MALDI Calibration

Thank you for choosing SpheriCal® as your calibrant. You will come to find that it outperforms comparable calibrants available on the market and that it offers you the precision needed by today's MALDI-TOF MS instruments. The following is a short and simple explanation on how to set up the necessary files for calibration and in extent the necessary files for using the MALDI-ToF. It is written to comply with Bruker instruments.

Mass Control List

To calibrate your MALDI instrument you will need two files. The first one is called a Mass Control List and is supplied by us at our webpage. You may download it at:

http://www.polymerfactory.com/spherical/instrument-files

Once downloaded, place it in the relevant folder which should be a folder called Mass Control Lists and the path to this folder should be D:\Methods\MassControlLists. Make sure it has the correct file extension (.mcl).

The SpheriCal® Average Mass Control List has all masses available in the SpheriCal® library as average Mw values whereas the Isotopic Mass list has the monoisotopic peaks listed and the one you chose, or both, should now be visible alternative in your calibrant list in your MALDI acquisition software (e.g. FlexControl). If this list is not visible, restart your control program and make sure the file has the right file extension and is in the right folder.

The calibration masses below are the monoisotopic masses for the peptide range and the average masses for the protein range **without the addition of a counter ion**. For additional masses, contact Polymer Factory or consult the Polymer Factory homepage.

Product Masses (M_w)

Spherical Range	Calibration Peaks	PFSA (mass1)	PFSB (mass2)	PFSC (mass3)	PFSD (mass4)
Peptide Low	Monoisotopic	732.314577 Da	952.388136 Da	1172.46169 Da	1478.60842 Da
Peptide Medium	Monoisotopic	1692.69254 Da	2232.89209 Da	2773.09164 Da	3399.36435 Da
Peptide High	Monoisotopic	3613.44847 Da	4793.90000 Da	5975.3074 Da*	7241.8790 Da*
Protein Low	Average Mass	7459.89 Da	9922.47 Da	12385.1 Da	14933.8 Da
Protein Medium	Average Mass	15148.0 Da	20173.3 Da	25198.5 Da	30309.9 Da

**The 2nd monoisotopic peak is chosen instead as the 1st monoisotopic peak is not in great abundance.*

User Method

The second file you need is a User Method which is created by you, the user. It is created by saving the parameters you are using into a method file that can be opened later for quick access to preferable parameters. These files are usually found in the folder called User Methods.

Suggested parameters for acquisition are (as optimized for Bruker Ultraflex):

SpheriCal® Peptide Low:	IS1: 25.00	IS2: 22.10	Lens: 9	PIE: 20 ns
SpheriCal® Peptide Medium:	IS1: 25.00	IS2: 21.85	Lens: 9	PIE: 20 ns
SpheriCal® Peptide High:	IS1: 25.00	IS2: 21.75	Lens: 11	PIE: 20 ns
SpheriCal® Protein Low:	IS1: 25.00	IS2: 21.55	Lens: 11	PIE: 80 ns
SpheriCal® Protein Medium:	IS1: 25.00	IS2: 21.45	Lens: 11	PIE: 120 ns

You may optimize by analyzing SpheriCal® and adjusting your values by small increments to yield a better spectrum. You should then have the optimized values for the molecular weight range you are interested in.

Calibration

The following is a guide on how to calibrate the MALDI-ToF MS after the previous two steps are completed. Note that these instructions are made in accordance with Bruker Instruments and that you should take care not do deviate from any instrument specific instructions you may have been given.

I. Start with preparing your sample and then acquiring a spectrum of the calibrant using your desired parameters. (NOTE: Changing parameters between calibration and analysis may change the precision considerably.)
   - For SpheriCal® Mix; the recommended procedure is to dissolve it in 50 µl of THF and then use 1 µl for your sample spot as explained below.
   - For SpheriCal® Neat; mix the dissolved SpheriCal® sample with the Matrix and Counter-Ion of choice or mix it with Counter-Ion and layer it with the sandwich method. The recommended amount of solvent is 50 µl THF, which would give you a concentration of 1 mg/ml. For consultation regarding solvents and quantities, consult PF at info@polymerfactory.com. Suggested ratios are:
     SpheriCal Peptide Range - S:C:M 1:1:20,
     SpheriCal Protein Range - S:C:M 1:1:10
   - Spot your calibrant on your MALDI-ToF sample plate, deposit up to 1 µl on the plate. THF should evaporate quickly leaving a thin film ready for analysis.
   - For best long term storage: aliquot the remaining sample into small vials and let the solvent evaporate. Store cool and dark.

II. In the MALDI software, calibrate your acquired peaks against the SpheriCal® mass list which can be found under calibration lists. They are given as either (AVG) average masses plus Na^+ or (ISO) monoisotopic masses plus Na^+.
   - When you feel confident you can calibrate against the monoisotopic peak do this since it will enhance the accuracy of your calibration. If you cannot find the monoisotopic peak, calibrate against the average mass.

III. Detailed instructions for calibration:
   - Select the mass you wish to calibrate from by first selecting the SpheriCal® Master Mass control list among your mass control lists and then selecting the relevant peak mass.
   - Then use the peak picking tool to select the corresponding peak.
     - For the monoisotopic peak select the relevant in the isotopic distribution in your spectra, look at Exhibits for clearer selection of peak.
     - For the average mass, using the ‘sum’-peak select tool, select the average mass of the peak distribution in your spectra.
       (NOTE: You may have to optimize your peak picking algorithm to make sure you get the right result. This can be done in the MALDI software.)
   - Do this for all relevant peaks giving you at least four points of calibration.
   - Apply your calibration to your method by clicking the relevant button.
   - Save the calibration by saving your User Method.

IV. Changing acquisition parameters can dramatically change the precision of your calibration, giving you dramatically different results. Frequent calibration gives you the accuracy you need.

