

Supplementary information for “Dimethyl sulfoxide as a gas phase charge-reducing agent for the determination of pegylated proteins’ intact mass”

Øystein Skjærvø, Alyssa Togle, Haley Sutton, Xuemei Han, Navin Rauniyar
Tanvex CDMO, 10394 Pacific Center Ct, San Diego, CA 92121, USA

Contents

Charge stripping dependence of triethylamine	1
Optimization of dimethyl sulfoxide content	2
Post-column infusion flowrate	3
Injection volume	4
Optimization of MS interface parameters	6
Deconvolution of PEG-filgrastim	7
Deconvolution of Neulasta®	8

Charge stripping dependence of triethylamine

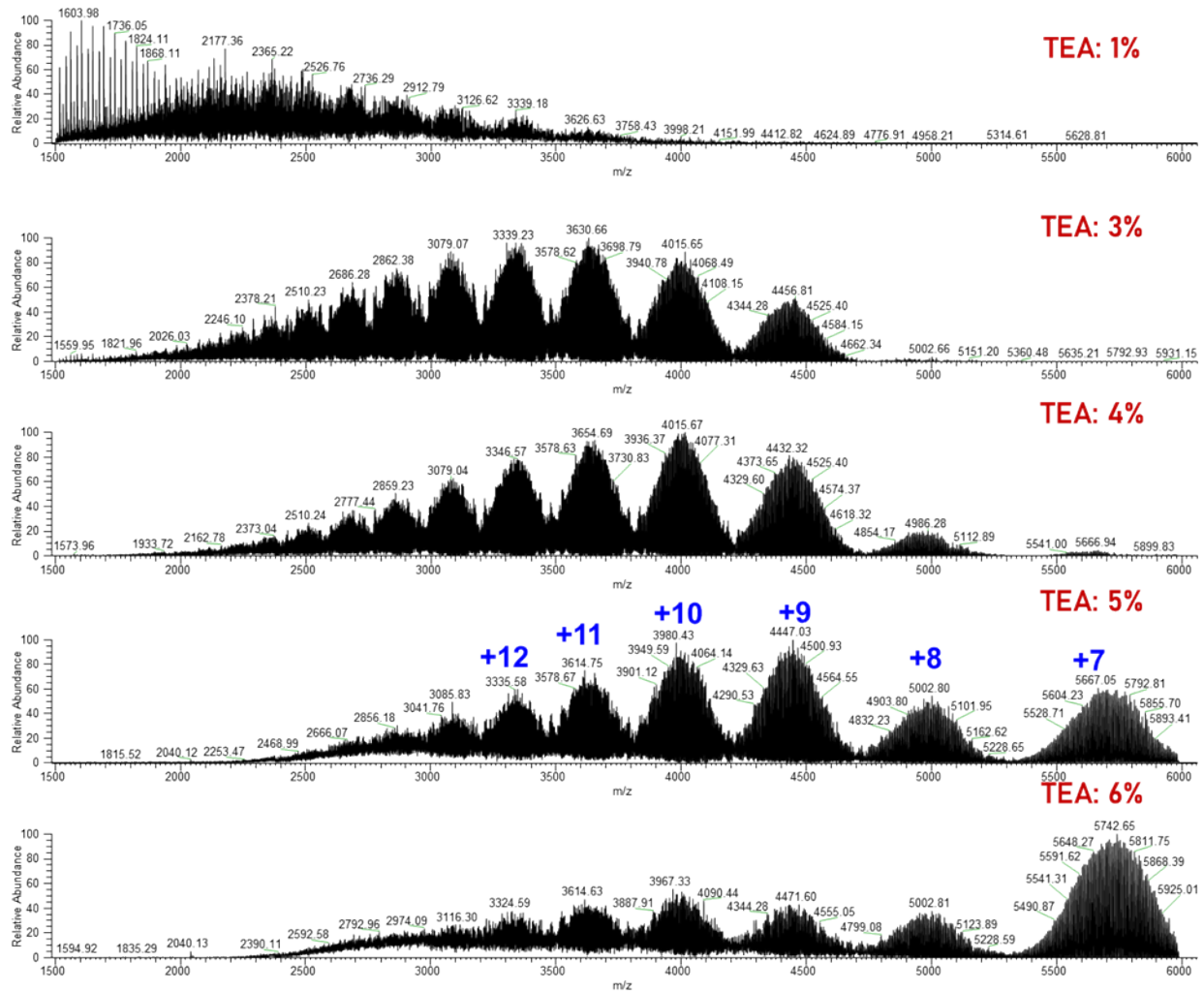


