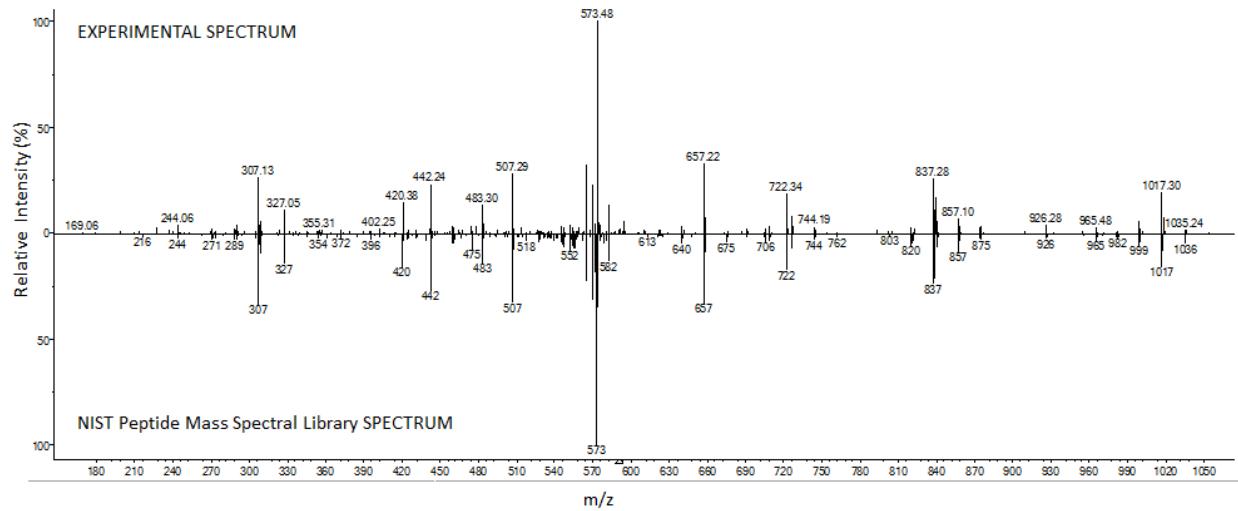


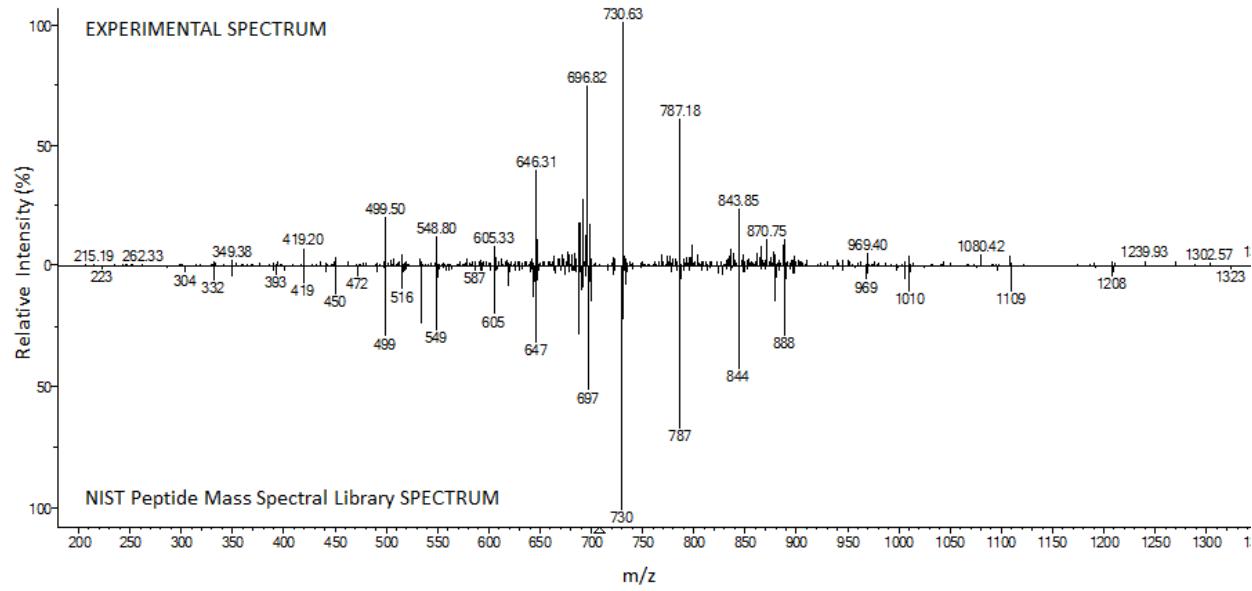
## SUPPORTING INFORMATION

**Figure S-1.** Experimental MS/MS spectra assigned for human SePP selenoprotein peptides in this study (top portion) and comparison with consensus spectra from the NIST Peptide Mass Spectral Library (bottom portion).

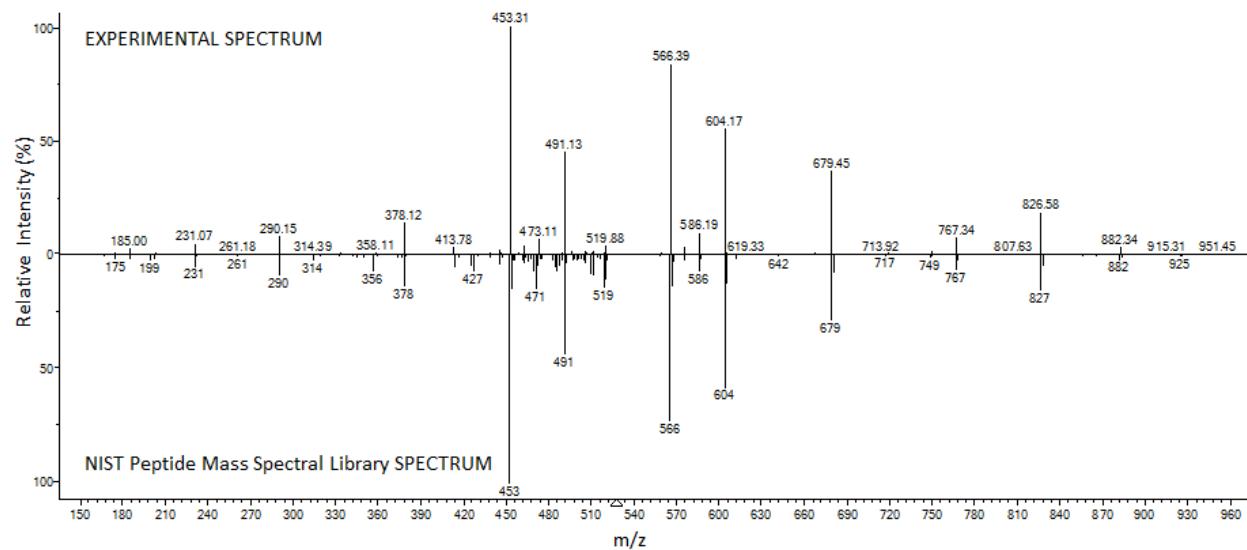
ESQDQSSLCK (Carbamidomethyl Cys, charge 2)



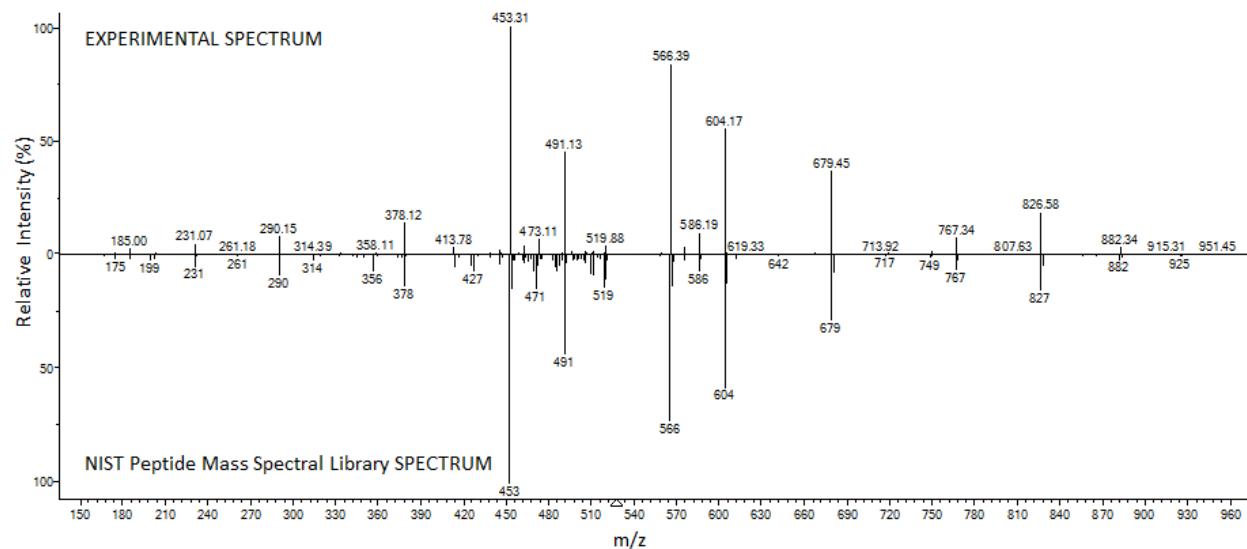
EGYSNISYIVVNHQGISSR (no modification, charge 3)