APPENDIX: List of molecular weights

Exhibit 1: SpheriCal® Peptide Low, "for the first 'monoisotopic' peak"

SpheriCal® 500-1,600 (Na^+ adduct): 755.304346 Da, 975.377905 Da, 1195.45146 Da, 1501.59819 Da

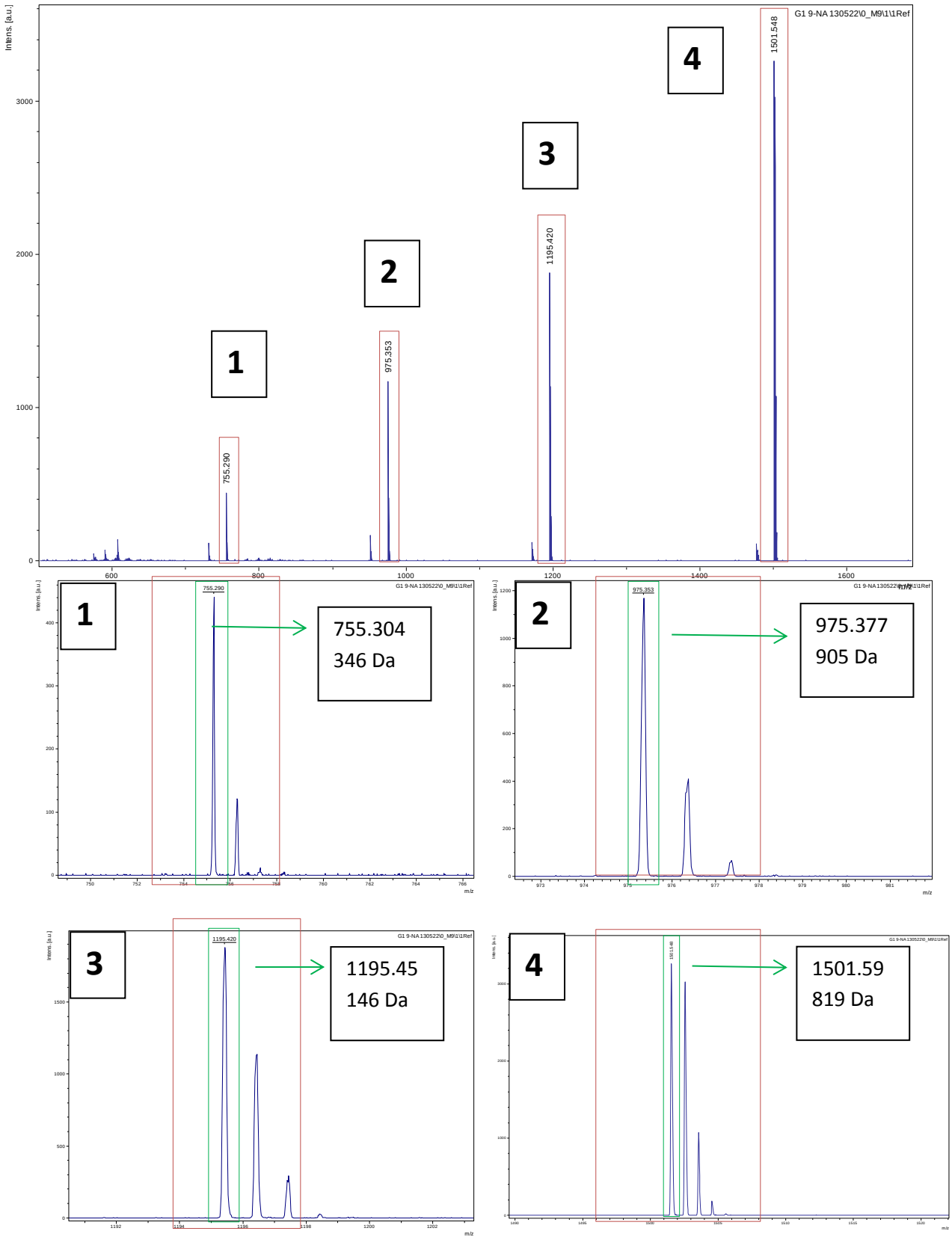
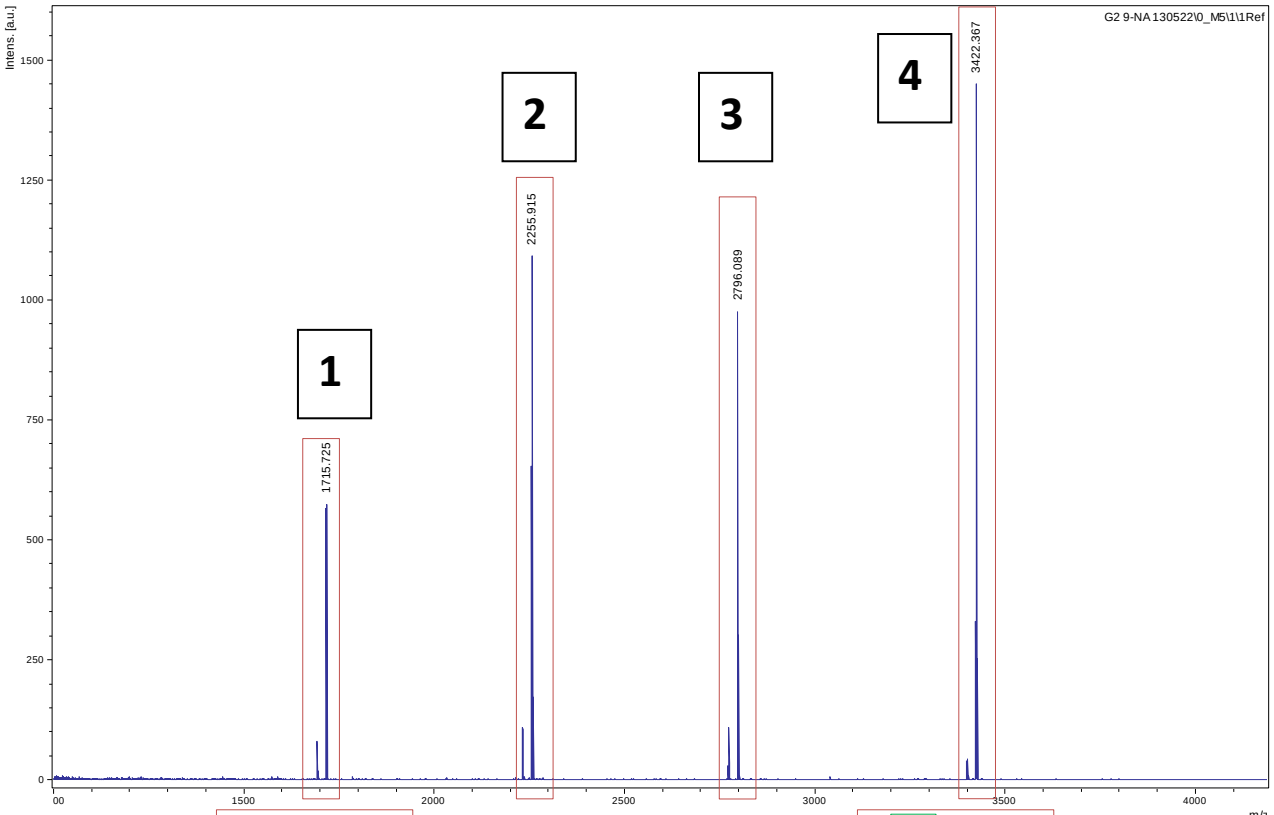
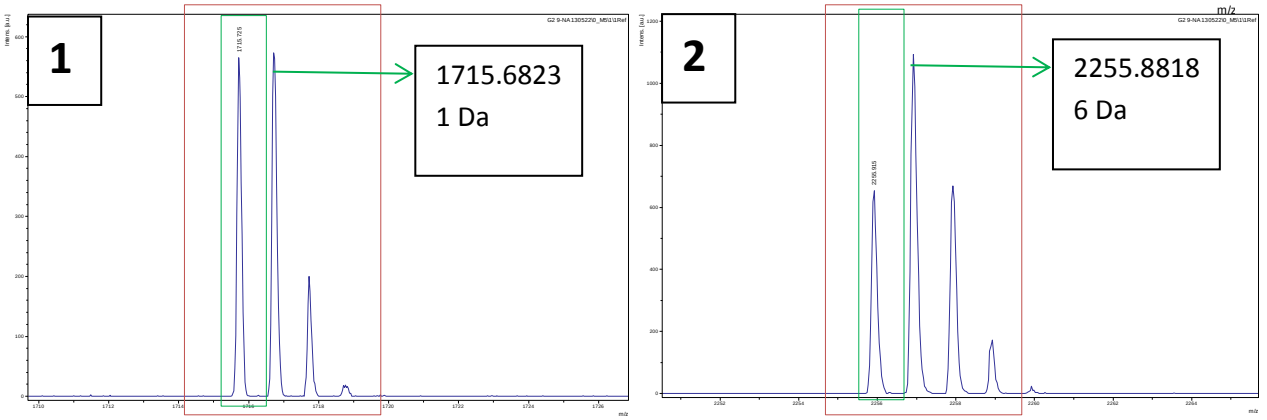


