

Establishing Ion Ratio Thresholds Based on Absolute Peak Area for Absolute Protein Quantification using Protein Cleavage Isotope Dilution Mass Spectrometry

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Table S1: Shown is the mean peptide concentration and relative standard deviation of 23 peptides from 3 wild type samples.

Peptide	Old Method		New Method	
	Mean [Protein] fmol/ μ g	Mean %RSD	Mean [Protein] fmol/ μ g	Mean %RSD
FDIGTLLGLIEK	276.22	31.44%	276.22	31.44%
FEIGSLLGLIEK	19.41	35.72%	19.63	35.85%
VCTLELFSPK	37.74	23.71%	38.32	24.97%
DYFVDER	26.98	8.46%	28.89	6.24%
DYFVEER	15.60	26.77%	18.36	37.31%
GGILGLGGVGHMGVK	109.12	23.96%	109.12	23.96%
FLEPGVPDFK	35.93	18.80%	35.81	19.07%
FMKPGVPDFK	7.94	25.01%	7.94	25.01%
FLKPGVPDFK	30.53	22.50%	30.23	23.11%
ENYELGLPVIQK	332.12	19.64%	334.84	20.13%
VGGLIGYDNTLWNGSVVAPPDAPMR	225.55	17.97%	225.40	18.22%
VGGLIGYDNTLWNGSVVAPADAPMR	234.65	13.58%	234.65	13.58%
FLSMLLK	31.10	32.21%	31.07	32.37%
DLGFEFTPVK	53.24	21.35%	53.65	22.62%
AGPGAFLSTSEIASHLPTK	2972.40	22.05%	2972.40	22.05%
SALDFLELQPDLSALVR	23.76	33.57%	23.84	33.32%
SALDYLELQPDLSALVR	12.63	25.01%	12.81	24.30%
EELGTGLLTGEK	17.22	30.31%	18.18	27.74%
EELGTILLTGEK	45.46	31.47%	46.56	29.86%
EELGTVLLTGEK	10.77	25.05%	10.90	25.60%
IGSFEEELK	63.51	12.47%	62.78	11.82%
DGIVSLGGPHIPLK	1.73	19.66%	1.76	15.59%
AFEIIEDLR	22.64	22.20%	22.64	22.20%

Table S2: Transitions measured and collision energy used for all peptides.

Peptide	Ion Type		NAT Ion <i>m/z</i>		SIL Ion <i>m/z</i>		CE (eV)
	Precursor	Product	Precursor	Product	Precursor	Product	
FDIGTLGLIEK	[M+2H ⁺]	y9 ⁺	659.884	943.582	663.393	950.599	23
		y7 ⁺		785.513		792.530	
		y6 ⁺		672.429		679.446	
		y5 ⁺		559.345		566.362	
		b2 ⁺		263.103		263.103	
FEIGSLLGLIEK	[M+2H ⁺]	y10 ⁺	659.885	1042.653	663.394	1049.670	23
		y9 ⁺		929.569		936.586	
		y6 ⁺		672.431		679.448	
		y5 ⁺		559.347		566.364	
		b2 ⁺		277.118		277.118	
VCTLELFSPK	[M+2H ⁺]	y8 ⁺	597.315	934.524	600.824	941.542	19
		y7 ⁺		833.477		840.494	
		y6 ⁺		720.393		727.410	
		y3 ⁺		331.198		331.198	
DYFVDER	[M+2H ⁺]	y5 ⁺	472.211	665.325	475.218	671.339	15
		y4 ⁺		518.257		524.271	
		y3 ⁺		419.188		419.188	
		b2 ⁺		279.098		279.098	
		a2 ⁺		251.103		251.103	
DYFVEER	[M+2H ⁺]	y5 ⁺	479.219	679.341	482.226	685.355	16
		y4 ⁺		532.273		538.286	
		y3 ⁺		433.204		433.204	
		a2 ⁺		251.103		251.103	
GGILGLGGVGHMGVK	[M+3H ⁺]	y12 ⁺⁺	451.255	562.816	453.260	565.823	15
		y11 ⁺⁺		506.274		509.281	
		y9 ⁺⁺		421.221		424.228	
YFGLDEPGK	[M+2H ⁺]	y8 ⁺	513.251	862.431	516.759	869.448	12
		y7 ⁺		715.362		722.379	
		y5 ⁺		545.257		545.257	
		y3 ⁺		301.187		301.187	
		b2 ⁺		311.139		311.139	
		a2 ⁺		283.144		283.144	
HIGIVLGGIGHVAVK	[M+3H ⁺]	y9 ⁺	509.644	837.494	511.983	844.511	21
		y6 ⁺		610.367		610.367	
		y9 ⁺⁺		419.251		422.759	
		b6 ⁺		577.346		577.346	
		b9 ⁺		804.473		804.473	
FLEPGVPDFK	[M+2H ⁺]	y8 ⁺	574.803	888.446	577.810	894.460	18
		y7 ⁺		759.404		765.417	
		y4 ⁺		506.261		506.261	
		b2 ⁺		261.160		261.160	