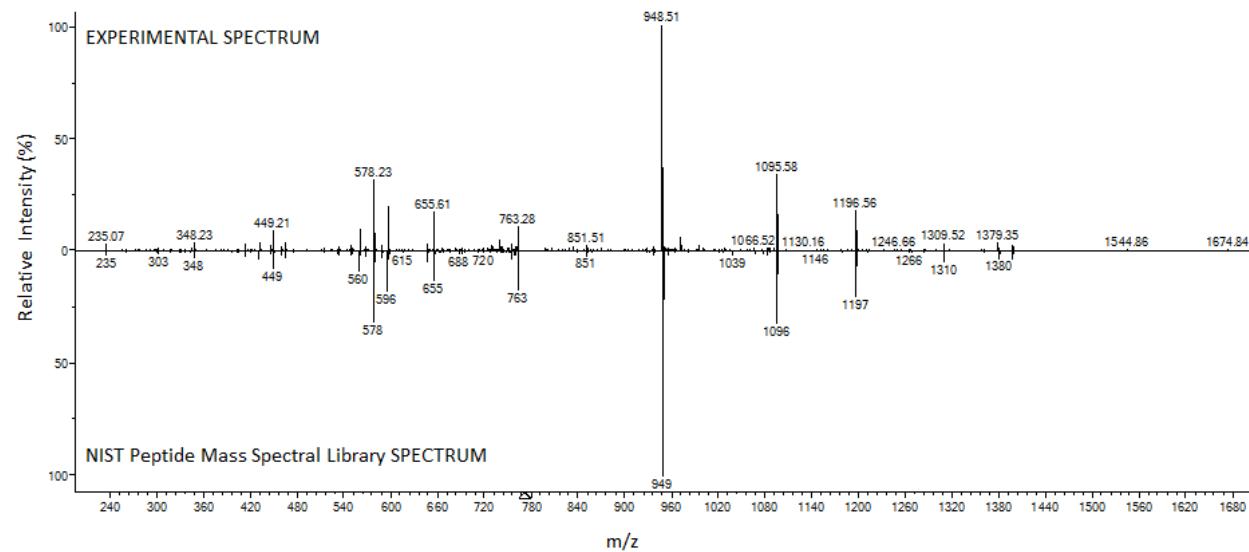
DDFLIYDR (no modification, charge 2)



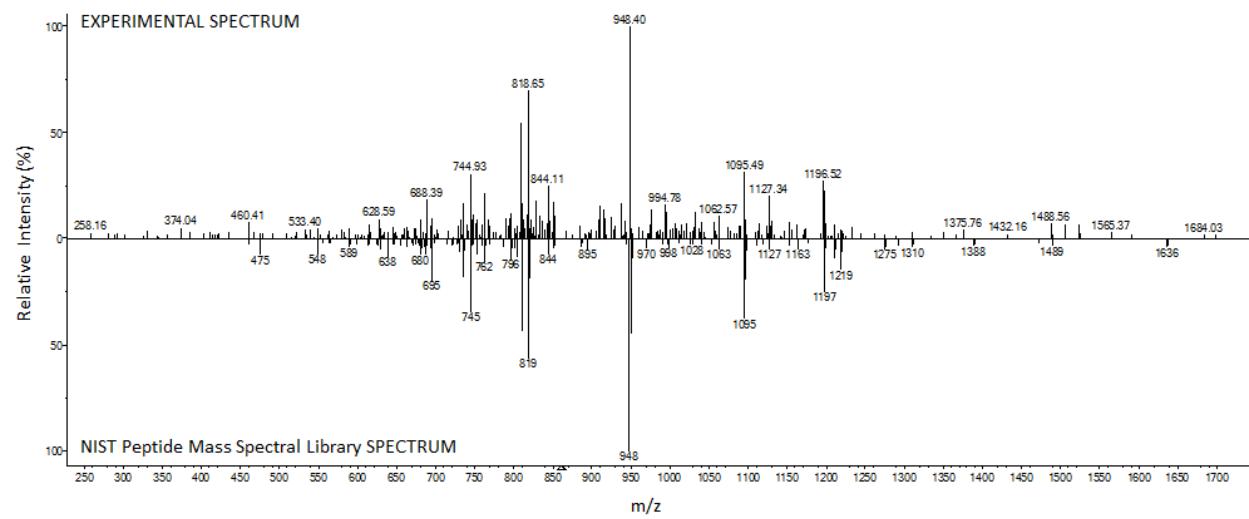
DDFLIYDR (no modification, charge 2)



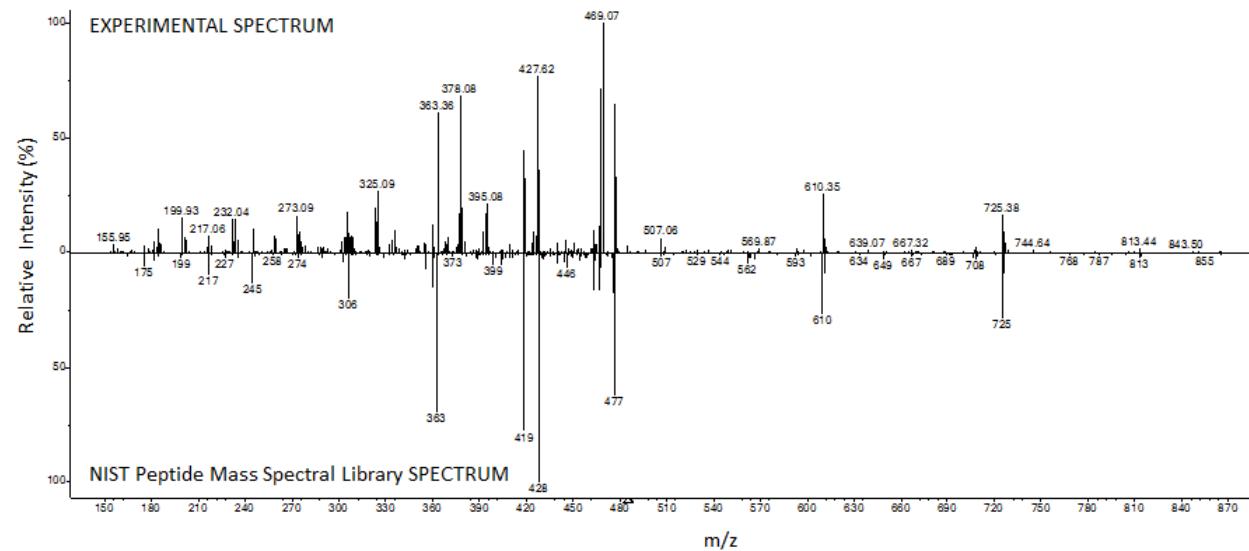
SFLTFPYVEEAIK (no modification, charge 2)