Exhibit 2: SpheriCal® Peptide Medium, "for the first 'monoisotopic' peak"

S*pheriCal® 1.600-3,500(Na+ adduct):* 1715.68231 Da, 2255.88186 Da, 2796.08141 Da, 3422.35412 Da





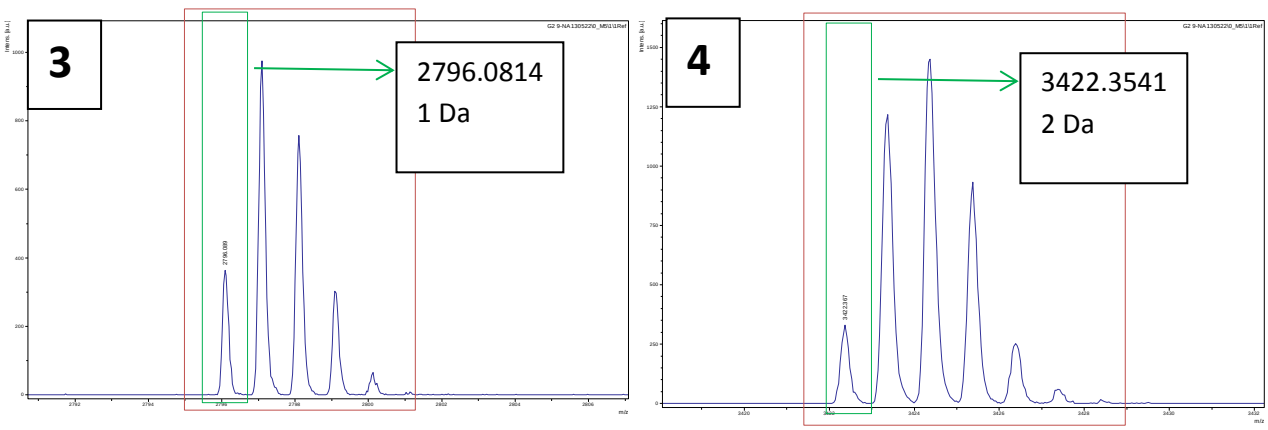
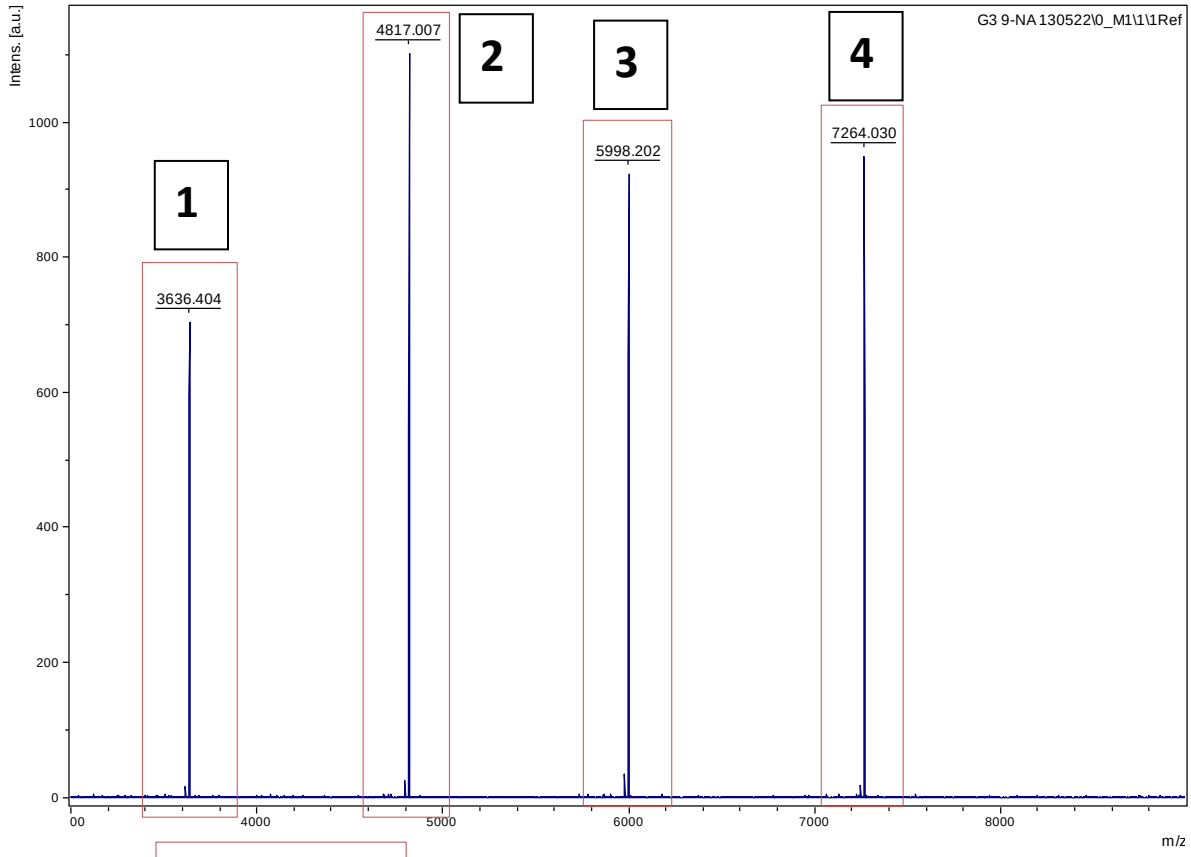
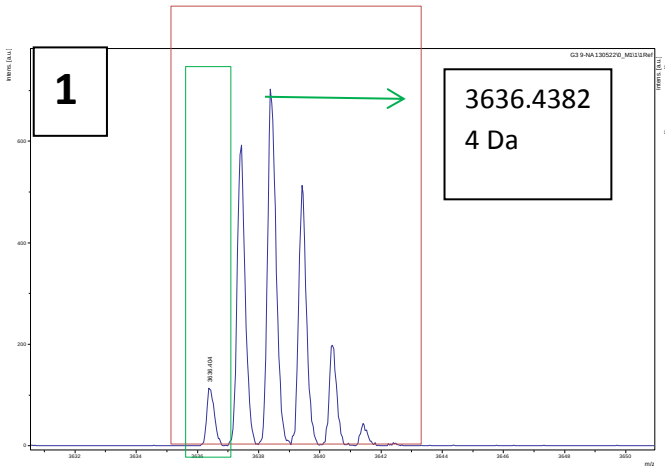
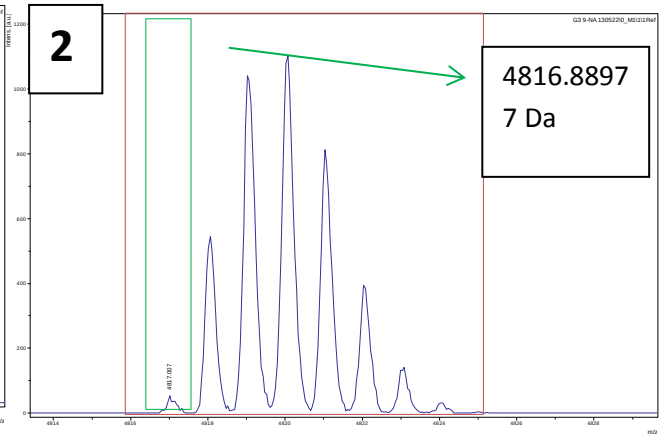


