

Supplemental material to:

Catalin E. Doneanu, Alex Xenopoulos, Keith Fadgen, Jim Murphy, St John

Skilton, Holly Prentice, et al. Analysis of host-cell proteins in biotherapeutic

proteins by comprehensive online two-dimensional liquid chromatography/mass

spectrometry. *mAbs* 2012; 4(1); DOI: 10.4161/mabs.4.1.18748;

<http://www.landesbioscience.com/journals/mabs/article/18748>

1: TOF MS ES+
BPI
800

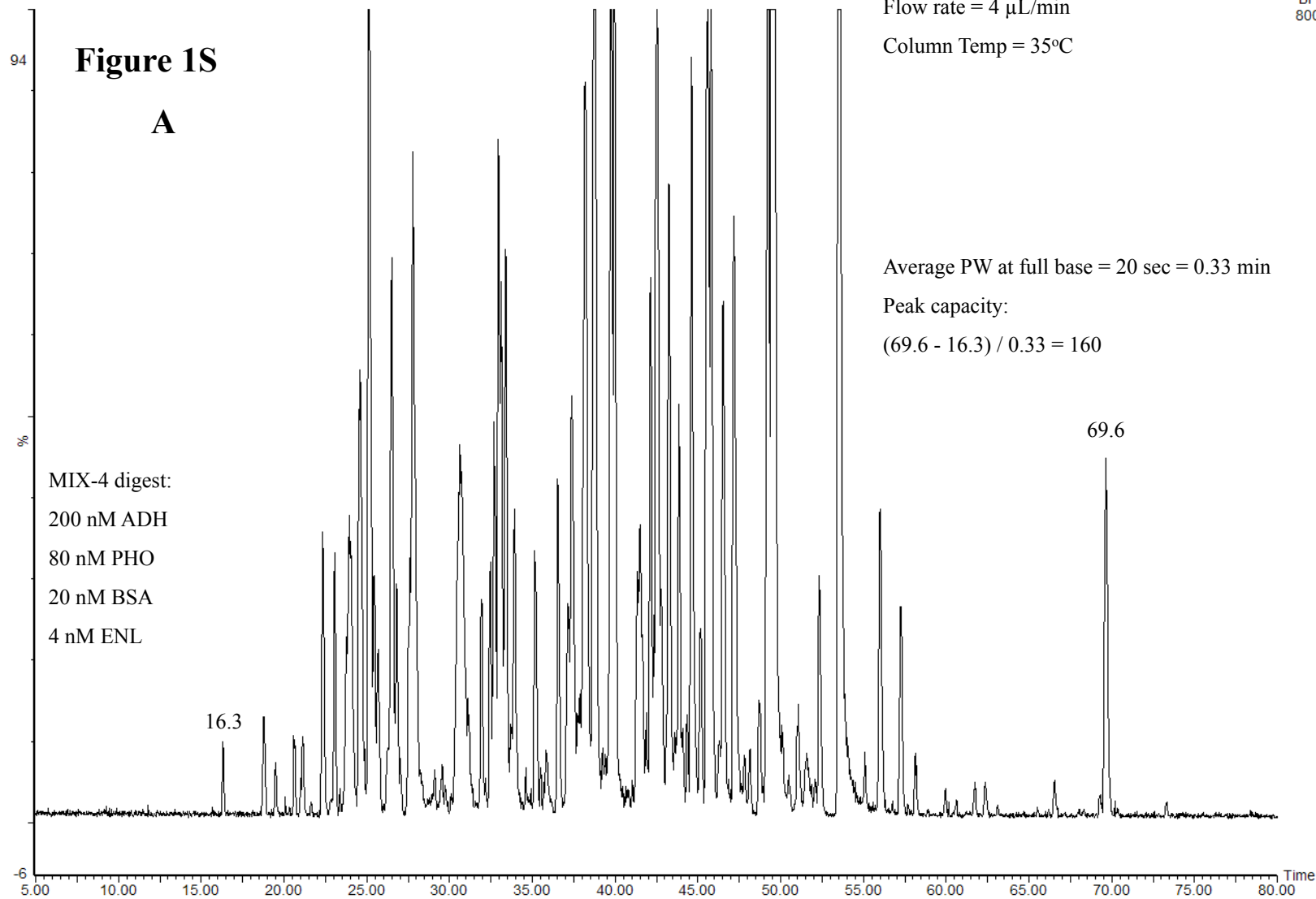
Flow rate = 4 μ L/min

Column Temp = 35°C

Average PW at full base = 20 sec = 0.33 min

Peak capacity:

$$(69.6 - 16.3) / 0.33 = 160$$



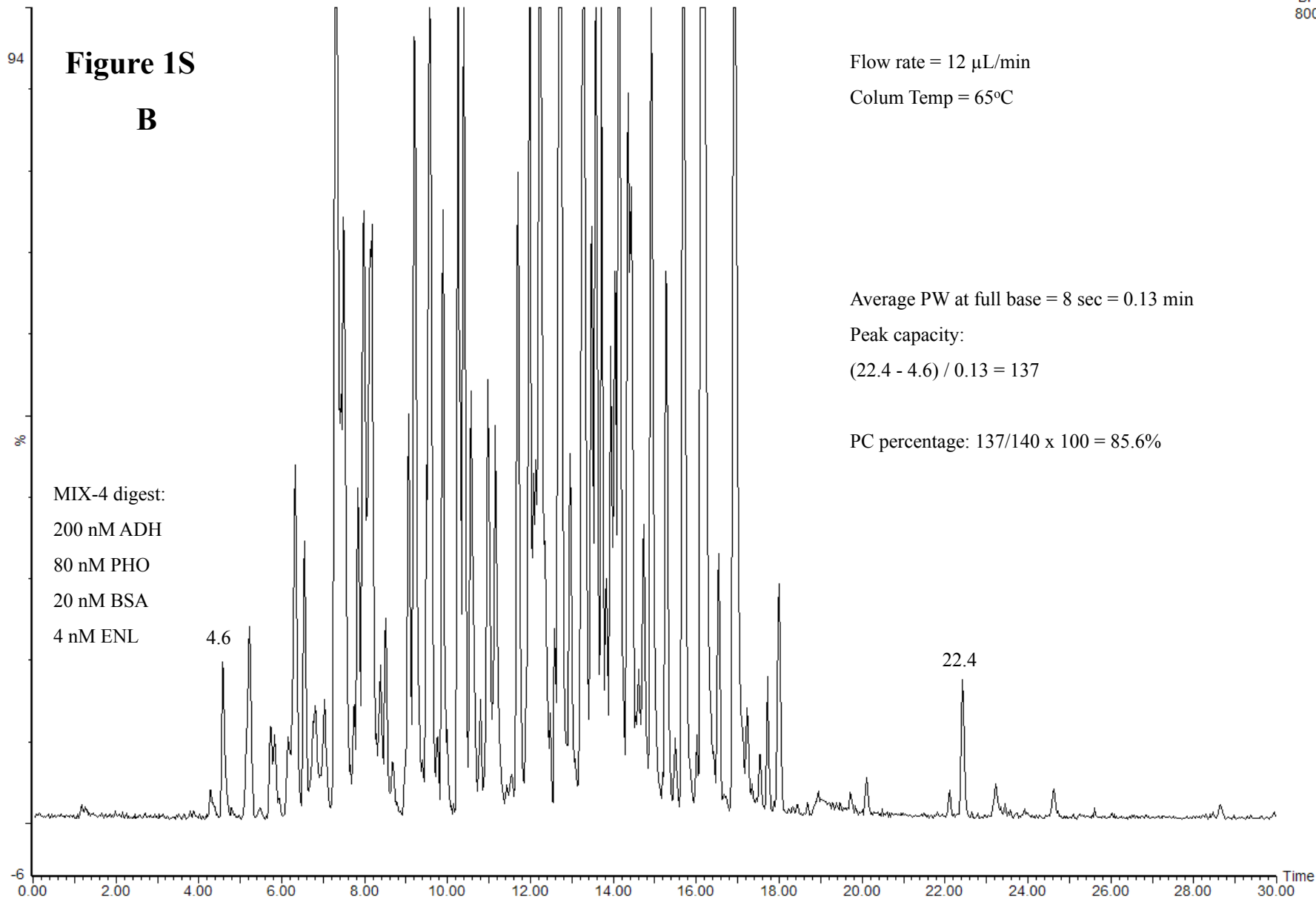


Figure 2S

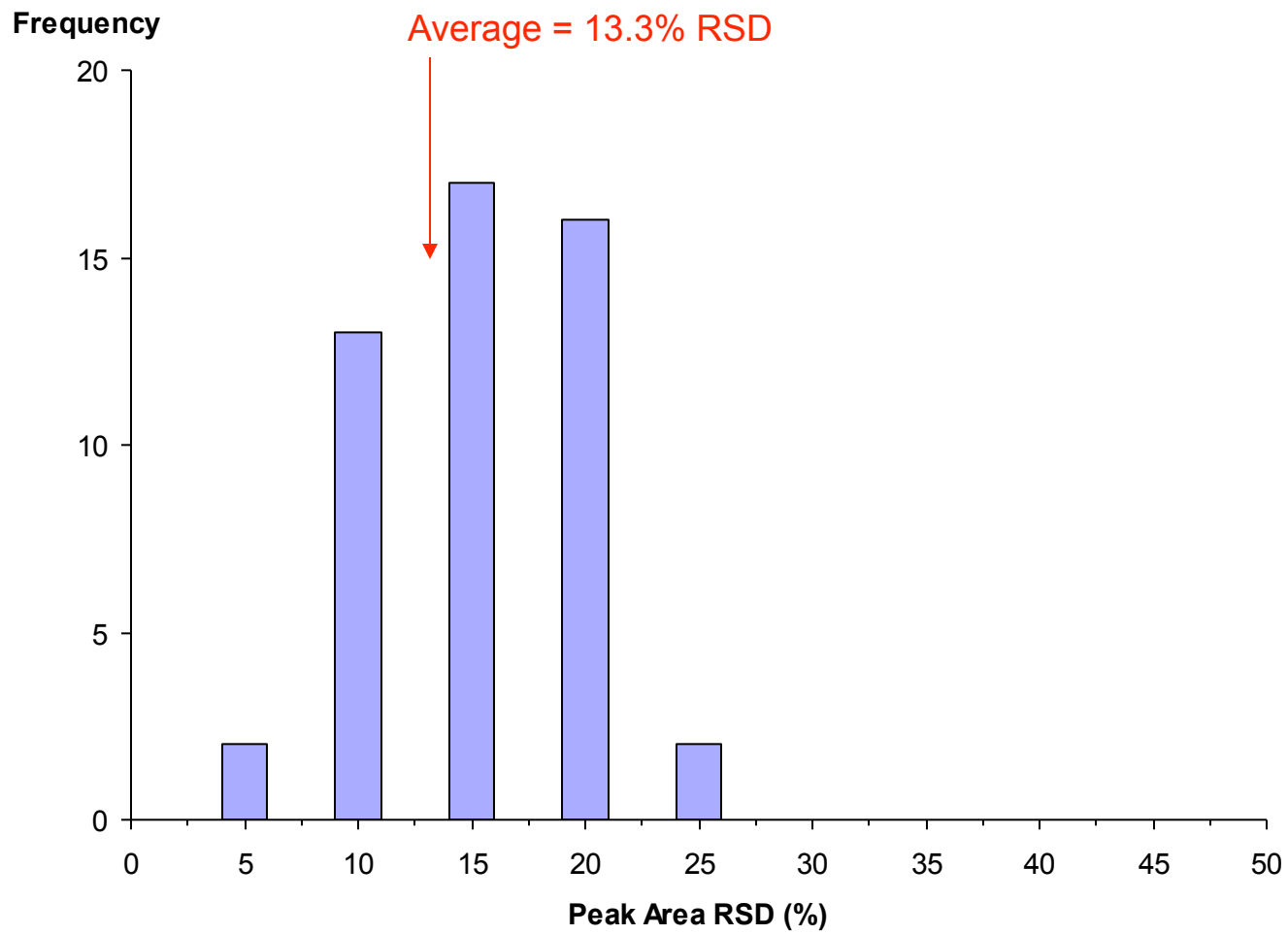


Table IS

No crt	Peptide sequence Sequence	Protein ID / species	SwissProt Accession no	Retention time (min)	Precursor / Charge state	Fragment I	Fragment II	Collision Energy (eV)	Dwell Time (ms)
1	GVEKPLR	Insulin-like growth factor binding protein 4 (mouse)	P47879	5.9	399.7 (+2)	385.2 (y3)	513.3 (y4)	16	20
2	GPSSVEDIK	Nucleophosmin (mouse)	Q61937	7.5	466.2 (+2)	603.3 (y5)	774.4 (y7)	18	20
3	STTTGHLIYK	Elongation factor 1-alpha (mouse)	P10126	7.7	560.8 (+2)	730.4 (y6)	831.5 (y7)	22	20
4	GFVLHK	Plasminogen activator/inhibitor 1 RNA bp (mouse)	Q9CY58	8.0	350.7 (+2)	397.3 (y3)	496.3 (y4)	15	20
5	VEIANDQGNR	78 kDa glucose regulated protein (hamster)	P07823	8.1	614.8 (+2)	774.3 (y7)	887.5 (y8)	24	20
6	GAAQNIIPASTGAAK	Glyceraldehyde 3-P dehydrogenase (hamster)	Q0QET9	9.1	685.4 (+2)	702.4 (y8)	815.5 (y9)	26	20
7	APIIAVTR	Pyruvate kinase isozyme M1/M2 (mouse)	P52480	9.4	420.8 (+2)	446.3 (y4)	559.3 (y5)	17	20
8	ELAEDGYSGVEVR	40S ribosomal protein S3 (mouse)	P62908	9.5	712.3 (+2)	646.4 (y6)	981.5 (y9)	27	25
9	SVSLYYTGEK	Nucleolin (hamster)	P08199	9.7	573.8 (+2)	760.3 (y6)	960.5 (y8)	22	20