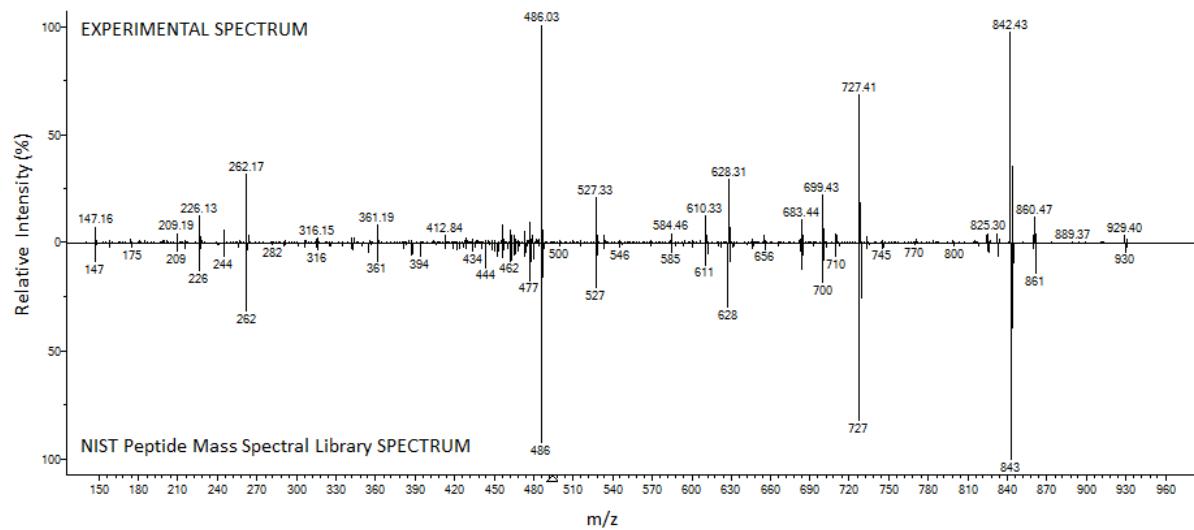
LVYHLGLPFSFLTFPYVEEAIK (no modification, charge 3)



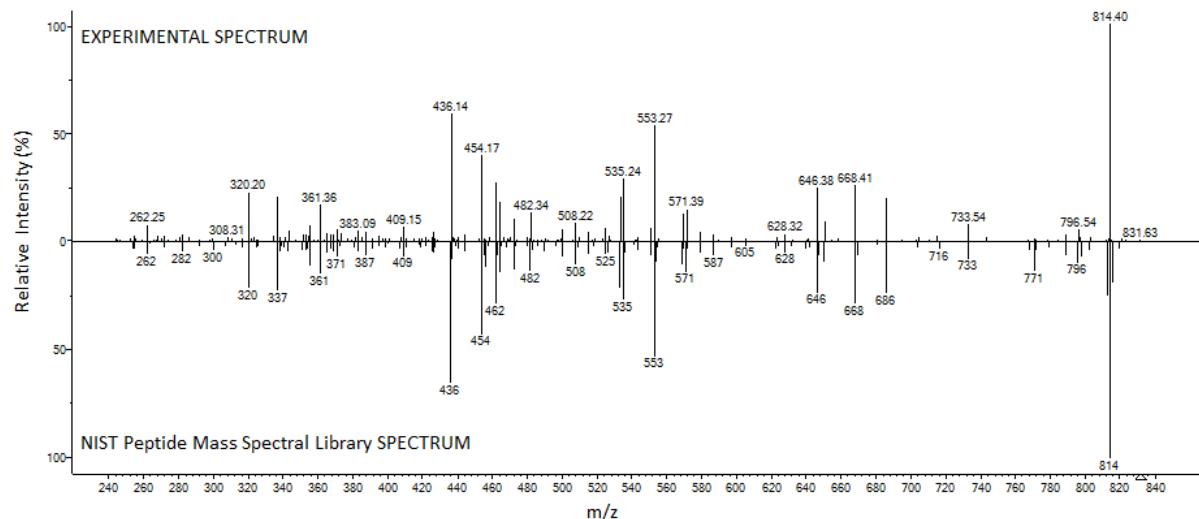
DED<sup>FC</sup>#KR (Carbamidomethyl Cys, charge 2)



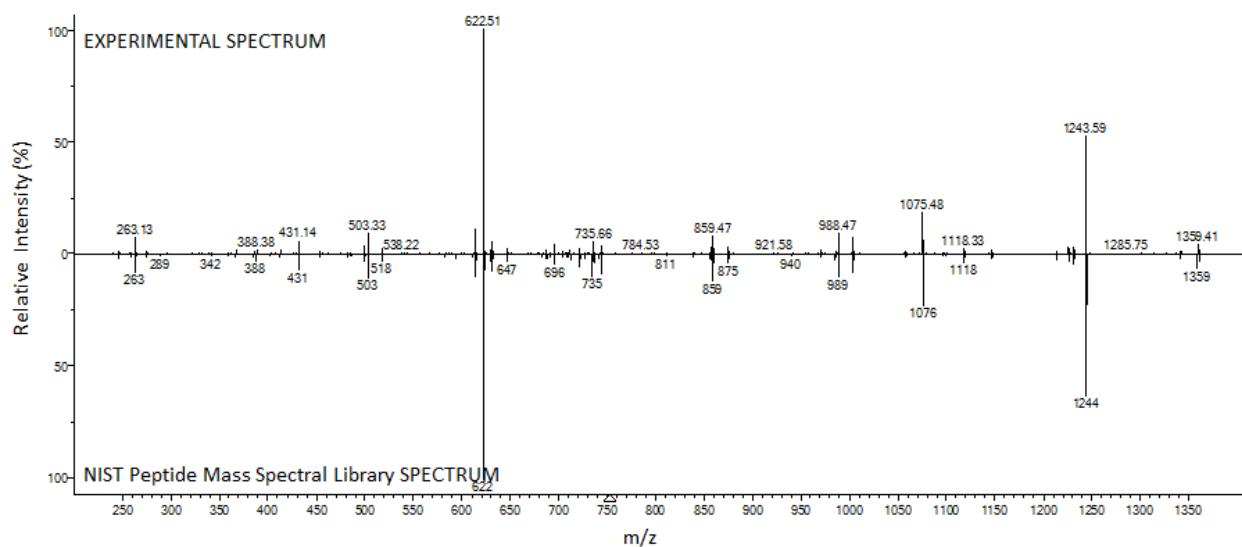
RVSLATVDK (no modification, charge 2)



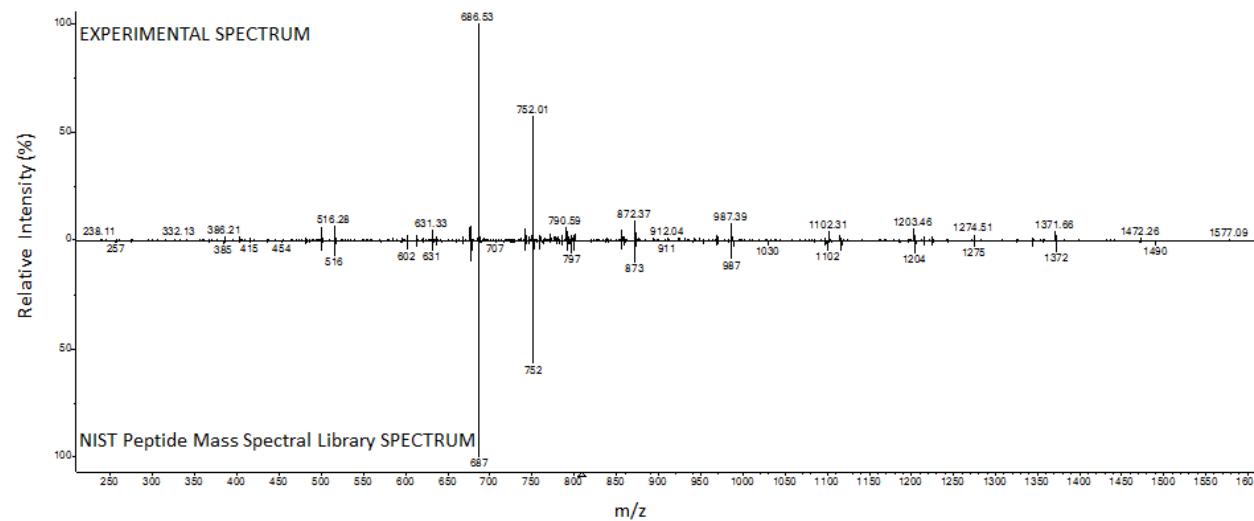
VSLATVDK (no modification, charge 1)



