Electronic Supplementary Material (ESI) for Analyst.
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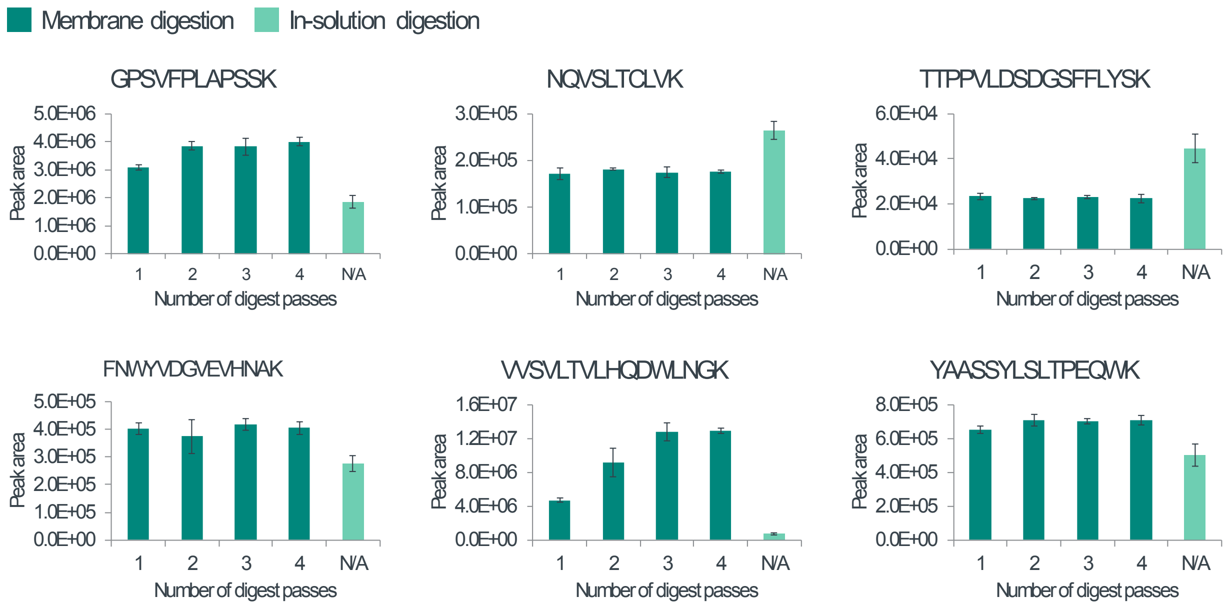
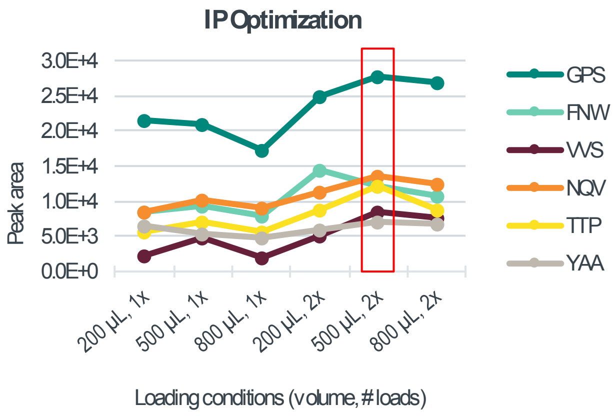
Supplemental Figures and Tables

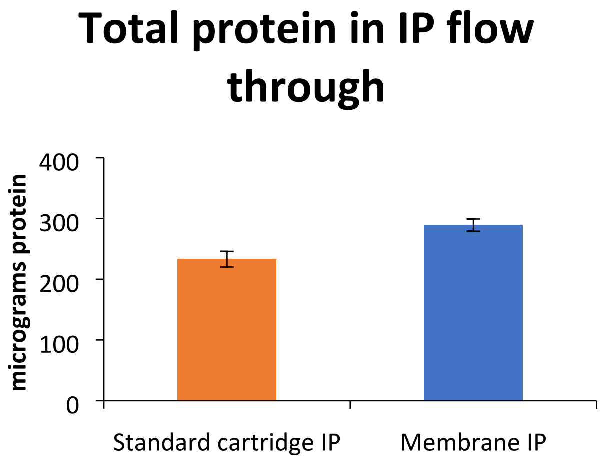
Supplemental Figure 1: Membrane based trypsin digest pass optimization for surrogate peptides selected for MRM