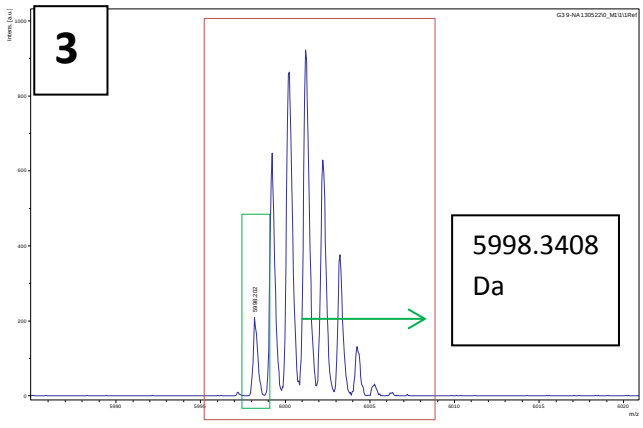
Exhibit 3: SpheriCal® Peptide High, "for the first or second 'monoisotopic' peak"

SpheriCal® 3.500-7,500 (Na^+ adduct): 3636.43824 Da, 4816.88977 Da, 5998.3408 Da*, 7264.8688 Da*









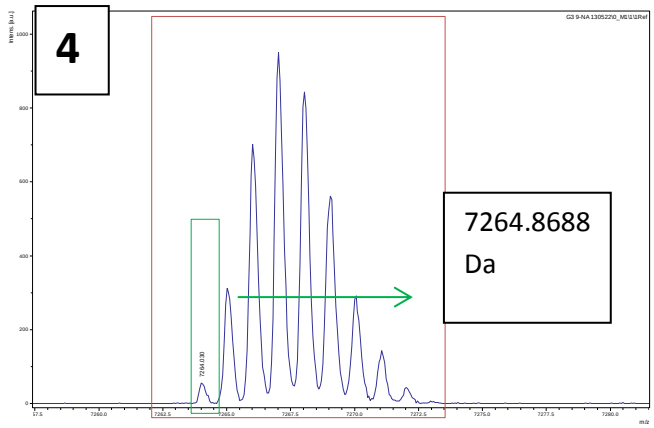


Exhibit 3B: SpheriCal® Peptide High, "for the 5997 second 'monoisotopic' peak"