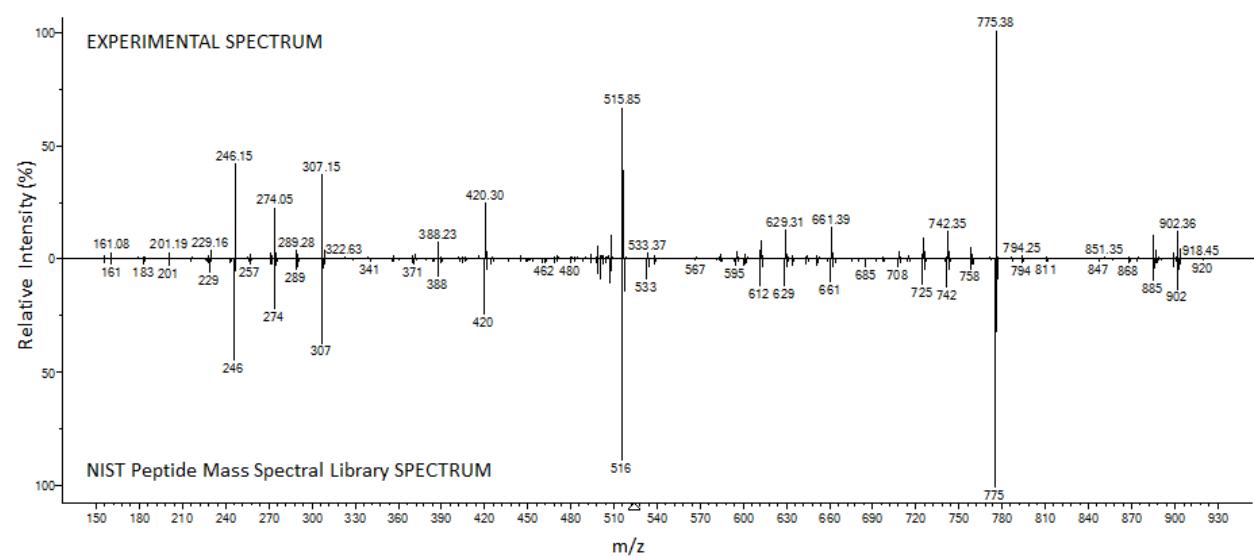
DM<sup>#</sup>PASEDLQDLQK (Met oxidized, charge 2)



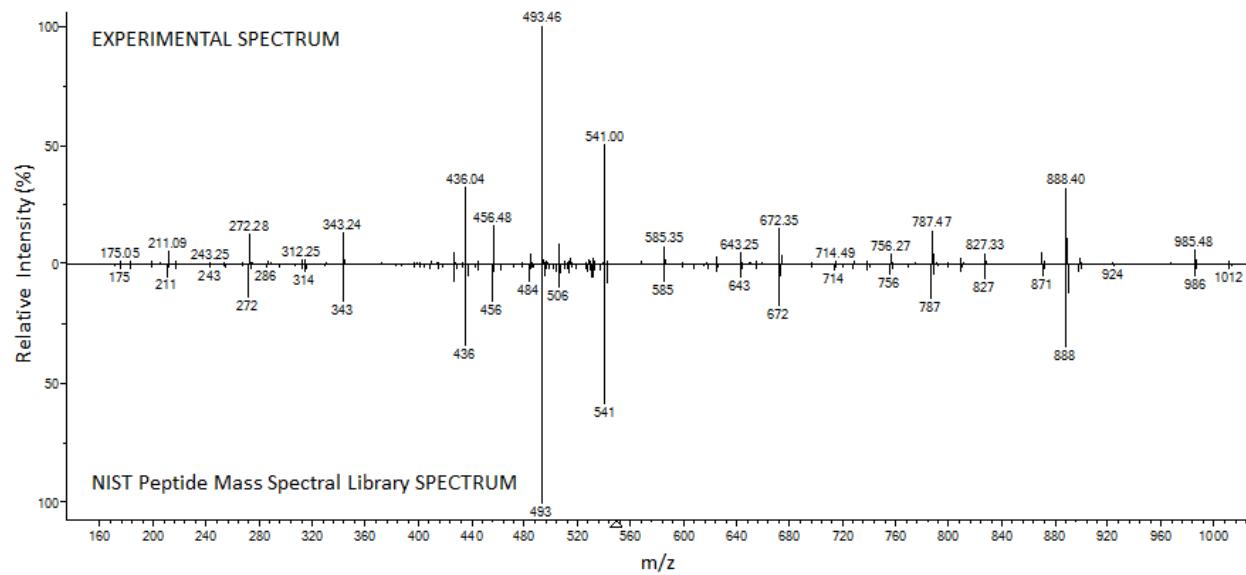
DMPASEDLQDLQKK (no modification, charge 2)



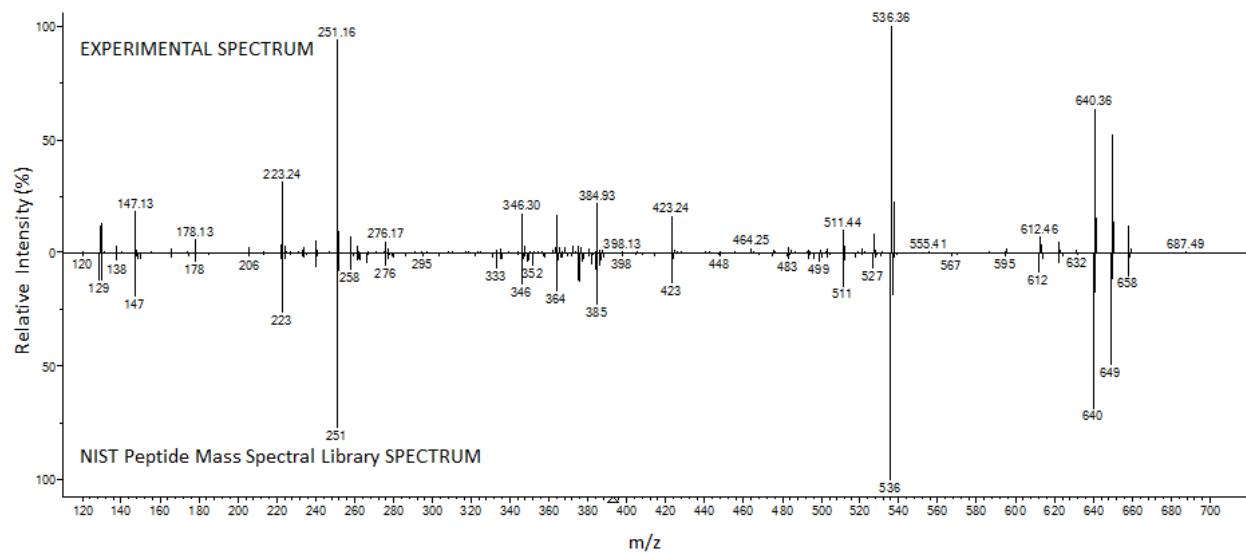
C#INQLLC#K (Carbamidomethyl Cys, charge 2)



LPTDSELAPR (no modification, charge 2)

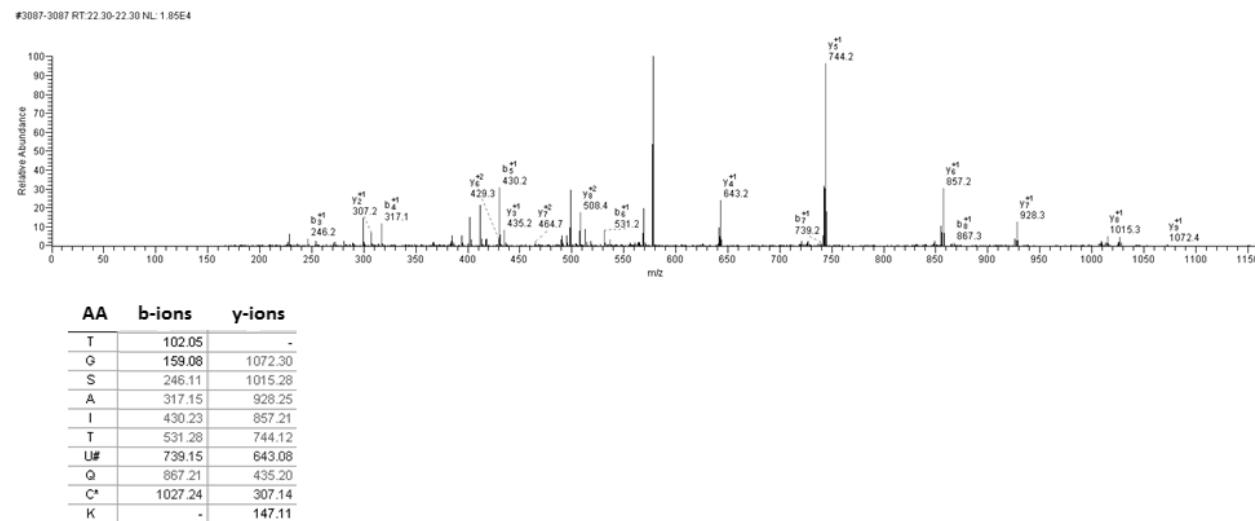


HLIFEK (no modification, charge 2)