10	IEDLSQQAQLAAAEK	Nascent polypeptide-associated complex alpha su (mouse)	P70670	9.8	807.9 (+2)	730.4 (y7)	929.5 (y9)	30	20
11	ALIAAQYSGAQR	Elongation factor 1-gamma (mouse)	Q9D8N0	9.9	674.4 (+2)	780.4 (y7)	979.5 (y9)	26	20
12	DLKDYFSK	Heterogeneous nuclear riboprotein D0 isoform (mouse)	Q60668	10.6	508.3 (+2)	659.3 (y5)	787.4 (y6)	20	20
13	IFVGGLSPDTPPEEK	Heterogeneous nuclear riboprotein D0 isoform (mouse)	Q60668	11.0	744.9 (+2)	815.4 (y7)	1228.6 (y12)	28	20
14	IFVGGLNPEATEEK	Heterogeneous nuclear riboprotein A/B isoform (mouse)	Q99020	11.3	752.4 (+2)	803.4 (y7)	1144.6 (y11)	28	20
15	AELNEFLTR	40S ribosomal protein S3 (mouse)	P62908	11.6	546.8 (+2)	779.4 (y6)	892.5 (y7)	21	15
16	LSSFIGAIAGDLVK	T complex protein 1 beta su (mouse)	P80314	11.8	752.4 (+2)	474.3 (y4)	531.3 (y5)	28	25
17	SLNLSLEEA	Clusterin (mouse)	Q06890	11.9	552.3 (+2)	790.4 (y7)	903.5 (y8)	21	25
18	SPASDTYIVFGEAK	Nascent polypeptide-associated complex alpha su (mouse)	P70670	11.9	742.9 (+2)	551.3 (y5)	1229.6 (y11)	28	15
19	VLFDTGLVNPR	Nidogen-1 (mouse)	P10493	12.5	615.8 (+2)	756.4 (y7)	1018.5 (y9)	24	30
20	LGGSAVISLEGKPL	Cofilin-1 (mouse)	P18760	12.7	670.9 (+2)	743.4 (y7)	856.5 (y8)	25	35