*NOTE: Because of the low abundance of the first monoisotopic peak for the third and fourth standard, it may be easier to identify one of the alternative peaks in this distribution. The second 'monisotopic' peak is thus chosen for the calibration for the control list. Below is a complete list of exact isotopes for the third standard in SpheriCal® 3,500-7,500 Da:

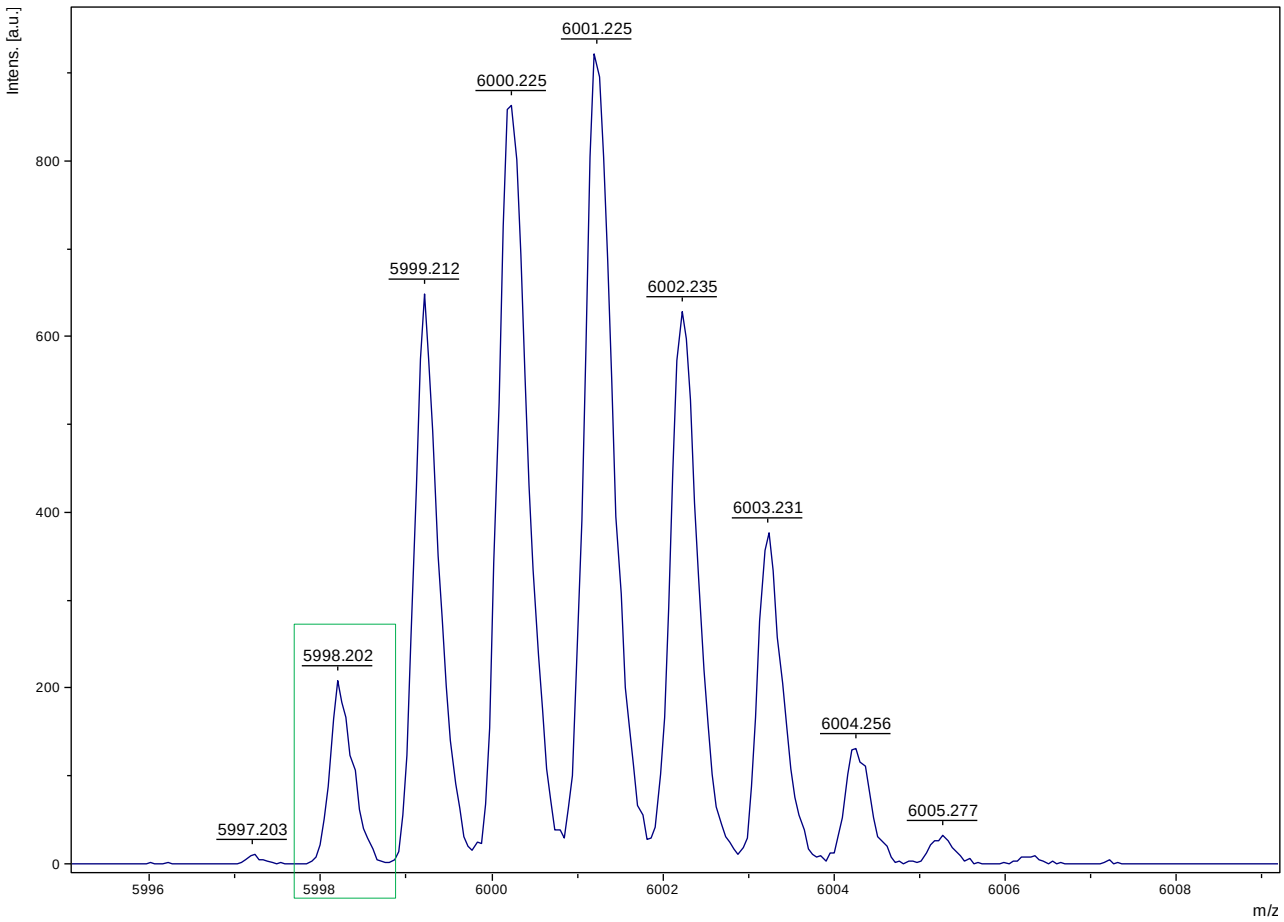


Figure 3B: Peak picking of third standard for SpheriCal® Peptide High after calibration.

Isotope 1 (Na+ Adduct)	**Isotope 2 (Na+ Adduct)**	Isotope 3 (Na+ Adduct)	Isotope 4 (Na+ Adduct)	Isotope 5 (Na+ Adduct)
12.2%: 5997.3412 Da	**43.4%: 5998.3408 Da**	79.5% 5999.3404 Da	100% 6000.3399 Da	96.8% 6001.3395 Da

Isotope 6 (Na+ Adduct)	Isotope 7 (Na+ Adduct)	Isotope 8 (Na+ Adduct)	Isotope 9 (Na+ Adduct)	Isotope 10 (Na+ Adduct)
77.0% 6002.3390 Da	52.2% 6003.3386 Da	31.0% 6004.3381 Da	16.4% 6005.3377 Da	7.9% 6006.3373 Da

Exhibit 3C: SpheriCal® Peptide High, "for the 7264 second 'monoisotopic' peak"

*NOTE: Because of the low abundance of the first monoisotopic peak for the third and fourth standard, it may be easier to identify one of the alternative peaks in this distribution. The second 'monisotopic' peak is thus chosen for the calibration for the control list. Below is a complete list of exact isotopes for the fourth standard in SpheriCal® 3,500-7,500 Da:

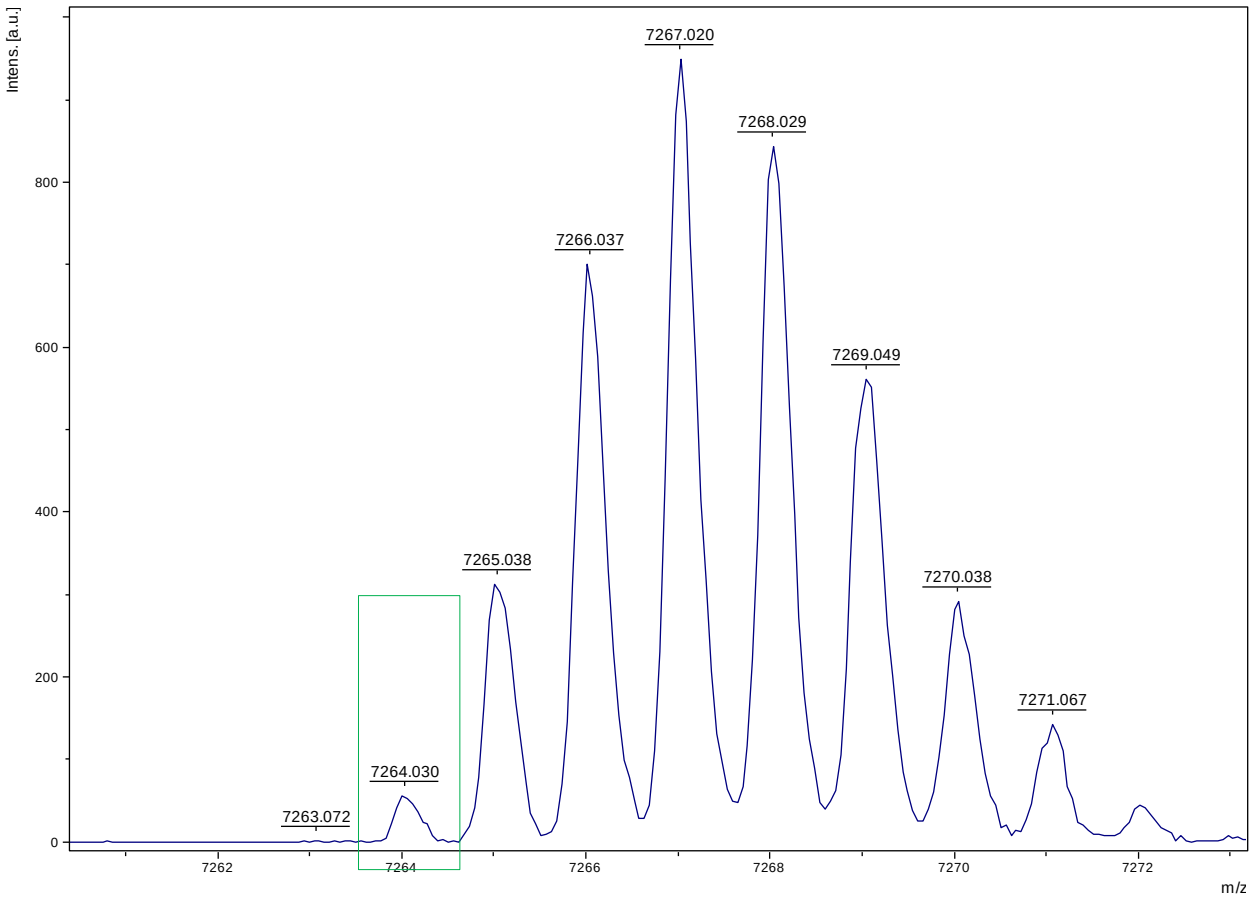


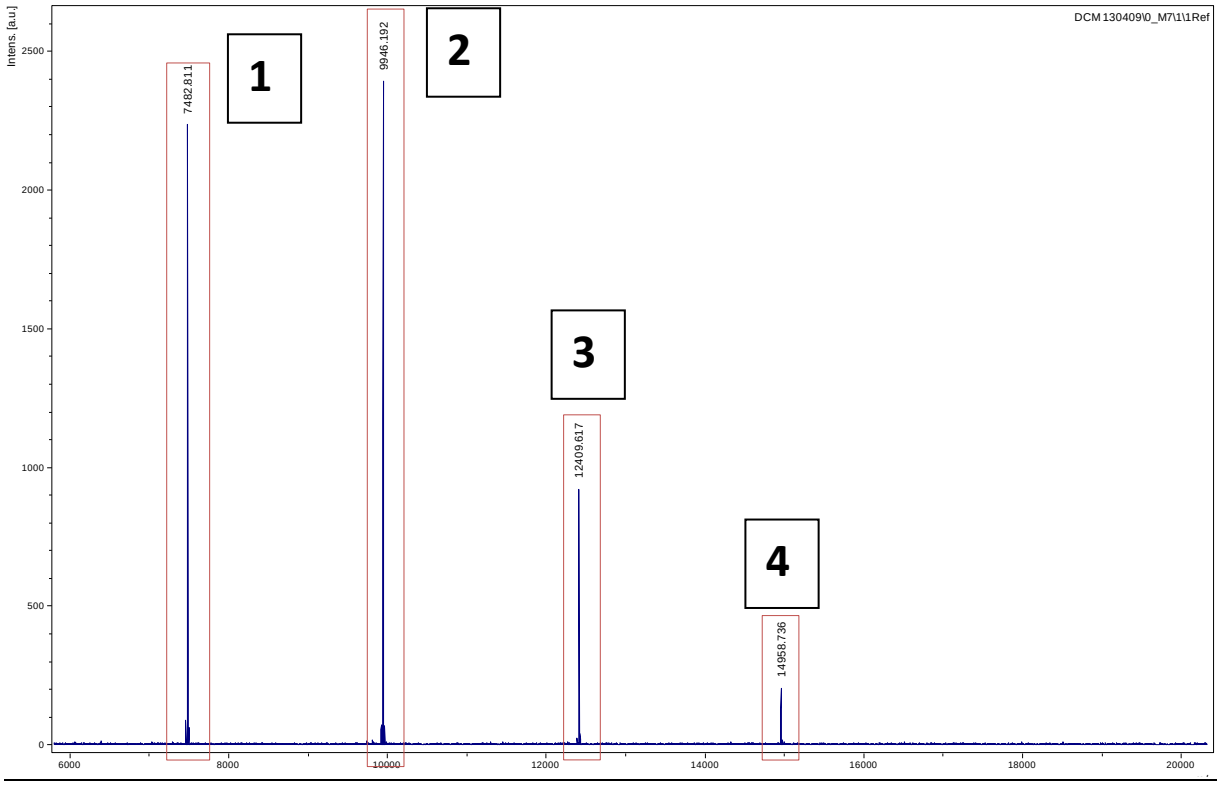
Figure 3C: Peak picking of fourth standard for SpheriCal® Peptide High after calibration.