FMKPGV <u>P</u> DFK	[M+3H ⁺] ³	b3 ⁺		390.202		390.202	
		y4 ⁺	389.207	506.261	391.212	506.261	14
		y2 ⁺		294.181		294.181	
		y4 ⁺⁺		253.634		253.634	
		b6 ⁺		660.354		666.368	
FLKPGV <u>P</u> DFK	[M+3H ⁺]	a6 ⁺⁺		316.683		319.690	
		y4 ⁺	383.222	506.261	385.226	506.261	14
		y2 ⁺		294.181		294.181	
		y4 ⁺⁺		253.634		253.634	
		b6 ⁺		642.397		648.411	
VGGLIGYDNTLWNGSV <u>V</u> APDAPMR	[M+3H ⁺]	y9 ⁺	867.104	953.487	869.109	959.501	31
		y7 ⁺		783.382		783.382	
		y7 ⁺⁺		392.195		392.195	
VGGLIGYDNTLWNGSV <u>V</u> APADAPMR	[M+3H ⁺]	y9 ⁺	858.432	927.472	860.437	933.485	31
		y8 ⁺		828.403		828.403	
		y7 ⁺		757.366		757.366	
EAYEIG <u>L</u> PFIQK	[M+2H ⁺]	y9 ⁺	704.380	1044.609	707.888	1051.626	14
		y8 ⁺		915.566		922.583	
		y7 ⁺		802.482		809.499	
		b4 ⁺		493.193		493.193	
		b5 ⁺		606.277		606.277	
VEISQISIGDGV <u>T</u> LCR	[M+2H ⁺]	y11 ⁺	873.956	1190.620	877.465	1197.637	27
		y10 ⁺		1077.536		1084.553	
		y9 ⁺		990.504		997.521	
		y8 ⁺		877.420		884.437	
		y4 ⁺		549.281		556.299	
DLGFEFT <u>P</u> VK	[M+2H ⁺]	y8 ⁺	576.800	924.483	579.807	930.496	20
		y6 ⁺		720.393		726.406	
		y5 ⁺		591.350		597.364	
		y3 ⁺		343.234		349.248	
		b2 ⁺		229.118		229.118	
		a2 ⁺		201.123		201.123	
AGPGAFL <u>S</u> TSEIASHLPTK	[M+3H ⁺]	y17 ⁺⁺	628.667	878.467	631.006	881.976	20
		y16 ⁺⁺		829.941		833.450	
		y14 ⁺⁺		765.912		769.420	
		y13 ⁺⁺		692.378		695.886	
		y18 ⁺⁺⁺		604.988		607.327	
		y17 ⁺⁺⁺		585.981		588.320	
SALDFLELQPD <u>L</u> SALVR	[M+2H ⁺]	y11 ⁺	944.015	1240.690	947.523	1247.707	28
		y10 ⁺		1111.647		1118.664	
		y9 ⁺		998.563		1005.580	
		y8 ⁺		870.504		877.522	
		b8 ⁺		889.467		889.467	
		b9 ⁺		1017.525		1017.525	
SALDYLELQPD <u>L</u> SALVR	[M+2H ⁺]	y11 ⁺	952.012	1240.690	955.521	1247.707	29
		y10 ⁺		1111.647		1118.664	

AAG <u>I</u> DSGFFELQPK	[M+2H ⁺]	y9 ⁺		998.563		1005.580	
		y8 ⁺		870.504		877.522	
		b8 ⁺		905.461		905.461	
		b9 ⁺		1033.520		1033.520	
		y10 ⁺	740.378	1167.568	743.886	1167.568	24
		y9 ⁺		1052.541		1052.541	
		y8 ⁺		965.509		965.509	
		y6 ⁺		761.419		761.419	
		y2 ⁺		244.166		244.166	
EELGTGL <u>L</u> TGEK	[M+2H ⁺]	a4 ⁺		285.192		292.209	
		y9 ⁺	623.830	875.483	627.338	882.500	15
		y7 ⁺		717.414		724.431	
		y6 ⁺		660.393		667.410	
		y5 ⁺		547.309		554.326	
		y4 ⁺		434.225		434.225	
EELGTIL <u>L</u> TGEK	[M+2H ⁺]	b2 ⁺		259.092		259.092	
		y9 ⁺	651.861	931.546	655.370	938.563	22
		y7 ⁺		773.477		780.494	
		y6 ⁺		660.393		667.410	
		y5 ⁺		547.309		554.326	
		y4 ⁺		434.225		434.225	
		b2 ⁺		259.092		259.092	
AAG <u>I</u> ESGFFELQPK	[M+2H ⁺]	y10 ⁺	747.385	1181.584	750.894	1181.584	24
		y9 ⁺		1052.541		1052.541	
		y8 ⁺		965.509		965.509	
		y6 ⁺		761.419		761.419	
		y2 ⁺		244.166		244.166	
		a4 ⁺		285.192		292.209	
EELGTVL <u>L</u> TGEK	[M+2H ⁺]	y9 ⁺	644.853	917.530	648.362	924.547	14
		y7 ⁺		759.461		766.478	
		y6 ⁺		660.393		667.410	
		y5 ⁺		547.309		554.326	
		y4 ⁺		434.225		434.225	
		b2 ⁺		259.092		259.092	
IGSFEEEL <u>L</u> K	[M+2H ⁺]	y8 ⁺	526.269	938.447	529.778	945.464	20
		y7 ⁺		881.425		888.442	
		y6 ⁺		794.393		801.410	
		y5 ⁺		647.325		654.342	
		y4 ⁺		518.282		525.299	
DGIVSLGGPH <u>I</u> PLK	[M+3H ⁺]	y8 ⁺	468.273	818.488	470.612	825.505	16
		y7 ⁺		761.467		768.484	
		y11 ⁺⁺		559.340		562.849	
		y10 ⁺⁺		509.806		513.314	
IYPTVDPTMDPDYAEYLK	[M+2H ⁺]	y12 ⁺	1066.001	1442.651	1069.008	1442.651	31

		y9 ⁺		1113.510		1113.510	
		y8 ⁺		998.483		998.483	
		y16 ⁺⁺		927.927		930.934	
		b6 ⁺		689.350		695.364	
		[y16-b6] ⁺		413.203 ^d		419.217 ^d	
MSSITGGQE <u>V</u> R	[M+2H] ⁺	y10 ⁺	582.788	1033.527	585.794	1039.541	19
		y9 ⁺		946.495		952.509	
		y8 ⁺		859.463		865.477	
		y7 ⁺		746.379		752.393	
		y6 ⁺		645.331		651.345	
AFEI <u>I</u> EDLR	[M+2H] ⁺	y7 ⁺	553.298	887.483	556.807	894.500	20
		y6 ⁺		758.441		765.458	
		y5 ⁺		645.357		652.374	
		y4 ⁺		532.273		532.273	
		b3 ⁺		348.155		348.155	

Stable isotope-labeled amino acids are underlined. All labeled amino acids were uniformly labeled with ¹³C and ¹⁵N isotopes.