Figure S1 Charge stripping effect of TEA on PEG-filgrastim. The assessment was carried out with a syringe flow rate of 5.0 $\mu\text{L}/\text{min}$.

Optimization of dimethyl sulfoxide content

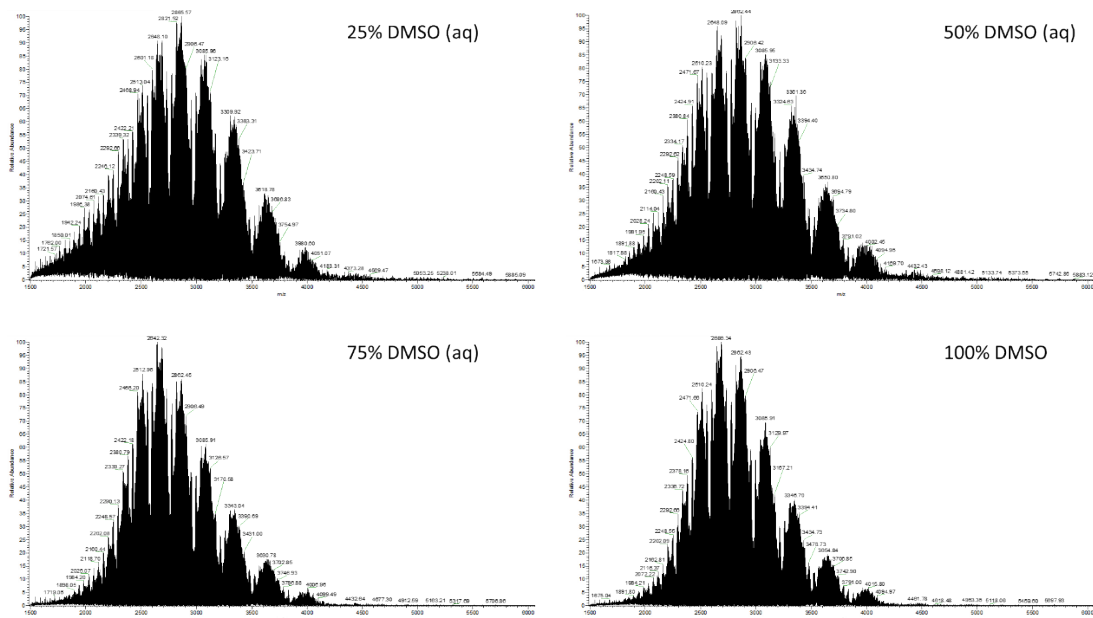


Figure S2 Mass spectrum of the in-house prepared PEG-filgrastim standard by post-column infusion of DMSO at 25% (aq), 50% (aq), 75% (aq), and 100%.

Table S1 Assessment of the DMSO concentration by post-column infusion. The table presents the deconvoluted mass with 483 EO units, molecular weight (MW), the number average molecular weight (Mn), polydispersity index (PDI), and PPM deviation compared to the theoretical mass with 483 EO units (Analyzed with post-column infusion at 4.5 μ L/min).