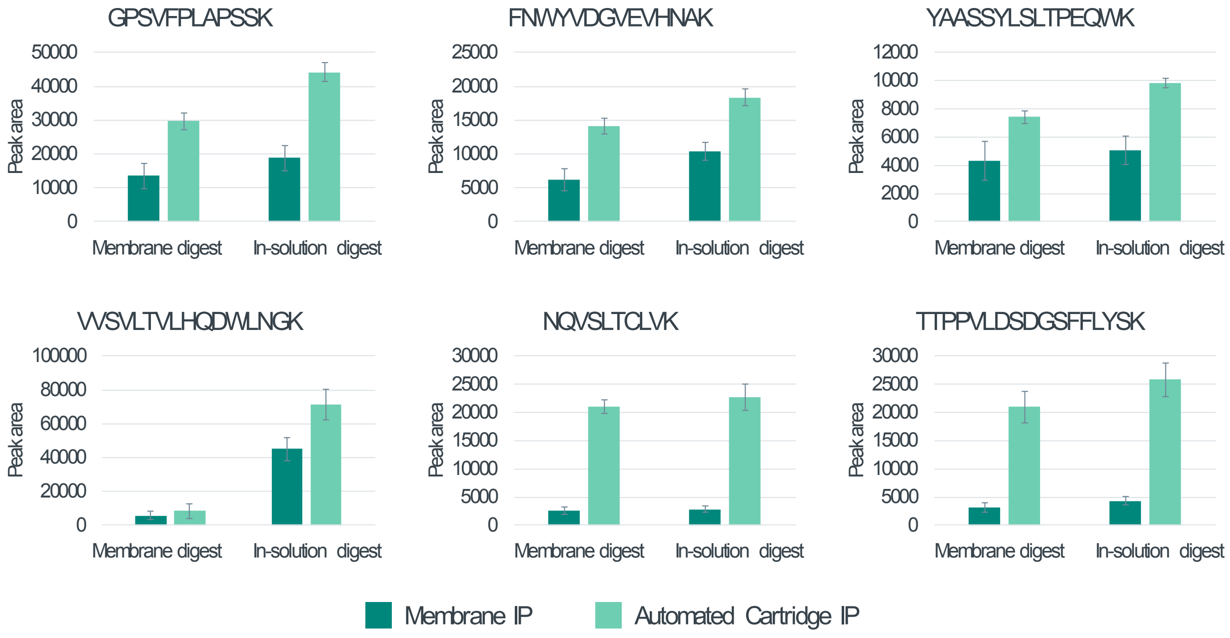
Supplemental Figure 2: Protein A IP optimization. AUC is plotted for six SILuLite peptides as a function of IP loading volume and number of loads. Results for the preferred condition, 500 µL loaded twice, are boxed in red.



Supplemental Figure 3: Total protein measurement in IP flow through from automated cartridge IP (light green) and membrane IP (dark green).



Supplemental Figure 4: Peak area results for surrogate peptides processed by mixed-mode, fully membrane, or fully standard methods. N=8 for each experimental condition.

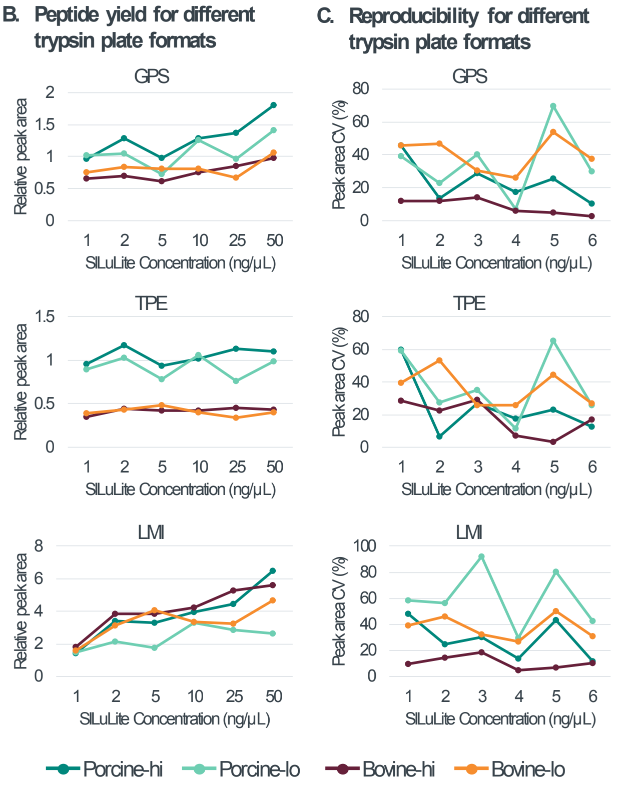


Supplemental Figure 5:

96-well digestion plates fabricated with high (hi) or low (lo) capacity membranes containing DM-porcine or bovine trypsin. The LLOQ is reported for 5 µL of plasma from n=4 standard curves processed by the membranes or the standard method (A). Digest efficiency was assessed using absolute peak areas without normalization to the SILuMAb heavy IS. Peak area results for each membrane format are plotted relative to results from the standard method to normalize across all levels of the curve (B). The CV in absolute peak area for n=4 standard curves is plotted for each membrane format (C).

A. LLOQ (ng/µL) for 5 µL plasma

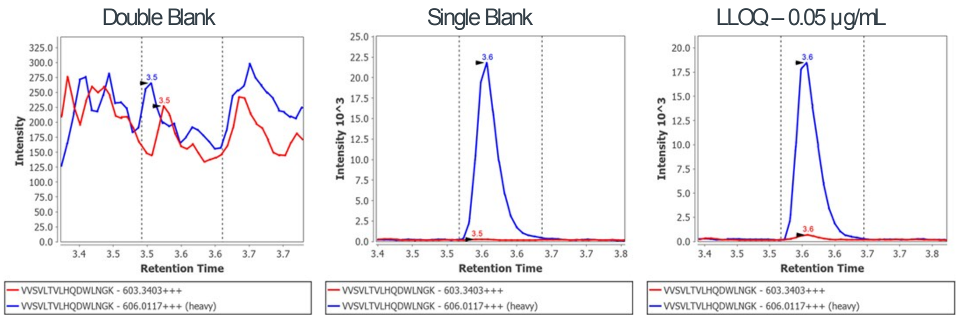
	Standard method	Porcine-hi	Porcine-lo	Bovine-hi	Bovine-lo
FNW	0.5	1	5	1	1
GPS	0.2	0.2	0.2	0.1	0.2
LMI	2	0.2	1	0.1	0.1
NQV	0.2	2	2	2	2
TPE	0.5	0.5	0.5	0.5	0.5
TTP	0.1	5	N/A	2	5
VVS	0.2	0.5	1	0.5	0.2
YAA	0.5	10	5	0.5	1