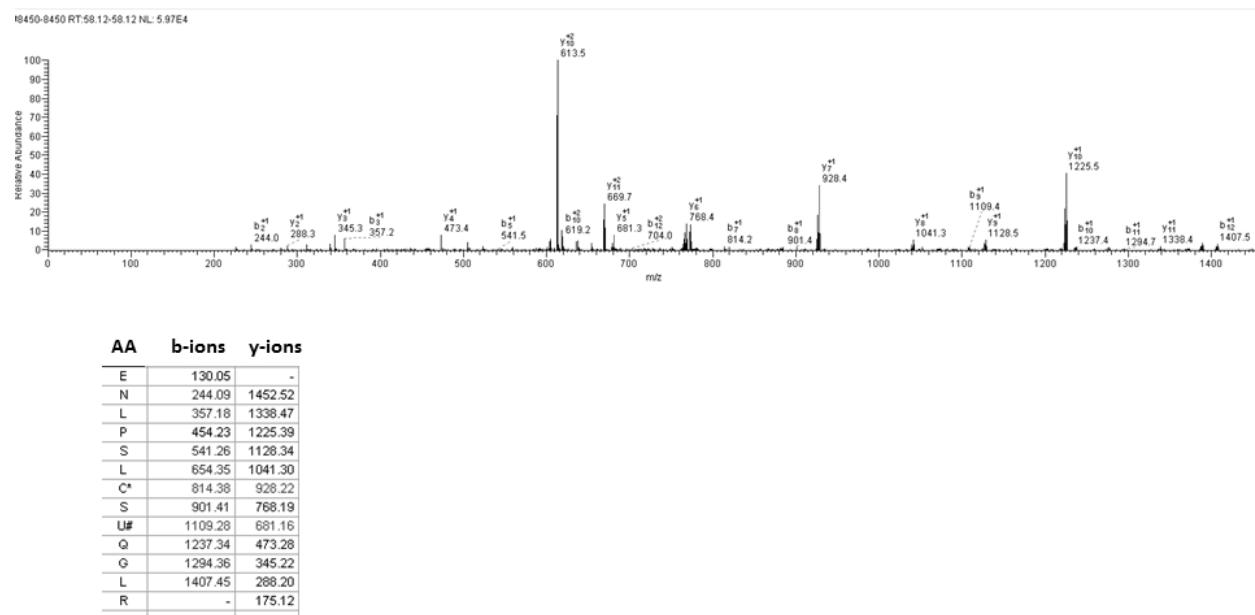


**Figure S-2.** SePP selenopeptides identified by sequence searching approach with SEQUEST search engine and a human protein database including the alkylated cysteine and selenocysteine. The tables list the theoretical masses (charge=+1) of b- and y- fragment ions for TGSAITUQCK, AEENITESCQUR and ENLPSLCSUQGLR selenopeptides.

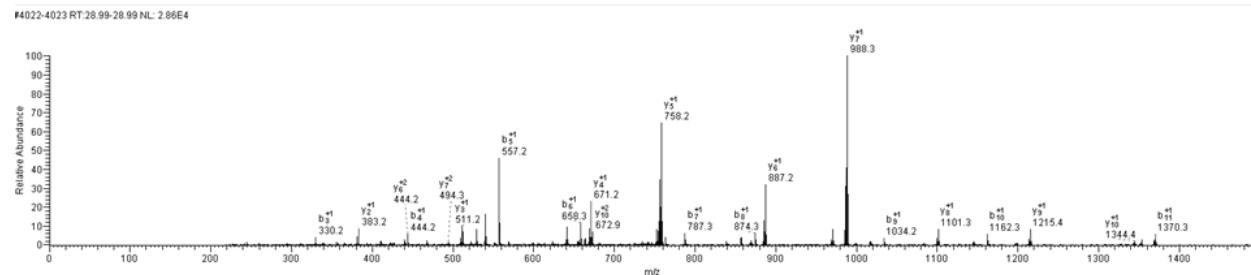
TGSAITU#QC\*K (Carbamidomethyl Cys and Sec, charge 2)



ENLPSLC\*SU#QGLR (Carbamidomethyl Cys and Sec, charge 2)

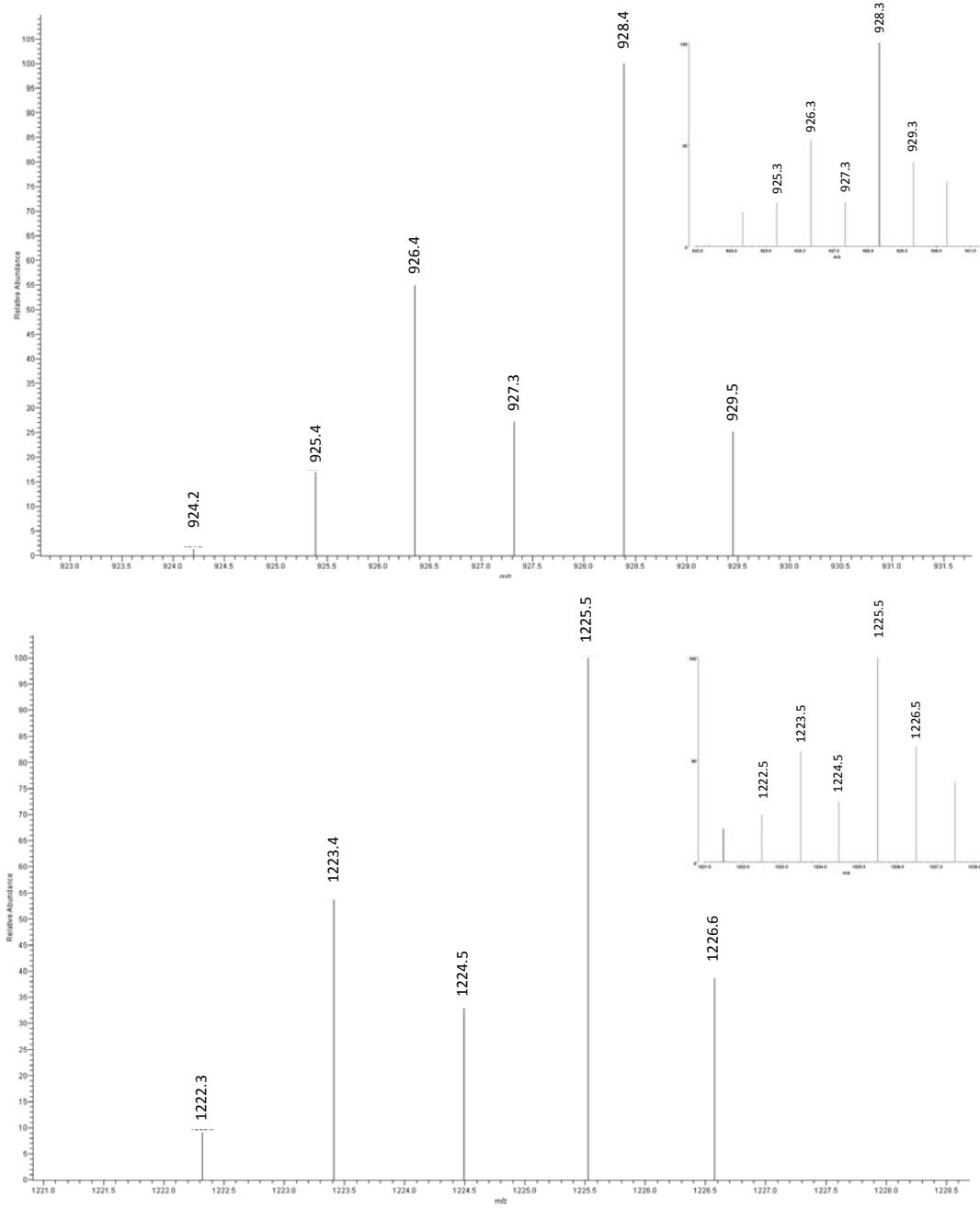


AEENITESC\*QU#R (Carbamidomethyl Cys and Sec, charge 2)