DMSO concentrations	Replicates	MW _{EO=483}	ppm error	Mn	Mw	PDI
25%	1	40148.1	17.4	40018.0	40030.0	1.0003
	2	40148.2	14.9	40153.2	40165.7	1.0003
	3	40148.9	2.5	40192.5	40204.1	1.0003
50%	1	40147.8	24.9	40010.7	40024.2	1.0003
	2	40148.0	19.9	40045.8	40059.8	1.0004
	3	40147.9	22.4	40057.6	40071.4	1.0003
75%	1	40148.2	14.9	40064.4	40077.8	1.0003
	2	40148.1	17.4	40071.4	40084.4	1.0003
	3	40148.0	19.9	40051.3	40064.3	1.0003
100%*	1	40148.1	17.4	40029.8	40042.9	1.0003
	2	40148.1	17.4	40040.0	40053.2	1.0003
	3	40147.9	22.4	40055.8	40069.1	1.0003

Post-column infusion flow rate

Table S2 Effect on molecular mass determination by post-column infusion of neat DMSO at different syringe pump flow rates. The table presents the deconvoluted mass with 483 EO units, molecular weight (MW), the number average molecular weight (Mn), polydispersity index (PDI), and PPM deviation compared to the theoretical mass with 483 EO units.

Syringe flow rate ($\mu\text{L}/\text{min}$)	Replicates	$\text{MW}_{\text{EO}=483}$	ppm error	Mn	Mw	PDI
4.0	1	40148.1	17.4	40085.3	40097.5	1.0003
	2	40147.9	22.4	40135.7	40148.3	1.0003
	3	40147.8	24.9	40141.5	40153.8	1.0003
4.5	1	40148.1	17.4	40029.8	40042.9	1.0003
	2	40148.1	17.4	40040.0	40053.2	1.0003
	3	40147.9	22.4	40055.8	40069.1	1.0003
5.0	1	40148.2	14.9	40211.8	40224.4	1.0003
	2	40148.1	17.4	40233.4	40245.4	1.0003
	3	40148.1	17.4	40194.2	40205.8	1.0003