B. Peptide yield for different trypsin plate formats

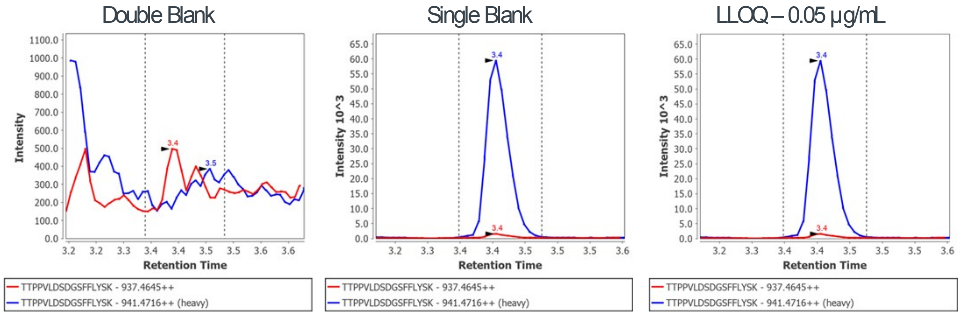
C. Reproducibility for different trypsin plate formats

Supplemental Figure 6: Representative chromatograms for VVS (A) and TTP (B) peptides in 20 µL of blank rat plasma (double blank), rat plasma containing only the internal standard (single blank), and rat plasma containing SILuLite at the LLOQ generated by the standard workflow.

A. Standard workflow VVS

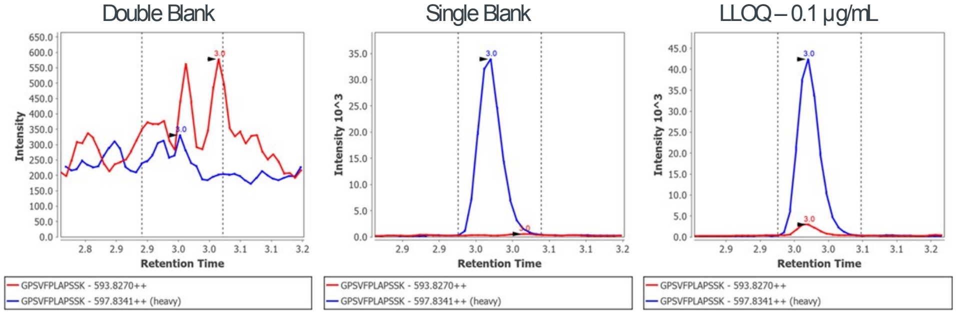


B. Standard workflow TTP

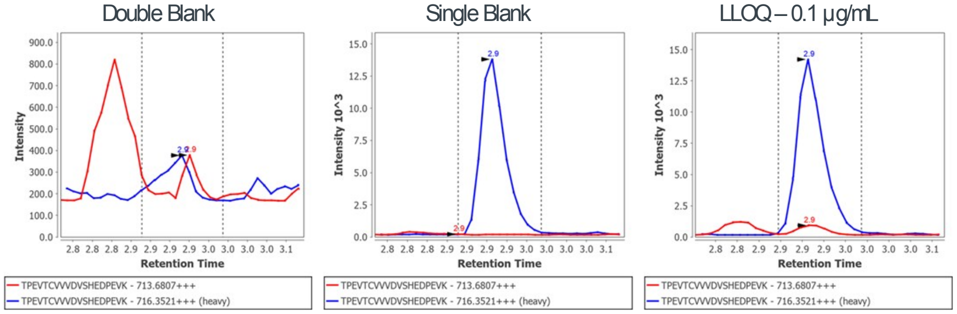


Supplemental Figure 7: Representative chromatograms for GPS (A,C) and TPE (B,D) peptides in 20 µL of blank rat plasma (double blank), rat plasma containing only the internal standard (single blank), and rat plasma containing SILuLite at the LLOQ generated by the membrane workflow using high (A-B) and low (C-D) capacity 96-well dimethyl-porcine trypsin plates.