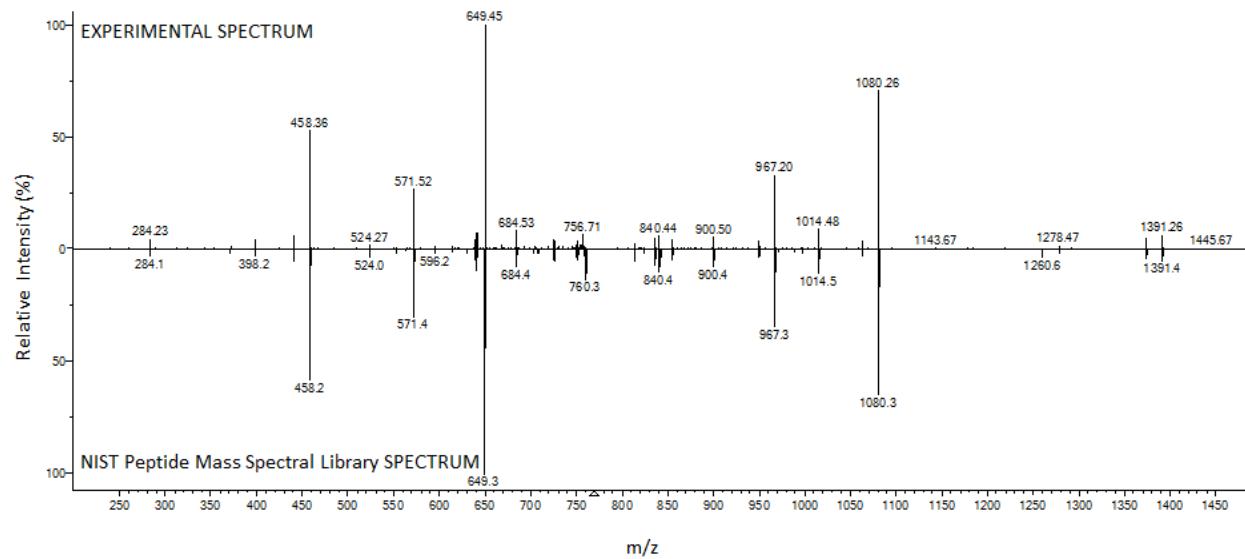
AA	b-ions	y-ions
A	72.04	-
E	201.09	1473.42
E	330.13	1344.38
N	444.17	1215.33
I	557.26	1101.29
T	658.30	988.21
E	787.35	887.16
S	874.38	758.12
C <sup>4</sup>	1034.41	671.08
Q	1162.47	511.05
U#	1370.34	382.99
R	-	175.12

**Figure S-3.**  $\gamma$ -fragment ions of alkylated ENLPSLCSUQGLR selenopeptide presenting the characteristic isotope pattern of Se. Top : MS spectrum of CSUQGLR  $\gamma$ -fragment ion; bottom: MS spectrum of PSLCSUQGLR  $\gamma$ -fragment ion. Inserts: Spectra representing the theoretical isotopic distribution of CSUQGLR and PSLCSUQGLR  $\gamma$ -fragment ions.

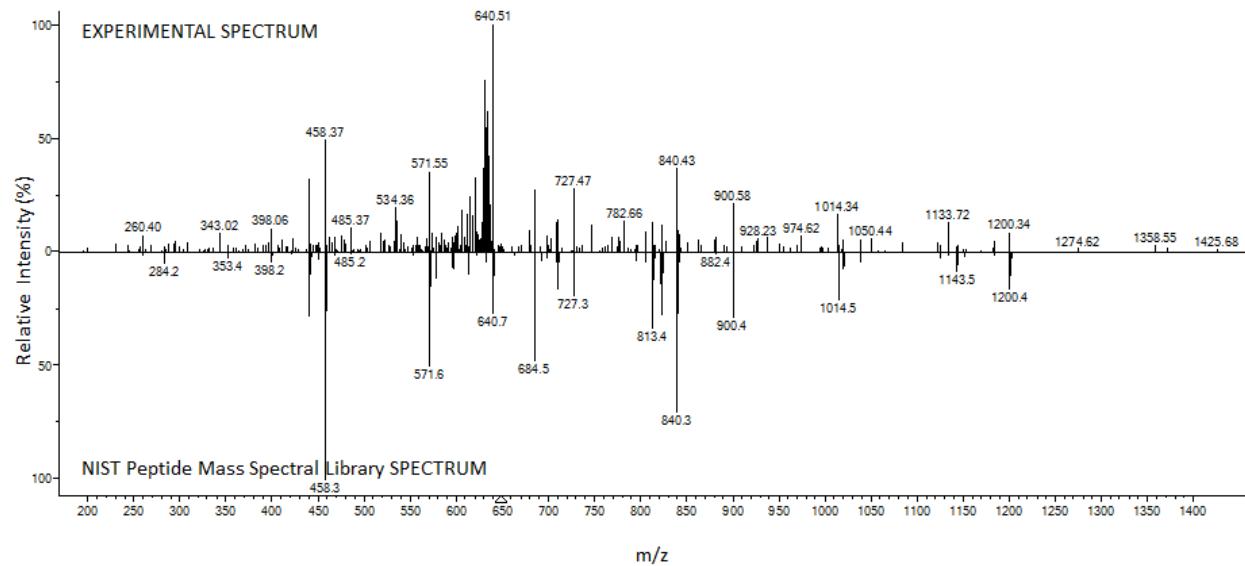


**Figure S-4.** Experimental MS/MS spectra assigned for human GPx3 selenoprotein peptides in this study (top portion) and comparison with consensus spectra from the NIST Peptide Mass Spectral Library (bottom portion).

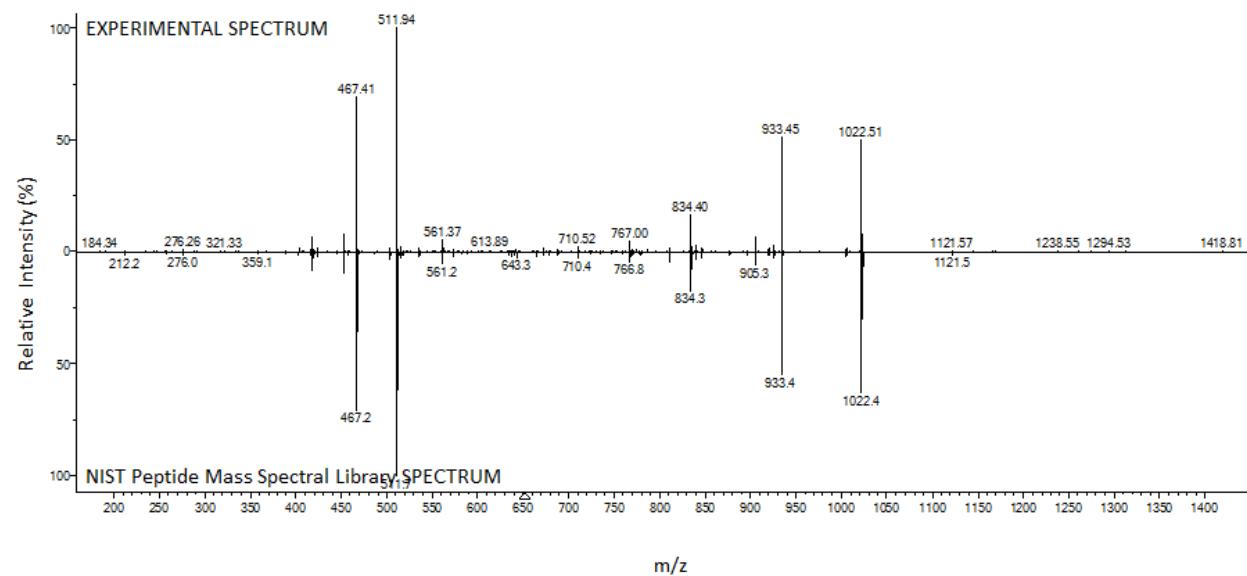
QEPGENSEILPTLK (Gln modified as pyro-Glu, charge 2)