Table S3: Average RA values and percent change in RA values for all transitions from 2011, 2012, 2013 and 2014. Significant changes ($\alpha = 0.05$) are highlighted in red.

Peptide & Sequence	Precursor Ion <i>m/z</i>	Product ion <i>m/z</i>	RA 9/11	RA 12/11	$\Delta\%$ RA 12'	$\Delta\%$ RA 13'	$\Delta\%$ RA 14'
AAGIDSGFFELQPK	743.886	244.166	0.316	0.285	NA	24.32%	3.58%
AAGIDSGFFELQPK	743.886	292.209	0.028	0.013	NA	111.33%	37.12%
AAGIDSGFFELQPK	743.886	761.419	0.105	0.103	NA	14.26%	1.05%
AAGIDSGFFELQPK	743.886	965.509	0.117	0.124	NA	-6.58%	-4.95%
AAGIDSGFFELQPK	743.886	1052.541	0.139	0.152	NA	-13.86%	-0.63%
AAGIDSGFFELQPK	743.886	1167.568	0.296	0.323	NA	-21.63%	-2.85%
AFEIIEDLR	556.807	348.155	0.175	0.161	-3.43%	2.95%	-10.85%
AFEIIEDLR	556.807	532.273	0.145	0.130	5.42%	8.37%	6.96%
AFEIIEDLR	556.807	652.374	0.269	0.272	1.94%	1.11%	2.75%
AFEIIEDLR	556.807	765.458	0.192	0.208	-0.64%	-3.12%	1.50%
AFEIIEDLR	556.807	894.500	0.219	0.229	-2.78%	-5.33%	-0.95%
AGPGAFSLTSEIASHLPTK	631.006	607.327	0.263	0.221	-8.08%	-4.20%	-36.48%
AGPGAFSLTSEIASHLPTK	631.006	695.886	0.139	0.153	4.24%	3.44%	5.09%
AGPGAFSLTSEIASHLPTK	631.006	769.420	0.306	0.318	4.24%	1.47%	7.11%
AGPGAFSLTSEIASHLPTK	631.006	833.450	0.177	0.198	-0.11%	0.60%	20.62%
AGPGAFSLTSEIASHLPTK	631.006	881.976	0.114	0.110	-3.85%	-1.67%	8.76%
DGIVSLGGPHIPLK	470.612	513.314	0.296	0.252	-1.14%	8.11%	-18.12%
DGIVSLGGPHIPLK	470.612	562.849	0.596	0.603	2.80%	-1.98%	1.20%
DGIVSLGGPHIPLK	470.612	768.484	0.049	0.064	-50.13%	-8.23%	16.88%
DGIVSLGGPHIPLK	470.612	825.505	0.059	0.082	-10.47%	-6.92%	33.71%
DLGFEFTPVK	579.807	201.123	0.307	0.322	-1.67%	6.28%	-2.40%
DLGFEFTPVK	579.807	229.118	0.131	0.124	-2.66%	-1.69%	8.10%
DLGFEFTPVK	579.807	349.248	0.100	0.103	1.51%	6.99%	8.05%
DLGFEFTPVK	579.807	597.364	0.057	0.056	13.47%	1.82%	1.86%
DLGFEFTPVK	579.807	726.406	0.121	0.118	-15.16%	-4.22%	-1.83%
DLGFEFTPVK	579.807	930.496	0.285	0.277	4.71%	-7.71%	-3.40%
DYFVDER	475.218	251.103	0.299	0.316	-8.37%	0.25%	-17.11%
DYFVDER	475.218	279.098	0.149	0.144	5.61%	-2.18%	7.14%
DYFVDER	475.218	419.188	0.077	0.075	9.64%	5.27%	10.97%
DYFVDER	475.218	524.271	0.131	0.122	7.34%	7.45%	12.92%
DYFVDER	475.218	671.339	0.344	0.344	-0.53%	-3.10%	5.76%
DYFVEER	482.226	251.103	0.337	0.350	-5.15%	0.23%	-10.11%
DYFVEER	482.226	433.204	0.104	0.103	1.80%	4.77%	7.97%
DYFVEER	482.226	538.286	0.181	0.170	4.45%	5.54%	10.50%
DYFVEER	482.226	685.355	0.378	0.377	1.90%	-4.02%	2.47%
EELGTILLTGEK	655.370	259.092	0.110	0.120	-2.10%	3.88%	-4.99%
EELGTILLTGEK	655.370	434.225	0.135	0.131	2.49%	9.30%	1.81%
EELGTILLTGEK	655.370	554.326	0.132	0.124	5.36%	9.21%	5.60%
EELGTILLTGEK	655.370	667.410	0.269	0.260	-0.98%	0.52%	1.70%
EELGTILLTGEK	655.370	780.494	0.164	0.161	-1.28%	-6.81%	-1.82%
EELGTILLTGEK	655.370	938.563	0.191	0.202	-1.68%	-9.26%	-2.39%
FDIGTLLGLIEK	663.393	263.103	0.205	0.219	-3.78%	2.52%	-7.69%
FDIGTLLGLIEK	663.393	566.362	0.229	0.204	0.22%	10.86%	0.76%
FDIGTLLGLIEK	663.393	679.446	0.161	0.163	-1.48%	2.56%	-2.75%
FDIGTLLGLIEK	663.393	792.530	0.106	0.112	2.53%	-3.57%	-1.60%