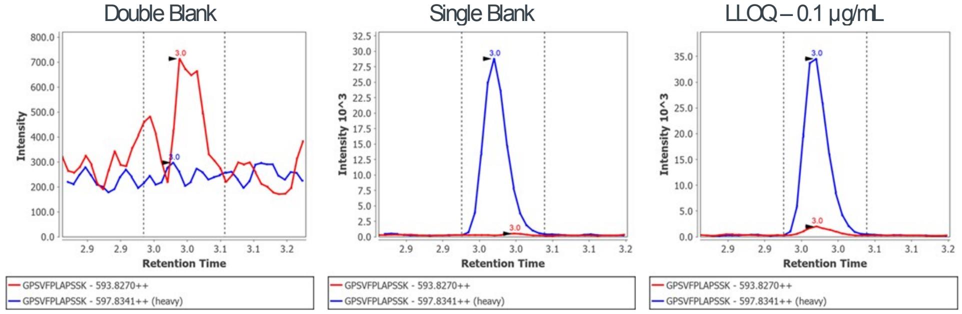
A. Porcine high capacity GPS



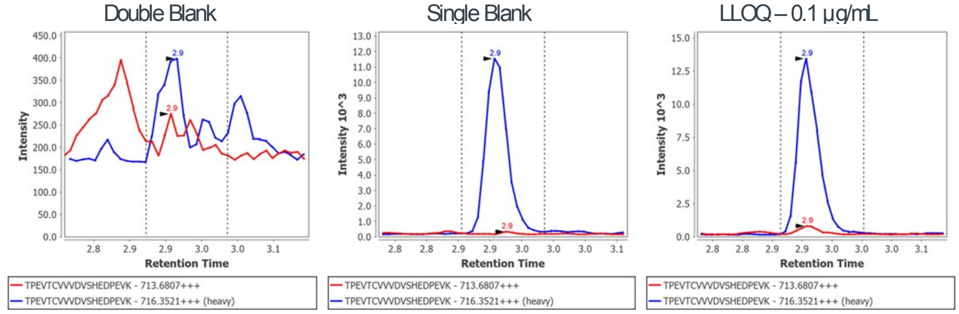
B. Porcine high capacity TPE



C. Porcine low capacity GPS

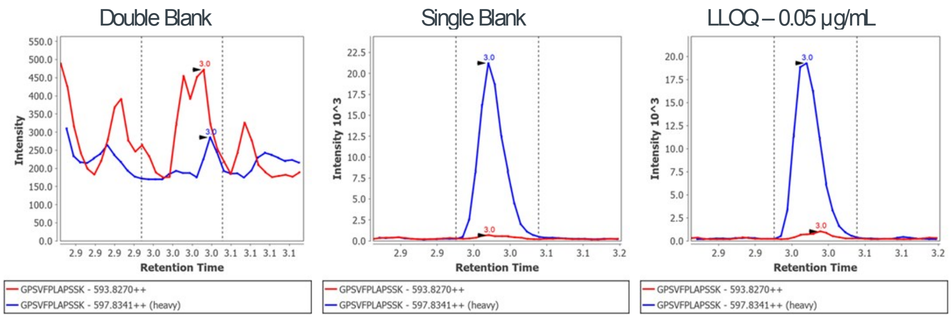


D. Porcine low capacity TPE

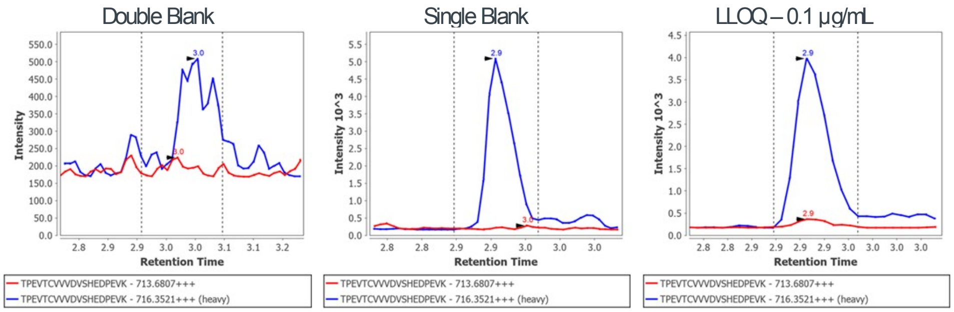


Supplemental figure 8: Representative chromatograms for GPS (A,C) and TPE (B,D) peptides in 20 µL of blank rat plasma (double blank), rat plasma containing only the internal standard (single blank), and rat plasma containing SILuLite at the LLOQ generated by the membrane workflow using high (A-B) and low (C-D) capacity 96-well bovine trypsin plates.