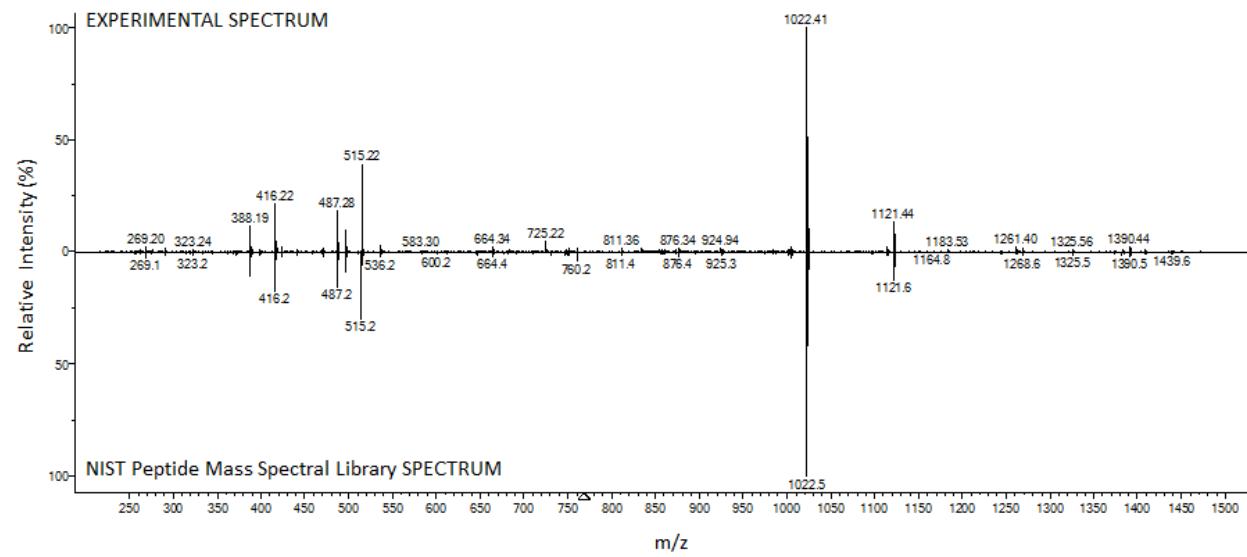
PGENSEILPTLK (no modification, charge 2)



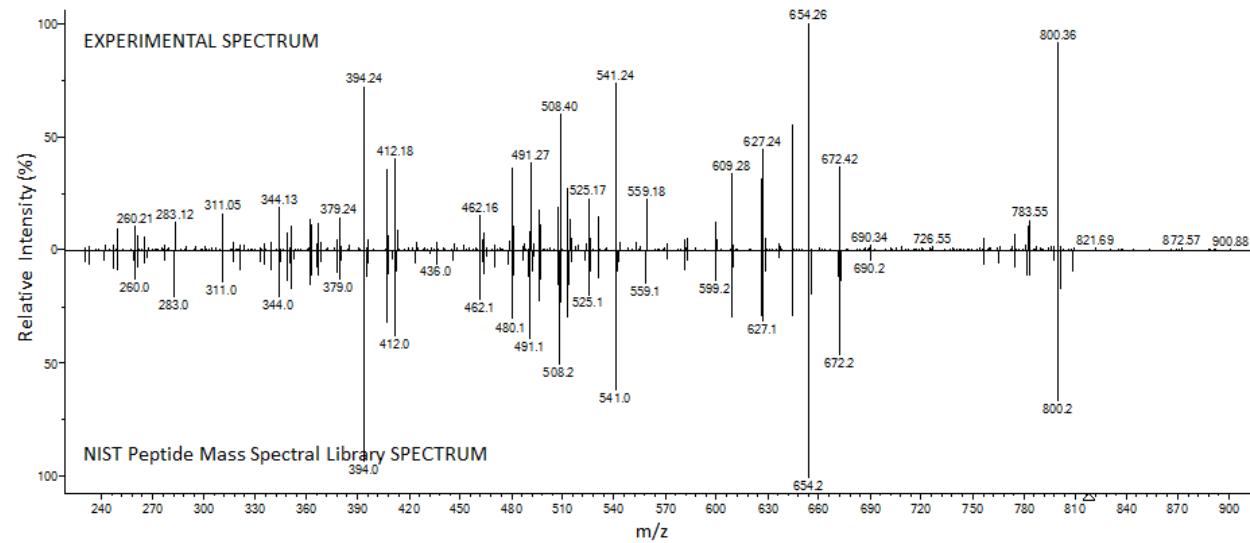
YVRPGGGFVPNFQLFEK (no modification, charge 3)



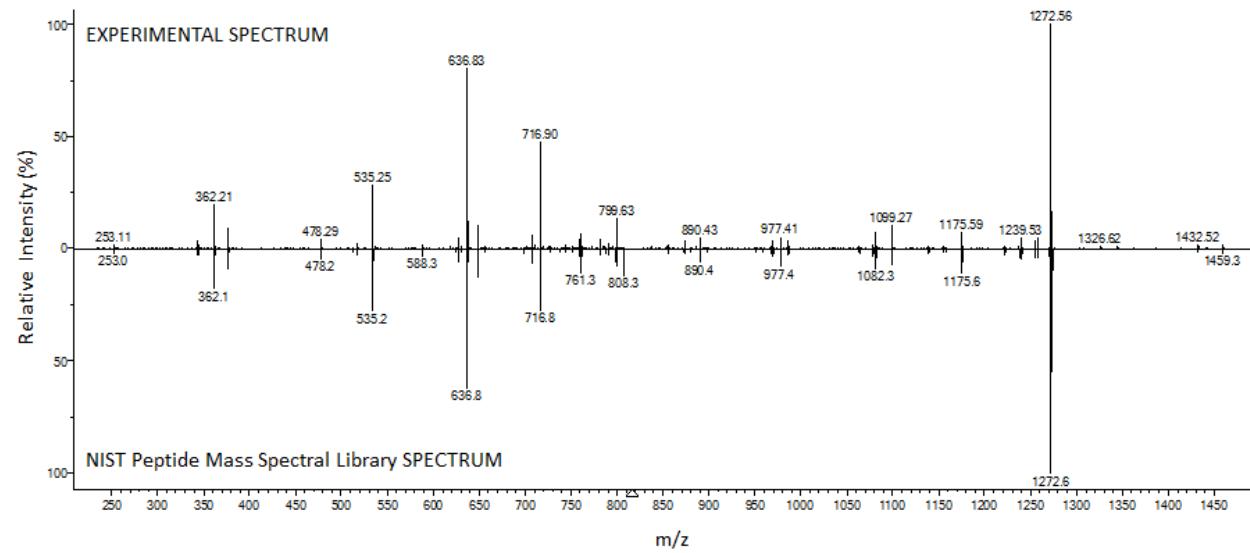
PGGGFVPNFQLFEK (no modification, charge 2)



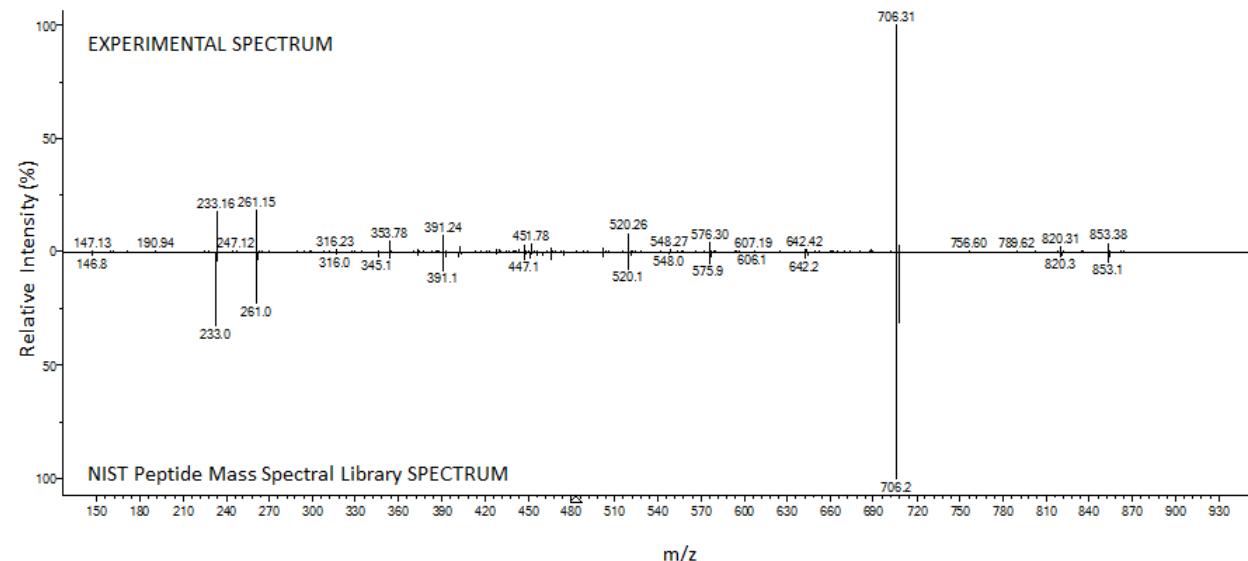
FYTFLK (no modification, charge 1)