Injection volume

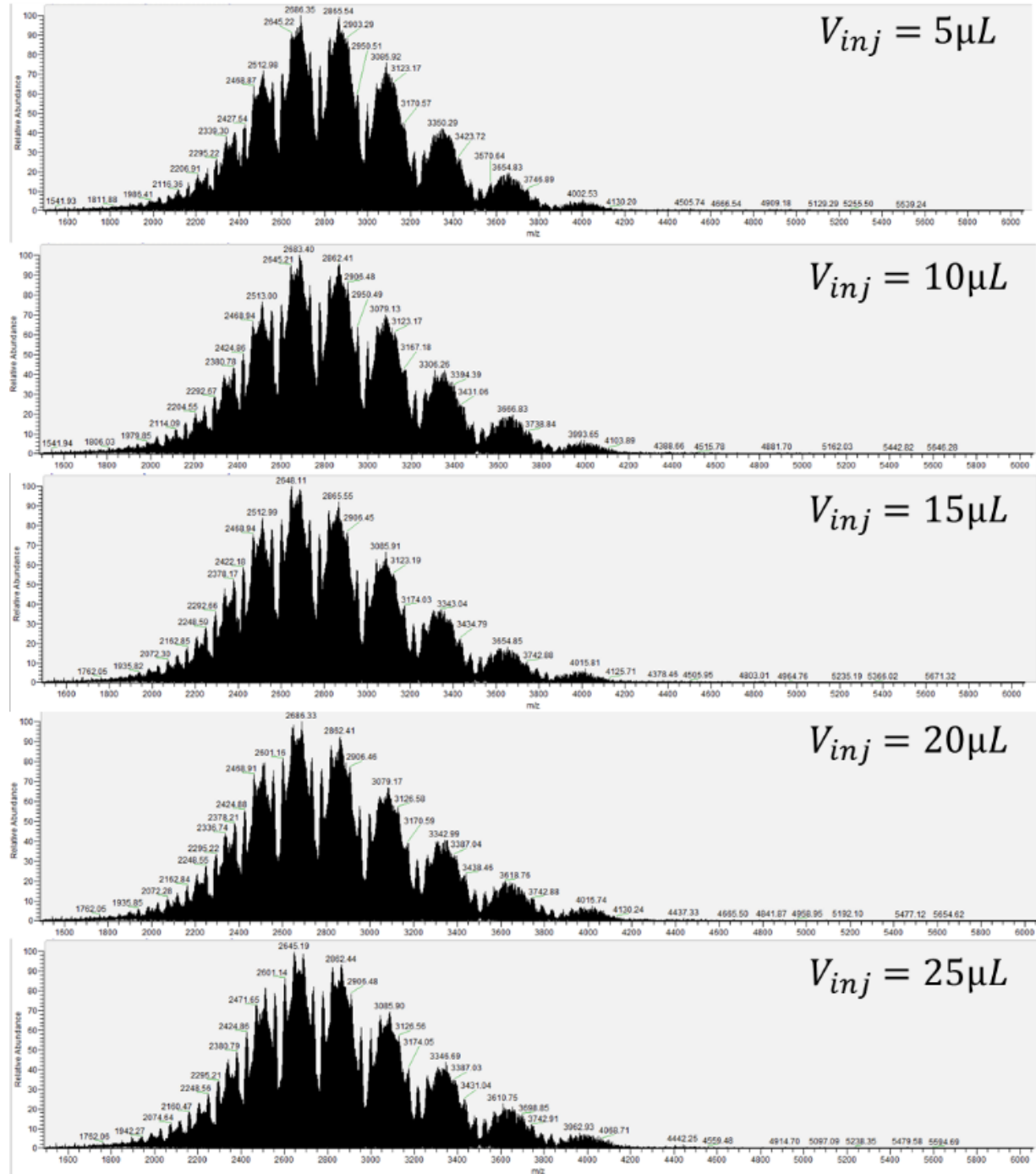


Figure S3 Mass spectra of PEG-filgrastim by different injection volumes.

Table S3: The table presents the deconvoluted mass with 483 EO units, molecular weight (MW), the number average molecular weight (Mn), polydispersity index (PDI), PPM deviation, and signal response compared to the theoretical mass with 483 EO units.

Injection	Replicates	MW _{n=483}	ppm error	Mn	Mw	PDI	Average Signal
-----------	------------	---------------------	-----------	----	----	-----	----------------

Volume, μL							intensity
5	1	40148.1	17.4	40166.24	40178.92	1.000316	2.66E+08 RSD=2.8%
	2	40148.0	19.9	40148.64	40161.14	1.000312	
	3	40148.1	17.4	40153.5	40166.41	1.000322	
10	1	40148.0	19.9	40116.6	40129.24	1.000315	3.43E+08 RSD=1.8%
	2	40148.0	19.9	40093.4	40105.74	1.000308	
	3	40148.1	17.4	40082.5	40094.41	1.000296	
15	1	40148.1	17.4	40096.21	40108.71	1.000312	3.96E+08 RSD=2.3%
	2	40148.1	17.4	40073.29	40085.76	1.000311	
	3	40148.2	14.9	40080.51	40093.44	1.000323	
20	1	40148.0	19.9	40030.89	40043.54	1.000316	3.91E+08 RSD=3.5%
	2	40148.1	17.4	40057.88	40070.92	1.000326	
	3	40148.0	19.9	40028.41	40041.06	1.000316	
25	1	40148.1	17.4	40018.96	40031.24	1.000307	3.87E+08 RSD=0.8%
	2	40148.1	17.4	40031.99	40044.97	1.000324	
	3	40148.0	19.9	40033.9	40046.63	1.000318	

Optimization of MS interface parameters

Table S4 Effect of ISF on the characterization of PEG-filgrastim. The table presents the deconvoluted mass with 483 EO units, molecular weight (MW), the number average molecular weight (Mn), polydispersity index (PDI), and PPM deviation compared to the theoretical mass with 483 EO units.