FDIGTLLGLIEK	663.393	950.599	0.299	0.302	2.26%	-9.20%	7.12%
FEIGSLLGLIEK	663.394	277.118	0.381	0.384	0.72%	5.68%	-5.17%
FEIGSLLGLIEK	663.394	566.364	0.116	0.107	7.90%	12.98%	5.76%
FEIGSLLGLIEK	663.394	679.448	0.077	0.078	4.35%	4.57%	7.16%
FEIGSLLGLIEK	663.394	936.586	0.207	0.212	-5.66%	-5.56%	3.19%
FEIGSLLGLIEK	663.394	1049.670	0.218	0.219	-1.92%	-12.52%	0.62%
FLEPGVPDFK	577.810	261.160	0.209	0.222	-4.70%	1.88%	-6.03%
FLEPGVPDFK	577.810	390.202	0.137	0.125	3.59%	0.03%	5.16%
FLEPGVPDFK	577.810	506.261	0.175	0.173	2.24%	9.77%	5.26%
FLEPGVPDFK	577.810	765.417	0.394	0.394	-0.58%	-3.45%	0.85%
FLEPGVPDFK	577.810	894.460	0.085	0.086	4.02%	-8.73%	-6.44%
FLKPGVPDFK	385.226	253.634	0.317	0.309	-9.64%	-2.38%	-22.04%
FLKPGVPDFK	385.226	294.181	0.306	0.358	-6.64%	-5.18%	-2.97%
FLKPGVPDFK	385.226	506.261	0.268	0.240	13.65%	9.49%	26.36%
FLKPGVPDFK	385.226	648.411	0.109	0.094	10.98%	3.34%	16.56%
FMKPGVPDFK	391.212	253.634	0.282	0.290	-0.47%	-3.90%	-34.19%
FMKPGVPDFK	391.212	294.181	0.347	0.368	-9.66%	-0.30%	15.07%
FMKPGVPDFK	391.212	506.261	0.257	0.237	7.73%	7.21%	18.14%
FMKPGVPDFK	391.212	666.368	0.114	0.105	11.70%	-4.51%	0.48%
GGILGLGGVGHMGVK	453.260	424.228	0.139	0.139	-3.29%	3.53%	-19.34%
GGILGLGGVGHMGVK	453.260	509.281	0.500	0.491	2.12%	1.11%	-2.56%
GGILGLGGVGHMGVK	453.260	565.823	0.360	0.370	-1.71%	-2.79%	10.67%
HIGIVLGGGLGHVAVK	511.983	422.759	NA	0.121	NA	NA	-31.44%
HIGIVLGGGLGHVAVK	511.983	577.346	NA	0.368	NA	NA	-3.85%
HIGIVLGGGLGHVAVK	511.983	610.367	NA	0.201	NA	NA	-21.65%
HIGIVLGGGLGHVAVK	511.983	804.473	NA	0.102	NA	NA	-27.46%
HIGIVLGGGLGHVAVK	511.983	844.511	NA	0.207	NA	NA	-17.18%
IGSFEEELK	529.778	525.299	0.059	0.057	-1.76%	17.77%	-15.22%
IGSFEEELK	529.778	654.342	0.125	0.131	1.53%	6.85%	0.73%
IGSFEEELK	529.778	801.410	0.103	0.103	8.14%	-0.02%	-1.35%
IGSFEEELK	529.778	888.442	0.260	0.246	1.14%	-2.17%	3.08%
IGSFEEELK	529.778	945.464	0.452	0.463	-2.90%	-2.96%	0.31%
IYPTVDPTMDPDYAEYLK	1069.008	695.364	NA	0.045	-21.67%	NA	17.50%
IYPTVDPTMDPDYAEYLK	1069.008	930.934	NA	0.609	12.09%	NA	5.59%
IYPTVDPTMDPDYAEYLK	1069.008	998.483	NA	0.172	-11.68%	NA	-1.44%
IYPTVDPTMDPDYAEYLK	1069.008	1113.510	NA	0.051	-18.30%	NA	-4.17%
IYPTVDPTMDPDYAEYLK	1069.008	1442.651	NA	0.122	-68.71%	NA	-30.59%
MSSITGGQEV	585.794	651.345	0.288	0.282	-5.60%	NA	-17.47%
MSSITGGQEV	585.794	752.393	0.463	0.461	2.59%	NA	6.09%
MSSITGGQEV	585.794	865.477	0.064	0.065	3.92%	NA	2.78%
MSSITGGQEV	585.794	952.509	0.123	0.124	-2.37%	NA	9.21%
MSSITGGQEV	585.794	1039.541	0.063	0.068	4.12%	NA	11.72%
SALDFLELQPDLSALVR	947.523	877.522	0.527	0.488	-3.09%	5.07%	-8.84%
SALDFLELQPDLSALVR	947.523	889.467	0.061	0.064	7.79%	1.54%	9.27%
SALDFLELQPDLSALVR	947.523	1005.580	0.170	0.169	5.12%	4.68%	12.29%
SALDFLELQPDLSALVR	947.523	1017.525	0.071	0.070	13.34%	-6.19%	14.74%
SALDFLELQPDLSALVR	947.523	1118.664	0.103	0.119	-0.88%	-10.66%	10.15%
SALDFLELQPDLSALVR	947.523	1247.707	0.069	0.089	-12.16%	-18.67%	-6.77%
SALDYLELQPDLSALVR	955.521	877.522	0.531	0.509	-0.51%	4.06%	-7.63%
SALDYLELQPDLSALVR	955.521	905.461	0.062	0.058	11.11%	3.69%	0.78%
SALDYLELQPDLSALVR	955.521	1005.580	0.165	0.167	4.47%	2.88%	13.13%
SALDYLELQPDLSALVR	955.521	1033.520	0.077	0.063	9.51%	-8.00%	3.75%
SALDYLELQPDLSALVR	955.521	1118.664	0.097	0.115	-3.93%	-6.88%	11.19%