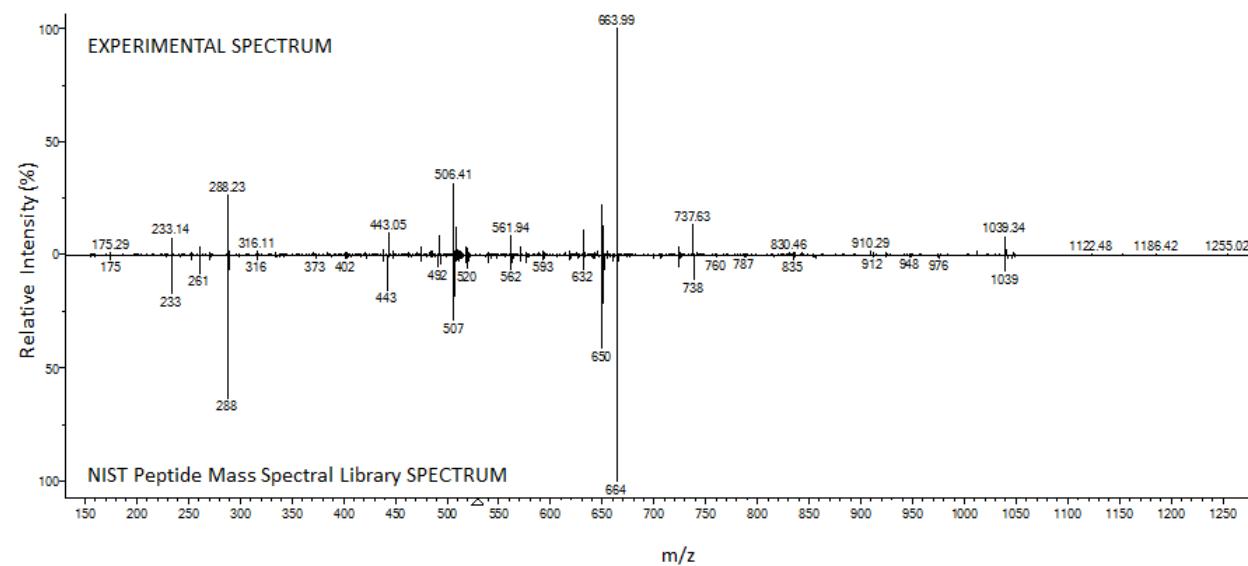
NSC#PPTSELLGTSDR (Carbamidomethyl Cys, charge 2)



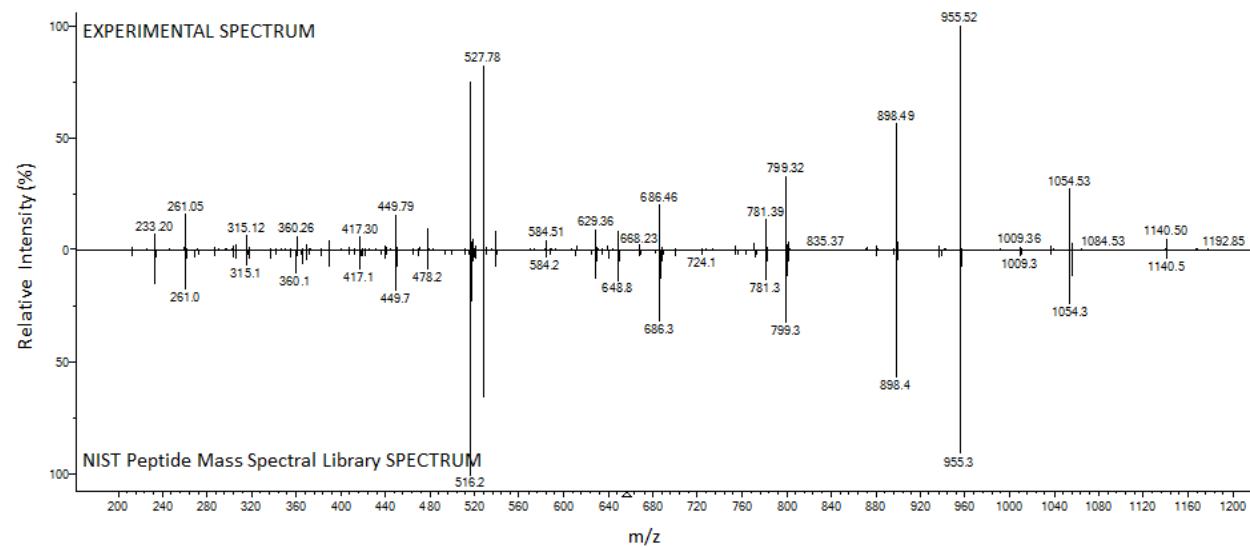
LFWEPMK (Met oxidized, charge 2)



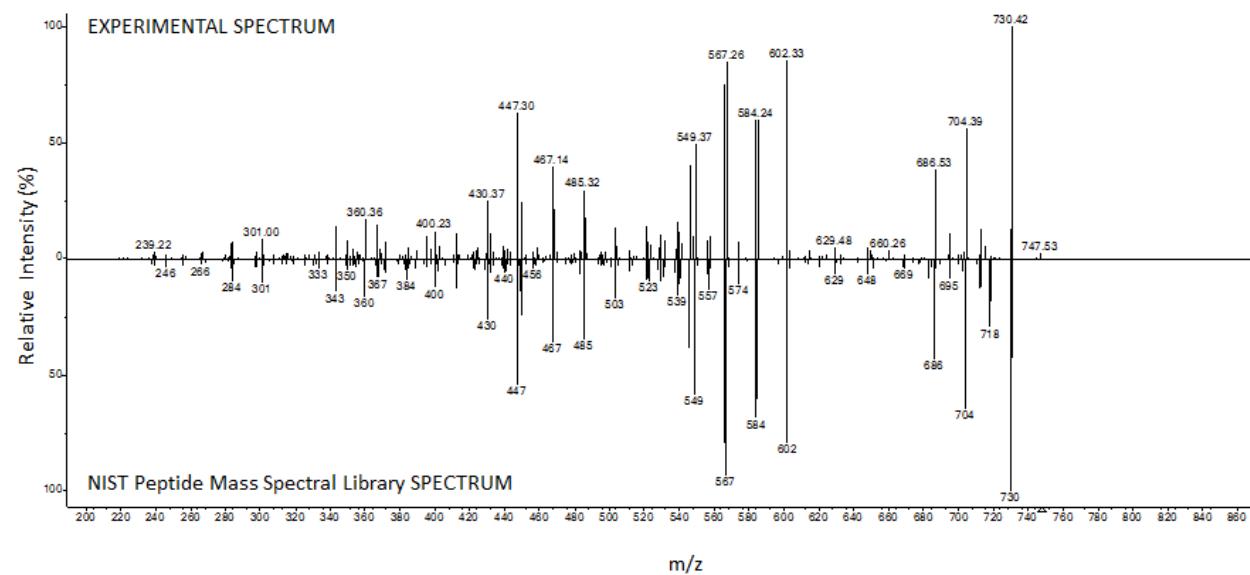
LFWEPM<sup>K</sup>VHDIR (Met oxidized, charge 3)



FLVGPDGIPIMR (no modification, charge 2)



TTVSNVK (no modification, charge 1)



M<sup>#</sup>DILSYMR (Met oxidized, charge 2)