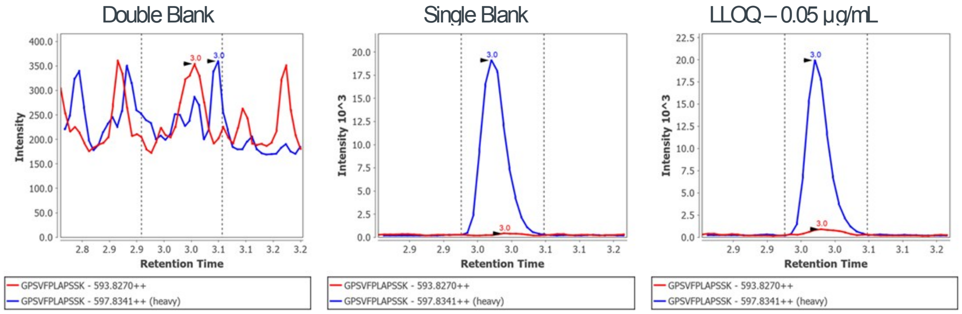
A. Bovine high capacity GPS



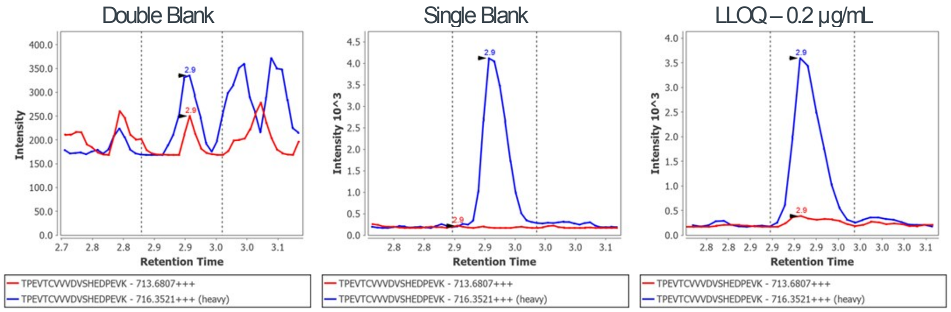
B. Bovine high capacity TPE



C. Bovine low capacity GPS



D. Bovine low capacity TPE



Supplemental Table 1: SILuLite heavy chain (A) and light chain (B) peptides identified by MaxQuant database search with intensities listed as a function of digest condition

A. Heavy chain peptides

Sequence	Missed cleavages	Intensity membrane pass 1	Intensity membrane pass 2	Intensity membrane pass 3	Intensity membrane pass 4	Intensity in solution digest
ALPAPIEK	0	0.00E+00	0.00E+00	9.45E+08	9.55E+08	1.52E+09
DSLYLQMNSLR	0	5.08E+09	1.14E+09	1.20E+09	1.13E+09	4.67E+08
DTLMISR	0	7.79E+08	8.43E+08	8.04E+08	7.83E+08	5.39E+08
EEQYNSTYR	0	1.07E+06	0.00E+00	0.00E+00	0.00E+00	0.00E+00
EPQVYTLPPSR	0	2.43E+06	5.22E+06	6.72E+06	9.42E+06	8.50E+06
EVQLVESGGGLVQPGGSLR	0	2.87E+09	3.06E+09	3.18E+09	3.11E+09	3.45E+09
FNWYVDGVEVHNAK	0	3.15E+09	3.03E+09	3.57E+09	3.14E+09	2.78E+09
GFYPSDIAVEWESNGQPENNYK	0	1.80E+08	2.11E+08	2.07E+08	1.80E+08	0.00E+00
GLEWVSK	0	4.12E+07	8.93E+07	1.15E+08	1.35E+08	1.80E+07
GPSVFPLAPSSK	0	1.67E+09	1.89E+09	1.70E+09	1.73E+09	1.75E+09
LSCVASGFTLNNYDMHWVR	0	1.88E+08	3.67E+08	5.85E+08	8.41E+08	2.29E+07
NQVSLTCLVK	0	1.57E+08	1.43E+08	1.55E+08	1.43E+08	2.60E+08
SLSLSPG	0	0.00E+00	0.00E+00	6.78E+07	0.00E+00	2.03E+08
STSGGTAALGCLVK	0	1.12E+09	1.38E+09	1.45E+09	1.29E+09	4.59E+08
TPEVTCVVVDVSHEDPEVK	0	3.67E+09	3.99E+09	3.85E+09	3.25E+09	3.21E+09
TTPPVLDSDGSFFLYSK	0	2.64E+08	3.02E+08	2.96E+08	2.42E+08	4.58E+08
VGDAAVYYCAR	0	1.28E+09	1.35E+09	1.36E+09	1.32E+09	2.05E+09
VVSVLTVLHQDWLNGK	0	6.79E+08	1.75E+09	2.25E+09	2.41E+09	8.95E+05
WAPLGAFDIWGQGTMVTVSSASTK	0	1.94E+09	2.27E+09	2.18E+09	2.36E+09	6.15E+08
WQQGNVFSCSVMHEALHNHYTQK	0	3.41E+08	2.95E+08	2.84E+08	2.67E+08	1.01E+08
YYAGSVK	0	6.92E+05	1.92E+06	2.18E+06	4.68E+06	1.13E+06
ALPAPIEKTISK	1	1.13E+06	1.27E+06	1.06E+06	5.89E+05	0.00E+00
DTLMISRTPEVTCVVVDVSHEDPEVK	1	3.87E+06	0.00E+00	0.00E+00	0.00E+00	0.00E+00
ENAKDSLYLQMNSLR	1	9.26E+08	6.78E+08	4.15E+08	2.40E+08	1.06E+09
EPQVYTLPPSRDELTK	1	1.99E+08	1.27E+08	2.26E+07	8.04E+07	2.16E+08
FTISRENAK	1	2.33E+08	2.39E+06	8.53E+05	2.08E+05	0.00E+00
GAGRWAPLGAFDIWGQGTMVTVSSASTK	1	4.15E+07	1.41E+07	9.16E+06	5.57E+06	7.95E+06
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK	1	2.91E+06	0.00E+00	0.00E+00	0.00E+00	0.00E+00
GPSVFPLAPSSKSTSGGTAALGCLVK	1	3.50E+06	7.76E+06	9.82E+06	7.98E+06	0.00E+00
GQPREPQVYTLPPSR	1	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.30E+06
GRFTISR	1	4.12E+06	2.51E+06	0.00E+00	0.00E+00	6.39E+06
IGTAGDRYYAGSVK	1	2.77E+07	4.21E+07	3.33E+07	2.48E+07	5.60E+07
LSCVASGFTLNNYDMHWVRQGIGK	1	0.00E+00	0.00E+00	5.12E+06	5.69E+06	0.00E+00
QGIGKGLEWVSK	1	0.00E+00	0.00E+00	0.00E+00	1.76E+06	0.00E+00
SRWQQGNVFSCSVMHEALHNHYTQK	1	1.17E+07	4.62E+06	2.12E+06	7.11E+05	9.62E+06
THTCPPCPAPELLGGPSVFLFPPKPK	1	7.71E+08	7.74E+08	8.38E+08	5.47E+08	5.03E+08

TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK	1	5.10E+07	2.14E+05	0.00E+00	0.00E+00	0.00E+00
VSNKALPAPIEK	1	1.50E+06	1.91E+06	1.54E+06	1.40E+06	6.71E+05
VVSVLTVLHQDWLNGKEYK	1	3.69E+09	3.54E+09	2.41E+09	1.67E+09	7.36E+08
WAPLGAFDIWGQGTMVTVSSASTKGPSVFPLAPSSK	1	1.54E+08	1.43E+07	7.48E+06	4.40E+06	5.62E+07
FNWYVDGVEVHNAKTKPR	2	2.43E+06	3.33E+06	2.92E+06	1.86E+06	0.00E+00
FTISRENAKDSLYLQMNSLR	2	9.02E+07	1.09E+07	0.00E+00	0.00E+00	4.42E+06
GAGRWAPLGAFDIWGQGTMVTVSSASTKGPSVFPLAPSSK	2	3.66E+07	2.97E+06	1.61E+06	0.00E+00	1.73E+07
GLEWVSKIGTAGDRYYAGSVK	2	0.00E+00	1.66E+06	0.00E+00	0.00E+00	0.00E+00
GQPREPQVYTLPPSRDELTK	2	1.60E+07	2.81E+06	5.40E+05	0.00E+00	4.67E+07
GRFTISRENAK	2	4.33E+07	1.24E+06	3.47E+05	0.00E+00	9.21E+06
LSCVASGFTLNNYDMHWVRQGIGKGLEWVSK	2	8.93E+07	2.39E+08	3.98E+08	5.41E+08	4.70E+07
SCDKTHTCPPCPAPELLGGPSVFLFPPKPK	2	9.16E+08	3.09E+08	1.06E+08	1.70E+07	4.79E+08
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISR	2	2.39E+07	0.00E+00	0.00E+00	0.00E+00	0.00E+00
TKPREEQYNSTYR	2	9.16E+06	1.72E+05	0.00E+00	0.00E+00	4.15E+06
VDKKVEPK	2	1.59E+05	0.00E+00	0.00E+00	0.00E+00	4.52E+05
VVSVLTVLHQDWLNGKEYKCK	2	0.00E+00	2.28E+06	0.00E+00	0.00E+00	0.00E+00
AKGQPREPQVYTLPPSRDELTK	3	1.19E+07	1.05E+06	0.00E+00	0.00E+00	6.54E+06
GRFTISRENAKDSLYLQMNSLR	3	8.01E+07	6.81E+06	0.00E+00	0.00E+00	1.96E+08
LSCVASGFTLNNYDMHWVRQGIGKGLEWVSKIGTAGDR	3	4.81E+08	7.24E+08	1.16E+09	8.39E+08	9.03E+07
QGIGKGLEWVSKIGTAGDRYYAGSVK	3	2.58E+06	0.00E+00	0.00E+00	0.00E+00	3.15E+07
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR	3	2.45E+07	0.00E+00	0.00E+00	0.00E+00	0.00E+00

B. Light chain peptides

Sequence	Missed cleavages	Intensity membrane pass 1	Intensity membrane pass 2	Intensity membrane pass 3	Intensity membrane pass 4	Intensity in-solution digest
AAPSVTLFPPSSEELQANK	0	6.57E+08	1.08E+09	1.27E+09	1.29E+09	5.64E+08
ADSSPVK	0	3.82E+06	5.16E+06	5.28E+06	4.92E+06	7.11E+06
AGVETTTPSK	0	1.71E+07	1.81E+07	1.72E+07	1.61E+07	3.16E+07
ATLVCLISDFYPGAVTVAWK	0	2.48E+09	3.50E+09	5.11E+09	3.83E+09	7.49E+08
LMIYDATK	0	9.87E+08	1.47E+09	1.28E+09	1.25E+09	7.39E+08
LTVLGQPK	0	4.76E+08	6.71E+08	5.69E+08	5.25E+08	1.13E+06
SGNTASLTISGLQAEDEADYYCCSYAGDYTPGVVFGGGTK	0	8.12E+08	9.22E+08	9.26E+08	8.78E+08	8.22E+08
SVSGSPGQSVTISCTGTSSDIGGYNFVSWYQQHPGK	0	1.23E+09	2.32E+09	2.75E+09	3.45E+09	2.55E+09
SYSCQVTHEGSTVEK	0	3.17E+09	3.39E+09	3.20E+09	3.03E+09	2.36E+09
TVAPTECS	0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.89E+06
YAASSYLSLTPEQWK	0	1.65E+09	1.80E+09	1.82E+09	1.63E+09	1.80E+09
AAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK	1	5.36E+08	1.38E+08	0.00E+00	2.10E+07	2.80E+08
ADSSPVKAGVETTTPSK	1	2.16E+07	2.63E+05	0.00E+00	1.51E+06	3.81E+07

