized peptide (GLSDGEWQQVLNVWGK) isomers via ZIC-HILIC (Black trace) and C18RP (Red trace).

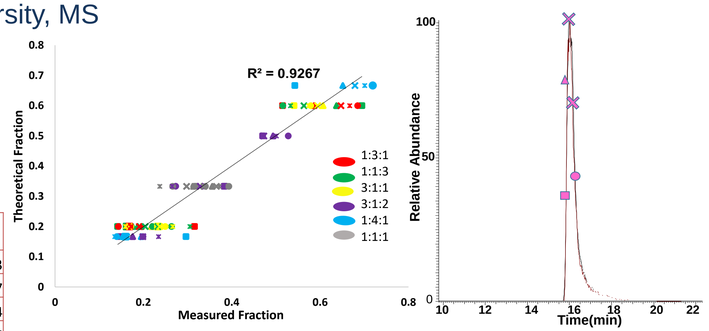


Figure 9. Measured oxidation of RPMFAIWK oxidation isomers calculated using ETD spectra at 5 different retention times via ZIC-HILIC. Each color and shape represent a specific molar ratio and retention time, respectively.

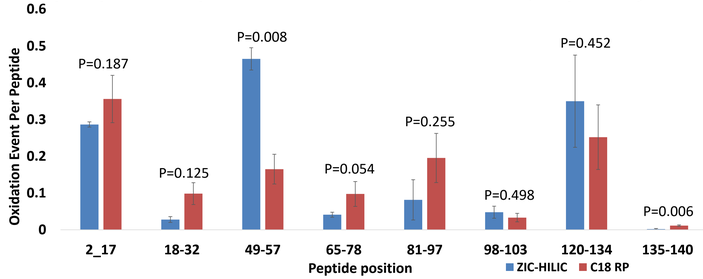


Figure 10. Peptide Level Comparison of Oxidized Myoglobin Using ZIC-HILIC and C18 RP Chromatography. For ZIC-HILIC 13 pmols and for C18 RP 25 pmols of digested myoglobin were injected.

Conclusions:

- **Co-elution of isomeric oxidized peptides and oxidized myoglobin isomeric peptides were achieved using 0.05%TFA in ACN and water buffers.**
- **The measurement site of the peak does not affect the oxidation ratio observed.**
- **Relative peptide oxidation of most myoglobin peptides was achieved by ZIC-HILIC in compare to C18 nano-RP column.**

Acknowledgements:

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Reference:

Xie, B., and Sharp, J. S. (2016) Relative Quantification of Sites of Peptide and Protein Modification Using Size Exclusion Chromatography Coupled with Electron Transfer Dissociation, *J Am Soc Mass Spectrom 27*, 1322-1327.