ISF eV	Replicates	MW _{EO=483}	ppm error	Mn	Mw	PDI
65	1	40147.9	22.4	40066.9	40080.6	1.0003
	2	40148.0	19.9	40023.9	40037.5	1.0003
	3	40147.9	22.4	40022.8	40037.0	1.0003
70	1	40147.8	24.9	40019.4	40032.6	1.0003
	2	40148.0	19.9	40033.4	40046.8	1.0003
	3	40148.0	19.9	40067.2	40080.3	1.0003
75	1	40148.1	17.4	40029.8	40042.9	1.0003
	2	40148.1	17.4	40040.0	40053.2	1.0003
	3	40147.9	22.4	40055.8	40069.1	1.0003

Table S5 Effect of S-lens RF level on the characterization of PEG-filgrastim. The table presents the deconvoluted mass with 483 EO units, molecular weight (MW), the number average molecular weight (Mn), polydispersity index (PDI), and PPM deviation compared to the theoretical mass with 483 EO units.

S-lens RF	Replicates	MW _{n=483}	ppm error	Mn	Mw	PDI
70	1	40147.9	22.4	40055.77	40069.1	1.000333
	2	40148.1	17.4	40040.04	40053.2	1.000328
	3	40148.0	19.9	40044.01	40056.3	1.000307
75	1	40148.0	19.9	40044.54	40057.1	1.000314
	2	40148.0	19.9	40038.52	40051.5	1.000324
	3	40148.0	19.9	40022.94	40036.1	1.000328
80	1	40148.0	19.9	40032.23	40045.4	1.000329
	2	40147.8	24.9	40042.65	40055.8	1.000328
	3	40148.1	17.4	40061.00	40073.9	1.000323

Deconvolution of PEG-filgrastim

To investigate the reproducibility of the method, six different preparations of PEG-filgrastim were prepared and analyzed (N=6, n=3). The analysis was performed with post-column infusion of DMSO at 4.5 $\mu\text{L}/\text{min}$. **Figure S4** presents an overlay of all injections where the average deconvoluted mass is plotted against its respective signal intensity.