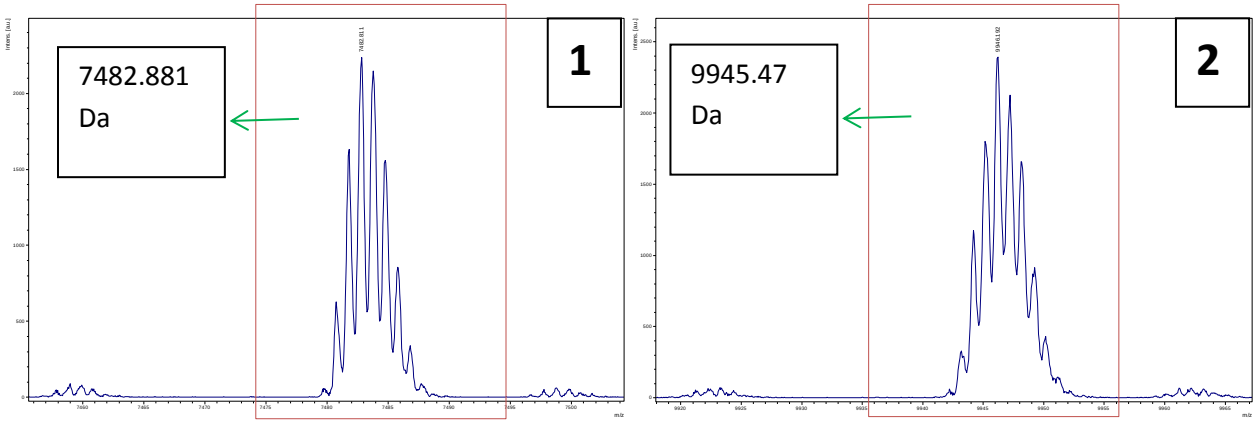
Isotope 1 (Na+ Adduct)	**Isotope 2 (Na+ Adduct)**	Isotope 3 (Na+ Adduct)	Isotope 4 (Na+ Adduct)	Isotope 5 (Na+ Adduct)
6.0%: 7263.8659 Da	**25.9%: 7264.8688 Da**	57.2% 7265.8721 Da	86.4% 7266.8755 Da	100% 7267.8789 Da

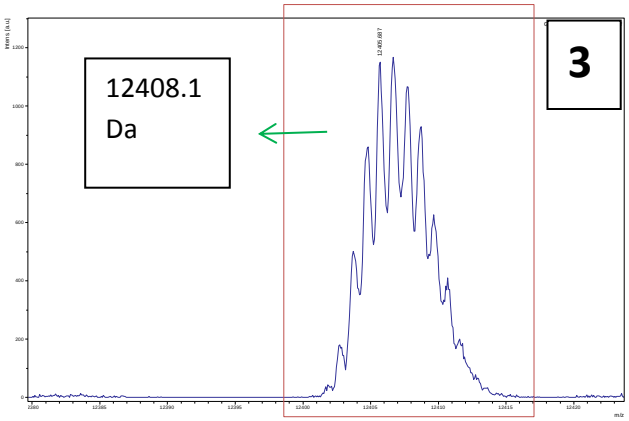
Isotope 6 (Na+ Adduct)	Isotope 7 (Na+ Adduct)	Isotope 8 (Na+ Adduct)	Isotope 9 (Na+ Adduct)	Isotope 10 (Na+ Adduct)
94.6% 7268.8822 Da	76.1% 7269.8856 Da	53.5% 7270.8889 Da	33.5% 7271.8923 Da	19.0% 7272.8956 Da

Exhibit 4: SphériCal® Protein Low, 'average mass peaks'

SphériCal® 7,500 – 15,000 (Na+ adduct): 7482.881 Da, 9945.47 Da, 12408.1Da, 14956.8 Da