APKLMIYDATK	1	0.00E+00	1.43E+06	1.92E+06	2.25E+06	0.00E+00
ATLVCLISDFYPGAVTVAWKADSSPVK	1	2.17E+08	5.59E+07	9.10E+06	3.61E+06	2.35E+07
LMIYDATKR	1	1.22E+07	1.69E+07	1.29E+07	9.17E+06	4.07E+06
LTVLGQPKAAPSVTLFPPSSEELQANK	1	9.08E+06	0.00E+00	0.00E+00	0.00E+00	0.00E+00
QSNNKYAASSYLSLTPEQWK	1	1.68E+08	6.78E+06	0.00E+00	3.07E+06	5.23E+07
RPSGVPDR	1	5.43E+06	9.54E+06	1.28E+07	1.32E+07	1.57E+07
SHRSYSCQVTHEGSTVEK	1	2.43E+06	2.90E+06	0.00E+00	0.00E+00	3.96E+06
SVSGSPGQSVTISCTGTSSDIGGYNFVSWYQQHPGKAPK	1	3.58E+09	3.23E+09	2.37E+09	1.48E+09	1.16E+09
SYSCQVTHEGSTVEKTVAPTECS	1	5.40E+06	4.56E+06	3.27E+06	3.01E+06	5.04E+06
YAASSYLSLTPEQWKSHR	1	4.25E+06	5.52E+06	5.29E+06	0.00E+00	0.00E+00
AGVETTTPSKQSNNKYAASSYLSLTPEQWK	2	4.11E+06	0.00E+00	0.00E+00	0.00E+00	0.00E+00
LMIYDATKRPSGVPDR	2	8.01E+08	4.21E+08	3.15E+07	4.90E+06	7.52E+08
RPSGVPDRFSGSK	2	1.37E+09	1.59E+09	9.19E+08	4.11E+08	9.48E+08
LMIYDATKRPSGVPDRFSGSK	3	6.14E+08	1.11E+08	4.85E+06	0.00E+00	2.89E+08

Supplemental Table 2: Surrogate peptide and MRM transition selection for targeted assay development

Peptide	Charge state	Transitions	Antibody Chain	Endogenous in rat?
GPSVFPLAPSSK	2+	b5, y4, y7, y8	Heavy	No
FNWYVDGVEVHNAK	3+	y6, y10, y11, y12	Heavy	No
VVSVLTVLHQDWLNGK	3+	y11, y12, y14	Heavy	No
NQVSLTCLVK	2+	b3, y5, y7, y8	Heavy	No
TTPPVLDSDGSFFLYSK	2+	y10, y11, y15	Heavy	No
YAASSYLSLTPEQWK	2+	y5, y6, y8, y9	Light	No

Supplemental Table 3: Precision and accuracy results for the best performing peptides in each workflow for replicate SILuLite standard curves in 20 µL of rat plasma processed using (A) the standard workflow, (B) the DM-porcine high capacity membrane, (C) the DM-porcine low capacity membrane, (D) the bovine high capacity membrane, and (E) the bovine low capacity membrane.

A. Standard Workflow

	VVS				TTP			
Concentration (ng/µL)	n	Mean	CV (%)	Bias	n	Mean	CV (%)	Bias

50	4	53.51	5.96	7.03	4	51.83	8.96	3.66
25	4	25.28	4.39	1.11	4	25.67	9.72	2.7
10	4	11.48	7.49	14.84	4	11.97	10.93	19.75
5	4	5.08	6.96	1.68	4	4.49	12.58	-10.13
2	4	1.89	5.52	-5.4	4	1.86	5.71	-7.12
1	4	0.94	1.97	-5.94	4	1.02	9.98	1.82
0.5	4	0.45	9.87	-9.08	4	0.45	15.35	-9.28
0.2	4	0.19	6.84	-6.87	4	0.19	16.78	-6.39
0.1	4	0.1	9.18	-0.66	4	0.1	21.45	4.79
0.05	4	0.05	14.59	3.29	4	0.05	16.84	0.21

B. DM-porcine trypsin high capacity membrane

	GPS				TPE			
Concentration (ng/μL)	n	Mean	CV (%)	Bias	n	Mean	CV (%)	Bias
50	4	56.73	5.44	13.46	4	55.43	13.32	10.87
25	4	24.64	9.24	-1.45	4	24.85	6.53	-0.61
10	4	10.69	13.84	6.88	4	11.11	9.24	11.07
5	4	4.89	12.46	-2.27	4	5.01	9.46	0.28
2	4	2	7.3	-0.17	4	1.93	4.44	-3.33
1	4	0.95	12.58	-4.54	4	0.93	9.57	-7.09
0.5	4	0.45	9.7	-9.54	4	0.48	8.29	-4.9
0.2	4	0.18	13.54	-9.4	4	0.17	5.71	-16.01
0.1	4	0.11	7.04	7.03	4	0.11	25.8	9.72
0.05	0	NA	NA	NA	0	NA	NA	NA