SALDYLELQPDLSALVR	955.521	1247.707	0.069	0.088	-19.96%	-16.57%	1.50%
VCTLELFSPK	600.824	331.198	0.149	0.157	6.83%	12.06%	-6.59%
VCTLELFSPK	600.824	727.410	0.136	0.145	-7.29%	-2.98%	-11.71%
VCTLELFSPK	600.824	840.494	0.149	0.150	-9.41%	1.00%	3.06%
VCTLELFSPK	600.824	941.542	0.566	0.547	2.02%	-2.95%	4.16%
VEISQISIGDGVTLCR	877.46495	556.299	0.117	0.110	NA	33.62%	-13.22%
VEISQISIGDGVTLCR	877.46495	884.437	0.350	0.332	NA	4.72%	-0.98%
VEISQISIGDGVTLCR	877.46495	997.521	0.095	0.089	NA	3.32%	3.63%
VEISQISIGDGVTLCR	877.46495	1084.553	0.335	0.358	NA	-9.40%	7.27%
VEISQISIGDGVTLCR	877.46495	1197.637	0.103	0.110	NA	-19.82%	-10.39%
VGGLIGYDNTLWNGSVVAPADAPMR	860.437	757.366	0.516	0.488	-3.30%	-1.08%	-10.13%
VGGLIGYDNTLWNGSVVAPADAPMR	860.437	828.403	0.210	0.225	3.96%	2.13%	9.24%
VGGLIGYDNTLWNGSVVAPADAPMR	860.437	933.485	0.274	0.287	2.15%	0.16%	9.95%
VGGLIGYDNTLWNGSVVAPPDAPMR	869.109	392.195	0.306	0.272	12.92%	7.90%	-8.46%
VGGLIGYDNTLWNGSVVAPPDAPMR	869.109	783.382	0.528	0.527	-8.91%	-2.91%	7.91%
VGGLIGYDNTLWNGSVVAPPDAPMR	869.109	959.501	0.167	0.201	1.39%	-3.03%	-9.30%

Table S4: Percent change in RSD of RA values for all transitions from 2011, 2012, 2013 and 2014. Significant changes ($\alpha = 0.05$) are highlighted in red.

Peptide & Sequence	Precursor Ion m/z	Product Ion m/z	$\Delta\%$ RSD 12/11	$\Delta\%$ RSD 2012	$\Delta\%$ RSD 2013	$\Delta\%$ RSD 2014
AAGIDSGFFELQPK	743.886	244.166	0.13%	NA	1.90%	2.07%
AAGIDSGFFELQPK	743.886	292.209	-3.11%	NA	7.67%	0.54%
AAGIDSGFFELQPK	743.886	761.419	-2.61%	NA	1.84%	0.32%
AAGIDSGFFELQPK	743.886	965.509	-3.25%	NA	2.48%	-0.23%
AAGIDSGFFELQPK	743.886	1052.541	-0.50%	NA	1.28%	4.26%
AAGIDSGFFELQPK	743.886	1167.568	0.33%	NA	2.87%	2.03%
AFEIIEDLR	556.807	348.155	0.01%	8.06%	2.92%	2.46%
AFEIIEDLR	556.807	532.273	-0.57%	2.96%	0.07%	0.86%
AFEIIEDLR	556.807	652.374	-0.62%	1.07%	0.26%	8.97%
AFEIIEDLR	556.807	765.458	-0.76%	0.73%	0.78%	1.59%
AFEIIEDLR	556.807	894.500	-0.95%	7.23%	0.92%	1.53%
AGPGAFLSTSEIASHLPTK	631.006	607.327	-0.80%	7.59%	-1.06%	0.06%
AGPGAFLSTSEIASHLPTK	631.006	695.886	-2.56%	4.18%	0.28%	3.11%
AGPGAFLSTSEIASHLPTK	631.006	769.420	-0.48%	-0.20%	-0.50%	0.62%
AGPGAFLSTSEIASHLPTK	631.006	833.450	-2.80%	5.97%	-0.18%	0.30%
AGPGAFLSTSEIASHLPTK	631.006	881.976	-0.02%	9.07%	0.25%	0.37%
DGIVSLGGPHIPLK	470.612	513.314	-0.12%	5.44%	2.83%	0.37%
DGIVSLGGPHIPLK	470.612	562.849	-0.20%	0.43%	3.65%	4.74%
DGIVSLGGPHIPLK	470.612	768.484	-0.01%	27.20%	7.28%	2.12%
DGIVSLGGPHIPLK	470.612	825.505	-0.18%	8.21%	10.80%	-3.26%
DLGFETPVK	579.807	201.123	-0.58%	6.47%	1.15%	-1.32%
DLGFETPVK	579.807	229.118	-0.38%	6.71%	1.47%	5.27%
DLGFETPVK	579.807	349.248	-0.50%	4.69%	1.42%	1.67%
DLGFETPVK	579.807	597.364	-2.48%	18.20%	1.12%	1.19%
DLGFETPVK	579.807	726.406	-0.40%	12.66%	1.05%	1.32%
DLGFETPVK	579.807	930.496	0.10%	9.11%	-0.03%	6.12%
DYFVDER	475.218	251.103	-0.20%	11.57%	4.07%	0.36%
DYFVDER	475.218	279.098	-0.38%	4.27%	5.39%	9.68%
DYFVDER	475.218	419.188	-0.05%	8.10%	1.76%	2.07%
DYFVDER	475.218	524.271	0.43%	4.94%	0.74%	0.54%
DYFVDER	475.218	671.339	0.03%	4.17%	0.54%	0.32%
DYFVEER	482.226	251.103	-0.92%	6.59%	3.94%	-0.23%
DYFVEER	482.226	433.204	0.08%	0.39%	1.26%	4.26%
DYFVEER	482.226	538.286	-0.41%	2.76%	1.83%	2.03%
DYFVEER	482.226	685.355	-0.81%	4.48%	2.38%	2.46%
EELGTILLTGEK	655.370	259.092	-1.94%	4.26%	1.39%	0.86%
EELGTILLTGEK	655.370	434.225	-0.87%	0.93%	-0.29%	8.97%
EELGTILLTGEK	655.370	554.326	-1.35%	1.09%	0.61%	1.59%
EELGTILLTGEK	655.370	667.410	-0.16%	2.22%	1.16%	1.53%
EELGTILLTGEK	655.370	780.494	0.22%	0.36%	-0.40%	0.06%
EELGTILLTGEK	655.370	938.563	0.17%	0.55%	1.04%	3.11%
FDIGTLLGLIEK	663.393	263.103	-1.33%	10.14%	4.89%	0.62%
FDIGTLLGLIEK	663.393	566.362	-1.44%	5.65%	0.79%	0.30%
FDIGTLLGLIEK	663.393	679.446	-1.31%	0.93%	0.44%	0.37%
FDIGTLLGLIEK	663.393	792.530	-0.37%	7.64%	0.40%	0.37%