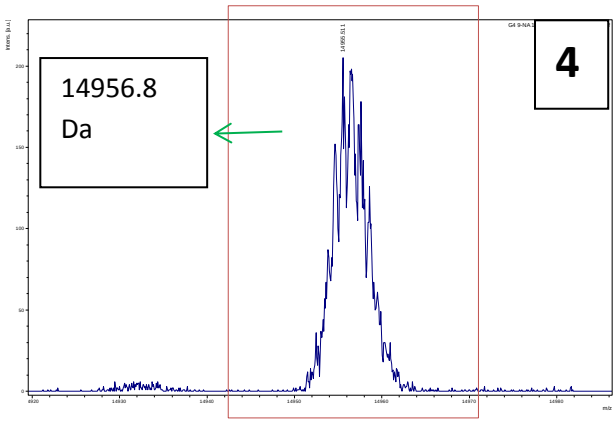
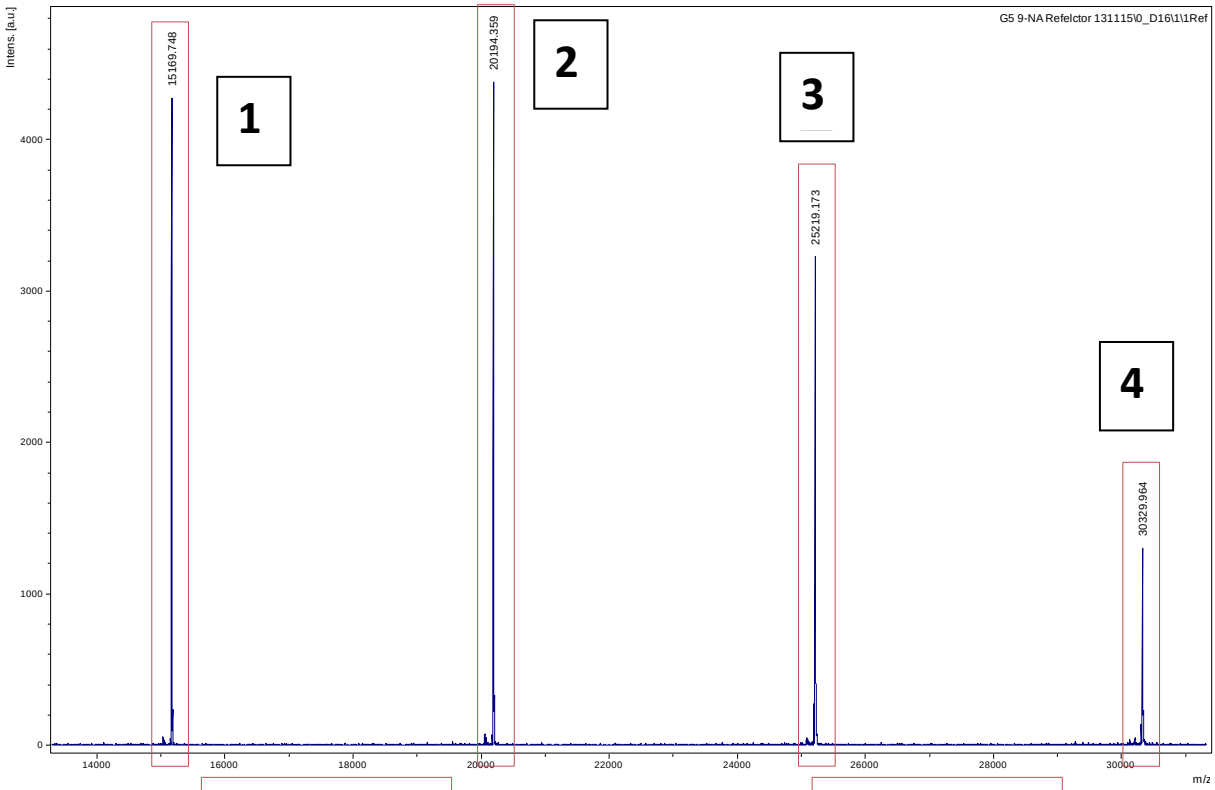
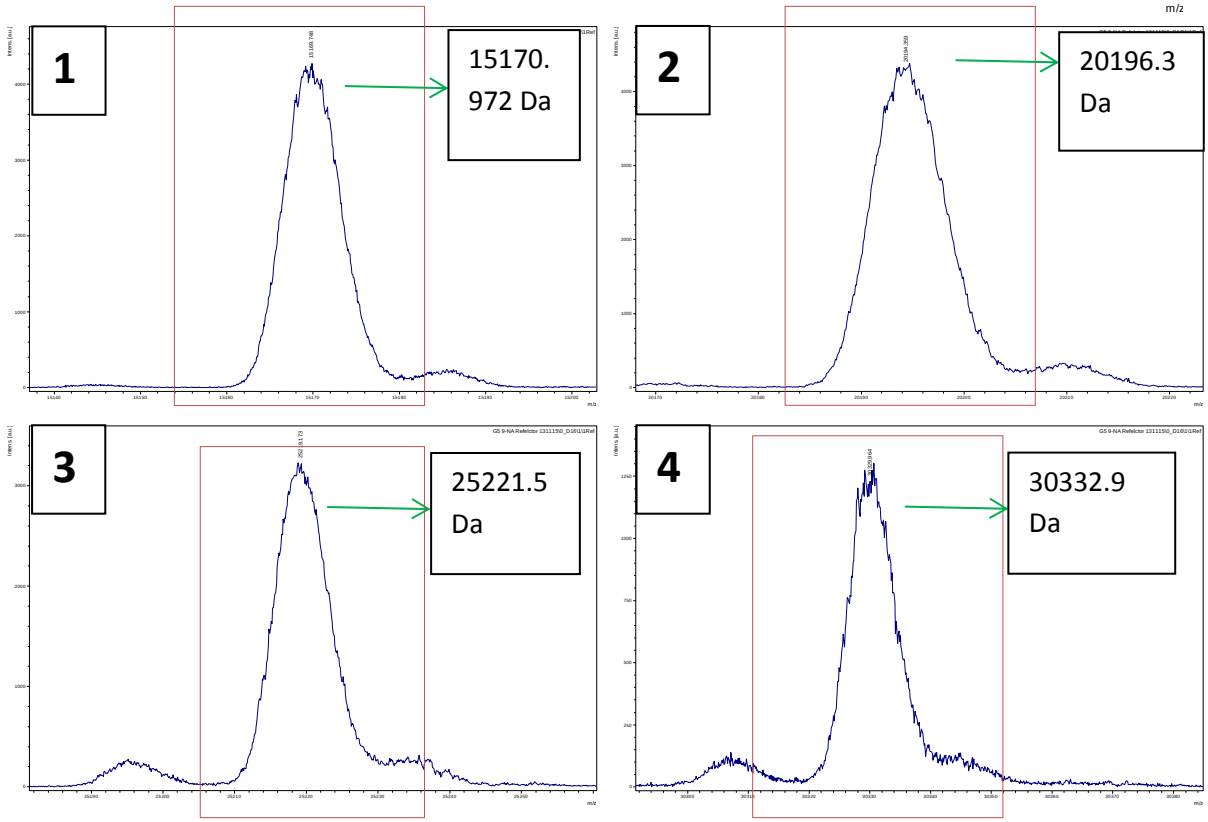


Exhibit 5: SpheriCal® Protein Medium 'average mass peaks'

SpheriCal® 15,000 –30,000 (Na+ adduct): 15170.972 Da, 20196.3 Da, 25221.5 Da, 30332.9Da





Contact

Please feel free to email me any inquiries about SpheriCal® at jamie.godfrey@polymerfactory.com.

Otherwise, Polymer Factory can be contacted at info@polymerfactory.com.