C. DM-porcine trypsin low capacity membrane

	GPS				TPE			
Concentration (ng/μL)	n	Mean	CV (%)	Bias	n	Mean	CV (%)	Bias
50	4	52.86	7.86	5.73	4	52.43	4.14	4.86
25	4	24.01	7.6	-3.96	4	26.21	7.57	4.86
10	4	11.29	10.66	12.91	4	10.41	2.48	4.08
5	4	4.96	4.28	-0.79	4	4.68	11.9	-6.43
2	4	1.83	9.61	-8.71	4	1.87	14.02	-6.66
1	4	1.04	3.98	4.01	4	0.93	7.78	-6.83
0.5	4	0.46	8.46	-8.75	4	0.52	12.28	4.42
0.2	4	0.19	13.77	-4.22	4	0.21	17.37	3.04
0.1	4	0.1	17.26	3.78	4	0.1	11.09	-1.33
0.05	0	NA	NA	NA	0	NA	NA	NA

D. Bovine trypsin high capacity membrane

	GPS				TPE			
Concentration (ng/μL)	n	Mean	CV (%)	Bias	n	Mean	CV (%)	Bias
50	4	50.72	7.75	1.43	4	54.45	9.08	8.9

25	4	27.88	14.87	11.51	4	25.42	13.4	1.67
10	4	10.31	12.31	3.13	4	11.14	8.56	11.42
5	4	4.96	11.29	-0.88	4	4.58	3.74	-8.41
2	4	1.9	14.54	-4.92	4	1.72	15.81	-14.1
1	4	1	11.5	0.44	4	1.1	15.4	10.06
0.5	4	0.45	5.86	-9.27	4	0.44	7.54	-11.58
0.2	0	NA	NA	NA	0	NA	NA	NA
0.1	4	0.1	23.45	-4.86	4	0.1	20.5	2.05
0.05	4	0.05	16.99	3.43	0	NA	NA	NA

E. Bovine trypsin low capacity membrane

	GPS				TPE			
Concentration (ng/µL)	n	Mean	CV (%)	Bias	n	Mean	CV (%)	Bias
50	4	52.81	2.45	5.62	4	54.52	9.08	9.04
25	4	25.9	9.5	3.61	4	25.38	9.67	1.5
10	4	10.66	15.61	6.6	4	10.49	16.12	4.91
5	4	4.96	3.38	-0.71	4	4.98	15.69	-0.33
2	4	2.01	9.8	0.68	4	1.61	5.17	-19.63
1	4	1.02	11.11	1.95	4	1.02	16.44	2.22
0.5	4	0.47	5.64	-6.21	4	0.51	13.4	1.51
0.2	4	0.17	10.72	-14.11	4	0.2	22.74	0.78
0.1	4	0.1	20.8	-2.87	0	NA	NA	NA
0.05	4	0.05	18.65	5.43	0	NA	NA	NA

Supplemental Table 4: Precision and accuracy results for the (A) GPS peptide and (B) TPE peptide in replicate SILuLite standard curves in 5 µL of rat plasma processed by the membrane or standard method for quantitation of PK samples.

A. GPSVFPLAPSSK

	Membrane				Standard method			
Concentration (ng/µL)	n	mean	CV (%)	Bias	n	mean	CV (%)	Bias
500	3	507.04	10.2	1.41	3	560.81	7.68	12.16

250	3	276.53	8.45	10.61	3	264.62	4	5.85
100	3	108.25	2.3	8.25	3	114.01	6.84	14.01
50	3	48.05	7.12	-3.9	3	50.35	4.98	0.71
25	3	24.13	2.36	-3.49	3	23.23	0.74	-7.09
10	3	10.01	3.94	0.06	3	9.63	4.02	-3.71
5	3	4.67	1.35	-6.61	3	4.22	13.1	-15.5
2.5	3	2.21	7.49	-11.75	3	2.24	2	-10.39
1	3	1.05	10.24	4.72	3	1	4.08	0.2
0.5	3	0.5	5.72	0.69	3	0.52	3.24	3.75

B. TPEVTCVVVDVSHEDPEVK

	Membrane				Standard method			
Concentration (ng/µL)	n	mean	CV (%)	Bias	n	mean	CV (%)	Bias
500	3	526.69	2.84	5.34	3	559.95	6.3	11.99
250	3	267.77	8.67	7.11	3	257.25	9.92	2.9
100	3	110.34	13.13	10.34	3	112.46	6.08	12.46
50	3	50.89	4.86	1.78	3	50.41	5.01	0.83
25	3	23.68	12.31	-5.28	3	23.82	9.92	-4.72
10	3	9.12	9.67	-8.83	3	9.95	8.37	-0.53
5	3	4.62	4.56	-7.64	3	4.05	15.22	-19.05
2.5	3	2.37	5.69	-5.05	3	2.27	12.5	-9.39
1	3	1	8.36	0.02	3	1.03	12.54	3.41
0.5	3	0.51	10.91	2.22	3	0.51	2.59	2.11