FDIGTLLGLIEK	663.393	950.599	-0.72%	8.17%	3.61%	4.74%
FEIGSLLGLIEK	663.394	277.118	-0.26%	6.57%	1.88%	2.12%
FEIGSLLGLIEK	663.394	566.364	-0.86%	1.10%	1.28%	-3.26%
FEIGSLLGLIEK	663.394	679.448	-1.16%	2.96%	2.18%	-1.32%
FEIGSLLGLIEK	663.394	936.586	-0.65%	2.73%	0.73%	5.27%
FEIGSLLGLIEK	663.394	1049.670	-0.03%	8.87%	1.17%	1.67%
FLEPGVPDFK	577.810	261.160	-0.15%	6.76%	1.83%	1.19%
FLEPGVPDFK	577.810	390.202	0.01%	6.57%	4.10%	1.32%
FLEPGVPDFK	577.810	506.261	-0.10%	2.55%	1.91%	6.12%
FLEPGVPDFK	577.810	765.417	0.22%	3.56%	0.74%	0.36%
FLEPGVPDFK	577.810	894.460	-0.38%	4.90%	0.68%	9.68%
FLKPGVPDFK	385.226	253.634	2.67%	8.80%	0.41%	2.07%
FLKPGVPDFK	385.226	294.181	-0.38%	3.24%	3.84%	0.54%
FLKPGVPDFK	385.226	506.261	1.46%	2.23%	-0.49%	0.32%
FLKPGVPDFK	385.226	648.411	3.11%	4.70%	3.64%	-0.23%
FMKPGVPDFK	391.212	253.634	-0.53%	16.34%	2.18%	4.26%
FMKPGVPDFK	391.212	294.181	-0.40%	9.03%	3.01%	2.03%
FMKPGVPDFK	391.212	506.261	-0.31%	10.23%	0.50%	2.46%
FMKPGVPDFK	391.212	666.368	0.17%	4.69%	6.10%	0.86%
GGILGLGGVGHMGVK	453.260	424.228	0.08%	7.44%	9.31%	8.97%
GGILGLGGVGHMGVK	453.260	509.281	-0.47%	3.21%	0.76%	1.59%
GGILGLGGVGHMGVK	453.260	565.823	-0.87%	7.40%	1.80%	1.53%
HIGIVLGGGLGHVAVK	511.983	422.759	NA	NA	NA	0.06%
HIGIVLGGGLGHVAVK	511.983	577.346	NA	NA	NA	3.11%
HIGIVLGGGLGHVAVK	511.983	610.367	NA	NA	NA	0.62%
HIGIVLGGGLGHVAVK	511.983	804.473	NA	NA	NA	0.30%
HIGIVLGGGLGHVAVK	511.983	844.511	NA	NA	NA	0.37%
IGSFEEELK	529.778	525.299	-0.48%	16.64%	6.89%	0.37%
IGSFEEELK	529.778	654.342	-0.16%	16.00%	1.81%	4.74%
IGSFEEELK	529.778	801.410	-0.71%	1.07%	1.09%	2.12%
IGSFEEELK	529.778	888.442	-0.47%	4.36%	1.82%	-3.26%
IGSFEEELK	529.778	945.464	-0.51%	4.55%	0.65%	-1.32%
IYPTVDPTMDPDYAEYLK	1069.008	695.364	NA	12.99%	NA	5.27%
IYPTVDPTMDPDYAEYLK	1069.008	930.934	NA	2.48%	NA	1.67%
IYPTVDPTMDPDYAEYLK	1069.008	998.483	NA	-7.78%	NA	1.19%
IYPTVDPTMDPDYAEYLK	1069.008	1113.510	NA	4.06%	NA	1.32%
IYPTVDPTMDPDYAEYLK	1069.008	1442.651	NA	1.98%	NA	6.12%
MSSITGGQEV	585.794	651.345	-0.21%	10.33%	NA	0.36%
MSSITGGQEV	585.794	752.393	-0.32%	3.50%	NA	9.68%
MSSITGGQEV	585.794	865.477	-0.40%	7.57%	NA	2.07%
MSSITGGQEV	585.794	952.509	-0.58%	2.01%	NA	0.54%
MSSITGGQEV	585.794	1039.541	-0.23%	11.62%	NA	0.32%
SALDFLELQPDLSALVR	947.523	877.522	-1.22%	5.20%	1.22%	-0.23%
SALDFLELQPDLSALVR	947.523	889.467	-7.68%	3.46%	-0.64%	4.26%
SALDFLELQPDLSALVR	947.523	1005.580	-0.61%	2.40%	0.86%	2.03%
SALDFLELQPDLSALVR	947.523	1017.525	-3.52%	7.50%	4.19%	2.46%
SALDFLELQPDLSALVR	947.523	1118.664	0.22%	7.94%	3.62%	0.86%
SALDFLELQPDLSALVR	947.523	1247.707	-3.18%	7.66%	2.51%	8.97%
SALDYLELQPDLSALVR	955.521	877.522	-0.58%	3.90%	2.51%	1.59%
SALDYLELQPDLSALVR	955.521	905.461	-1.14%	6.41%	4.85%	1.53%
SALDYLELQPDLSALVR	955.521	1005.580	0.36%	4.53%	1.05%	0.06%
SALDYLELQPDLSALVR	955.521	1033.520	-0.74%	7.94%	3.73%	3.11%
SALDYLELQPDLSALVR	955.521	1118.664	-0.62%	1.82%	2.42%	0.62%