21	GFGFVDFNSEEDAK	Nucleolin (hamster)	P08199	13.1	781.3 (+2)	1054.4 (y9)	1153.5 (y10)	29	15
22	IYVDDGLISLQVK	Pyruvate kinase isozyme M1/M2 (mouse)	P52480	13.6	731.9 (+2)	1087.6 (y10)	1186.7 (y11)	28	20
23	VHLVGIDIFTGK	Eukaryotic translation/initiation factor 5A-1 (mouse)	P63242	13.7	649.9 (+2)	680.4 (y6)	1062.6 (y10)	25	25
24	LSAEDLVLEGAGLR	Laminin gamma-1 subunit (mouse)	P02468	13.8	721.9 (+2)	715.4 (y7)	814.5 (y8)	27	30
25	LVPGGGATEIELAK	T complex protein 1 theta su (mouse)	P42932	14.7	677.9 (+2)	684.4 (y6-H2O)	1045.6 (y11)	26	30

Table IIS

Peptide sequence	Protein ID	RT (min)	MRM Transition		CV (V)	CE (eV)
			Native	¹³ C ¹⁵ N		
SLLNSLEEAK	Clusterin	12.2	552.3 -> 903.5	556.3 -> 911.5	35	21
STTTGHLIYK	Elongation factor	8.3	560.8 -> 831.5	564.8 -> 839.5	35	22
GAAQNIIPASTGAAK	GAD3-PDH	9.6	685.4 -> 702.4	689.4 -> 710.4	35	26
VLFD TGLVNP R	Nidogen-1	12.9	615.8 -> 1018.5	620.8 -> 1028.5	35	24

Table IIS

Sample no	Sample ID	CHO Cell Line	Protein conc (mg/mL)	No of HCPs Identified	Common HCPs	HCP conc (ppm)	PTG1 purity (%)
1	A1	DG44	5.00	21	4	5945	94.1
2	A2	DG44	7.37	23	4	7190	92.8
3	B1	DG44	5.79	30	4	12897	87.1
4	B2	DG44	4.47	30	4	15302	84.3
5	C	CHO-S	3.88	5	4	1151	98.8
6	D	CHO-S	5.00	9	4	1020	99.0