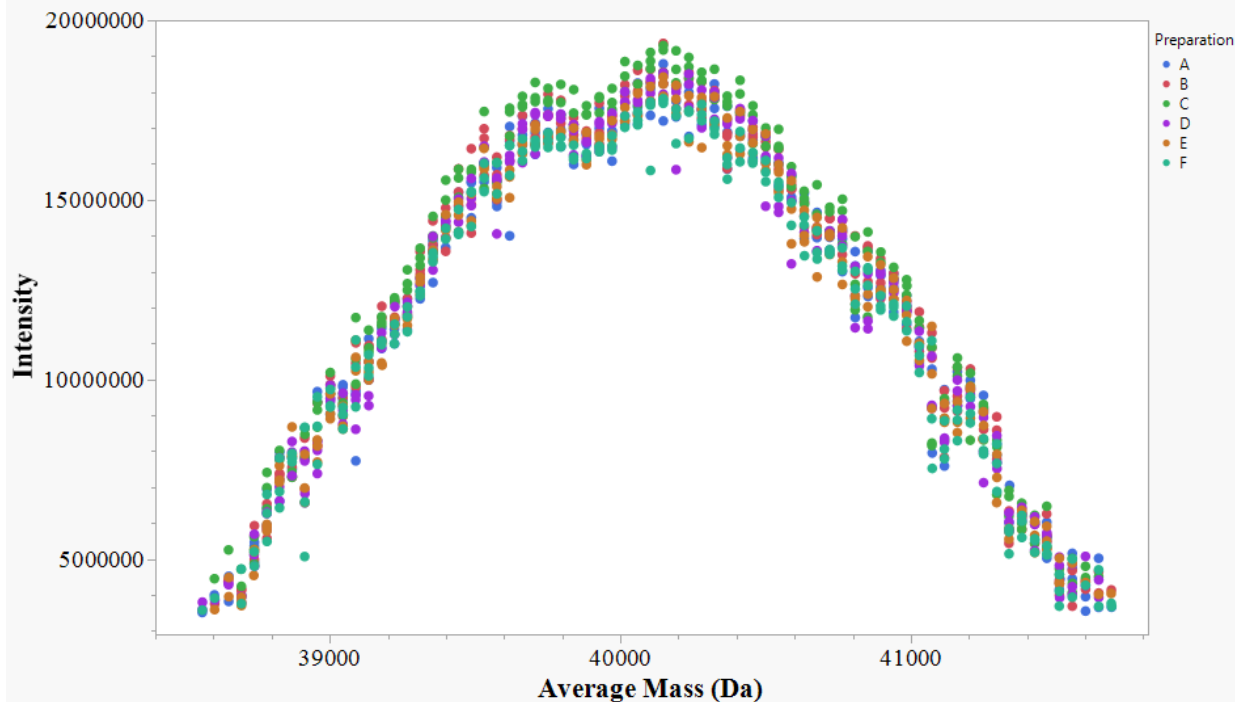


Figure S4 Deconvoluted average mass of the in-house prepared PEG-filgrastim standard plotted concerning its signal intensity. The overlay represents the reproducibility experiment of (N=6, n=3). The Nomenclature A, B, C, D, E, and F represent different preparations, each with 3 replicate injections.

Figure S5 presents the deconvoluted average mass plotted against its respective signal intensity for eight different Neulasta® LOTs.

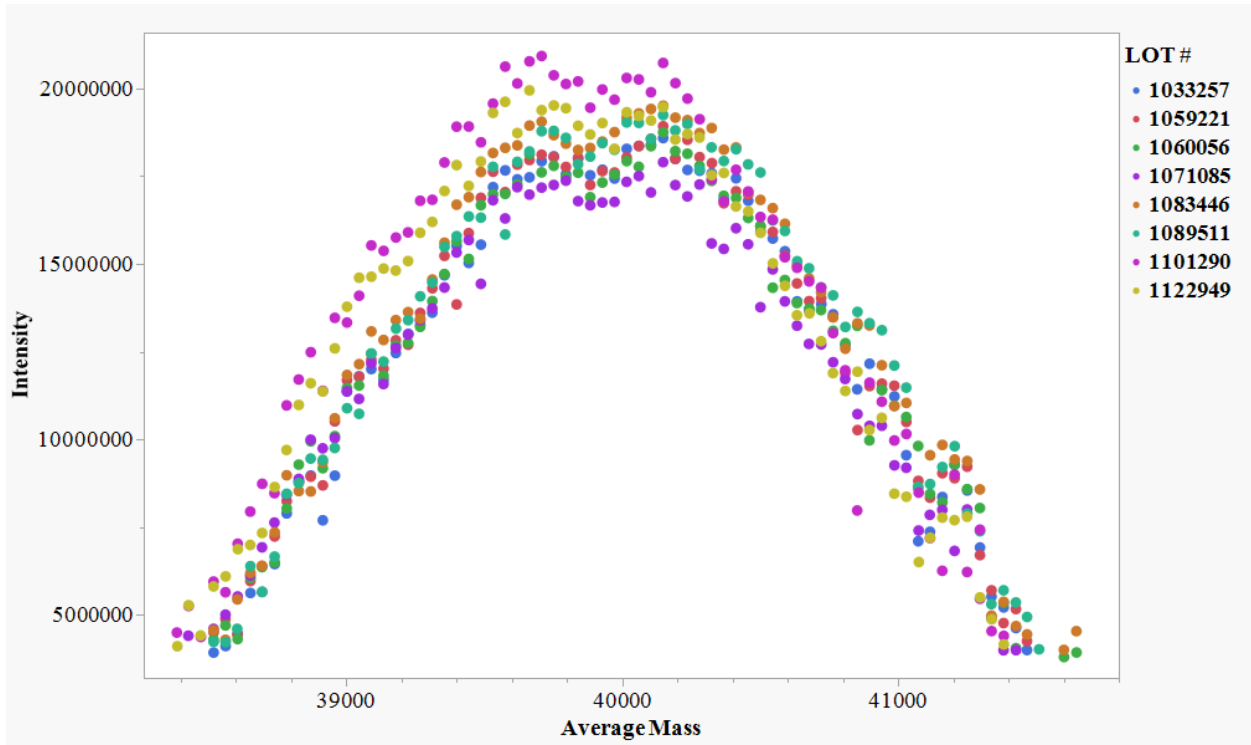


Figure S5 Deconvoluted average mass of eight different Neulasta® lots plotted concerning its signal intensity.