SALDYLELQPDLSALVR	955.521	1247.707	-1.59%	3.20%	2.49%	0.30%
VCTLELFSPK	600.824	331.198	-0.27%	6.24%	0.68%	0.37%
VCTLELFSPK	600.824	727.410	-0.43%	3.83%	0.80%	0.37%
VCTLELFSPK	600.824	840.494	-0.97%	2.76%	0.59%	4.74%
VCTLELFSPK	600.824	941.542	-0.15%	1.58%	0.47%	2.12%
VEISQISIGDGVTLCR	877.46495	556.299	-6.44%	NA	1.00%	-3.26%
VEISQISIGDGVTLCR	877.46495	884.437	-0.58%	NA	0.61%	-1.32%
VEISQISIGDGVTLCR	877.46495	997.521	-2.74%	NA	1.50%	5.27%
VEISQISIGDGVTLCR	877.46495	1084.553	-0.47%	NA	0.97%	1.67%
VEISQISIGDGVTLCR	877.46495	1197.637	-1.97%	NA	3.04%	1.19%
VGGLIGYDNTLWNGSVVAPADAPMR	860.437	757.366	-0.99%	4.97%	3.44%	1.32%
VGGLIGYDNTLWNGSVVAPADAPMR	860.437	828.403	-1.43%	3.92%	3.36%	6.12%
VGGLIGYDNTLWNGSVVAPADAPMR	860.437	933.485	-0.80%	4.81%	3.12%	0.36%
VGGLIGYDNTLWNGSVVAPPDAPMR	869.109	392.195	-1.69%	1.79%	2.57%	9.68%
VGGLIGYDNTLWNGSVVAPPDAPMR	869.109	783.382	-0.52%	6.81%	0.27%	2.07%
VGGLIGYDNTLWNGSVVAPPDAPMR	869.109	959.501	-3.17%	13.64%	3.23%	9.68%

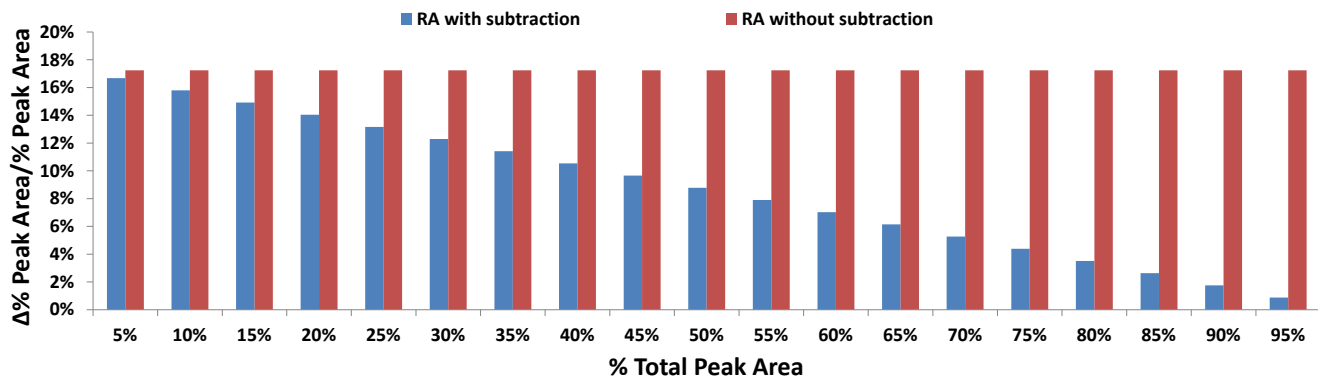


Figure S1: Shows theoretical data using RA calculations with (equation 2) and without subtraction (equation 3) of the transition in question. Thresholds were calculated for each method as described in Figure 3C using their respective equations. The advantage of subtracting the transition in question allows for a more sensitive method as shown, detecting smaller changes in peak area. However, the method which subtracts the area of the transition of interest fluctuates in the percent peak area change allowed. Subtracting the transition results in a bias against transitions with higher RA values. RA calculation without subtraction allows for uniform change in the percent peak area of any given transition, regardless of its composition of the total area.

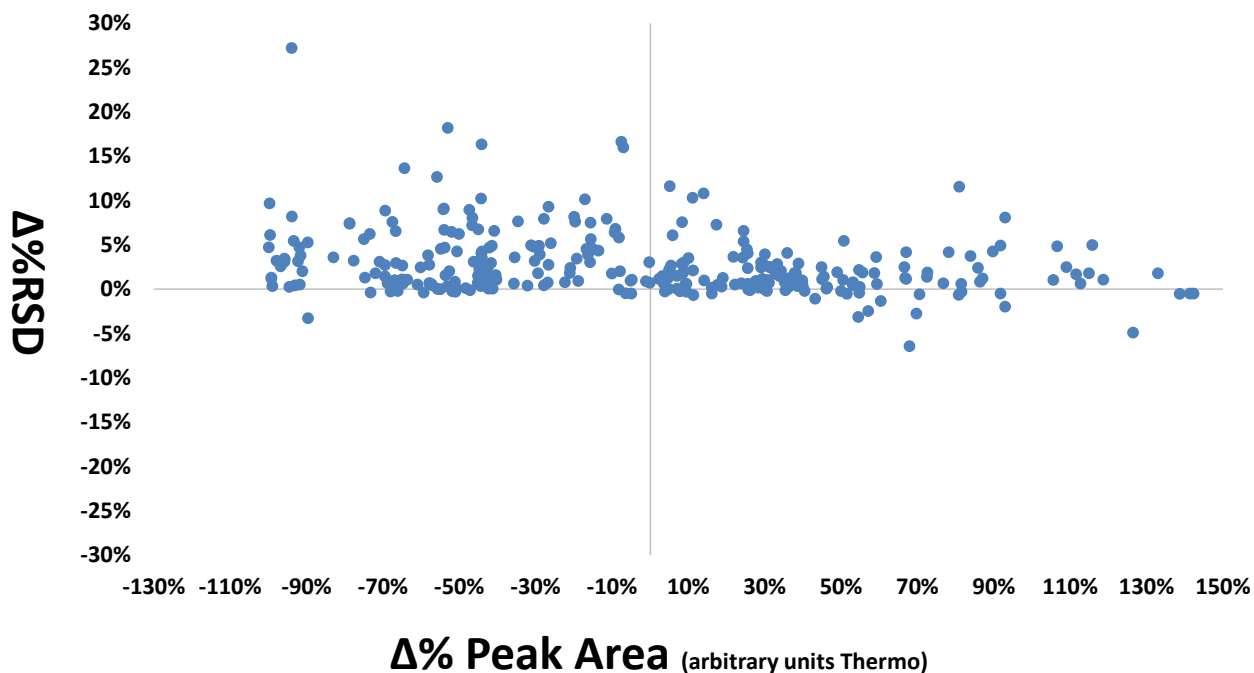


Figure S2: A scatter plot showing the change in the relative standard deviation of transitions from 2011-2014 as a function of the percent change in peak area. Data with increasing RSD were equally likely to increase as they were to decrease in peak area. Several of the points which had a decreased RSD were found to have an increased peak area.

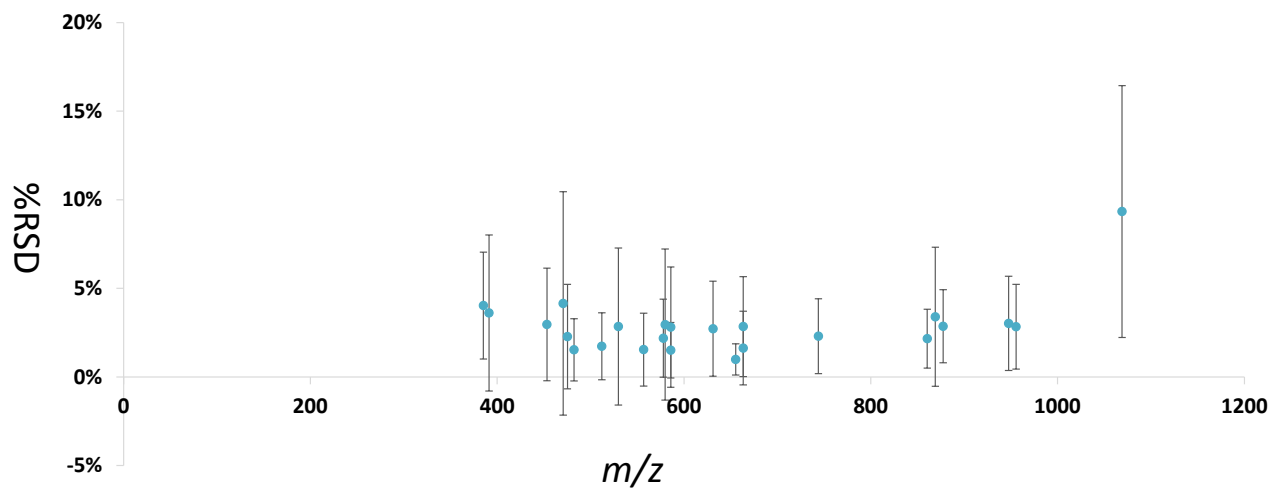


Figure S4: A scatter plot showing the mean %RSD and standard deviation for peptides as a function of precursor m/z . There was little overall correlation of %RSD with precursor m/z however, the peptide with the highest m/z “IYPTVDPTMDPDYAEYLK” was observed to have a significantly higher %RSD and variation in %RSD than all other peptides shown.