

MALDI Calibration

Thank you for choosing SpheriCal®Aqua as your choice of 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-aqua/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®Aqua Average Mass Control List has all masses available in the SpheriCal®Aqua 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®Aqua Range	Calibration Peaks	PFSA (mass1)	PFSB (mass2)	PFSC (mass3)	PFSD (mass4)
Peptide Low	Monoisotopic	468.220677 Da	600.232935 Da	732.305194 Da	1164.50474 Da
Peptide Medium	Monoisotopic	1164.50474 Da	1528.64149 Da	1892.77864 Da	2342.98875 Da
Peptide High	Monoisotopic	2557.07287 Da	3385.39919 Da	4213.72552 Da	5128.12501 Da
Protein Low	Average Mass	5345.33 Da	7103.06 Da	8860.79 Da	10704.6 Da

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

You may optimize by analyzing SpheriCal® Aqua 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 to 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® Aqua 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 deionized water, which would give you a concentration of 1 mg/ml. For consultation regarding solvents and quantities, consult us at info@polymerfactory.com. Suggested mass 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. For best long term storage: aliquot the remaining sample into small vials and freeze.
- 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 simply select the first peak in the isotopic distribution in your spectra.
 - 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 2: SpheriCal® Aqua Peptide Low, "for the first 'monoisotopic' peak"

SpheriCal® Aqua 500 Da - 1,000 Da (Na^+ adduct): 491.210446 Da, 623.252705 Da, 755.294963 Da, 1187.49451 Da

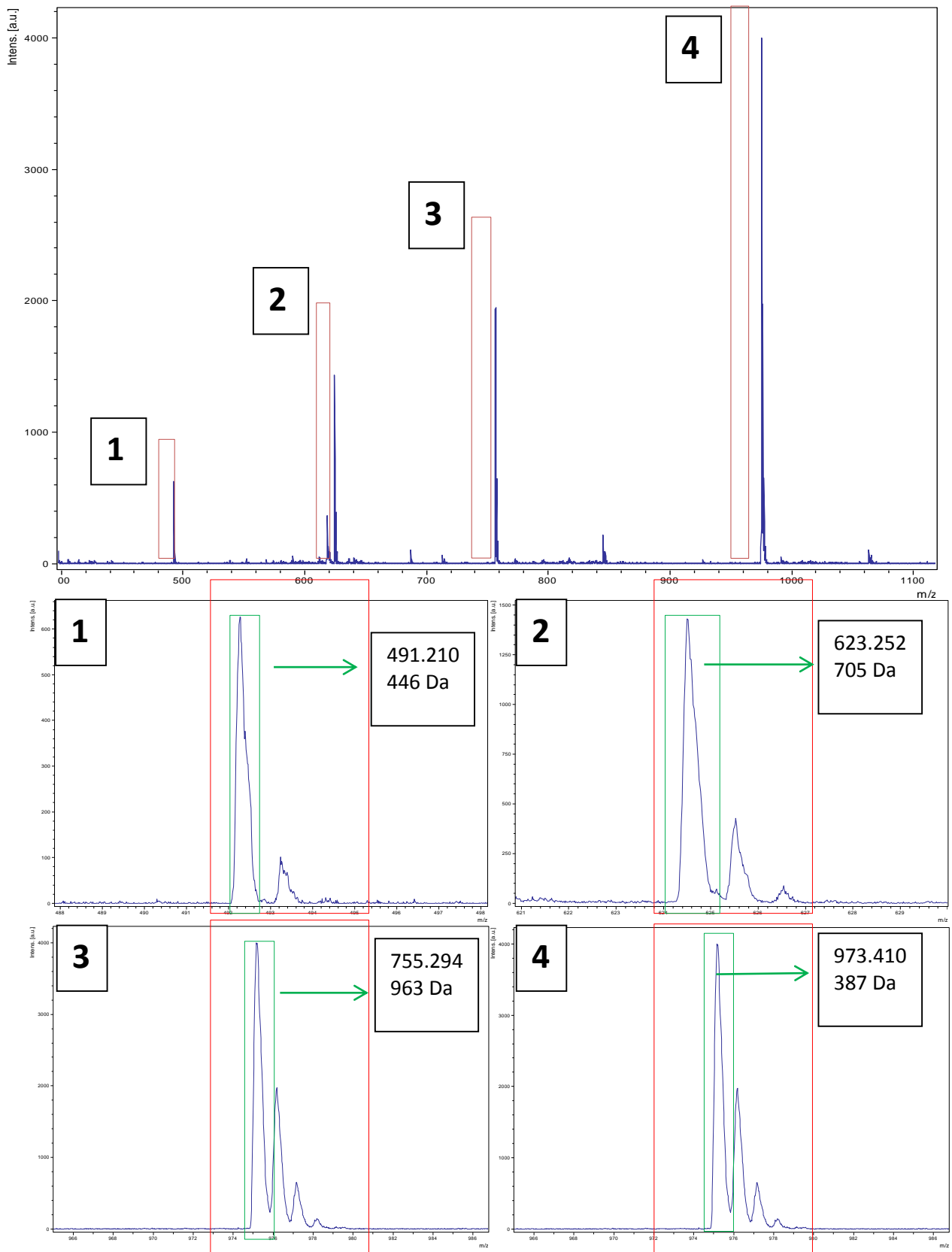


Exhibit 2: SpheriCal® Aqua Peptide Medium, “for the first ‘monoisotopic’ peak”

SpheriCal® Aqua 1,000 Da -2,500 Da (Na⁺ adduct): 1187.49451 Da, 1551.63146 Da, 1915.7684 Da, 2365.97852 Da

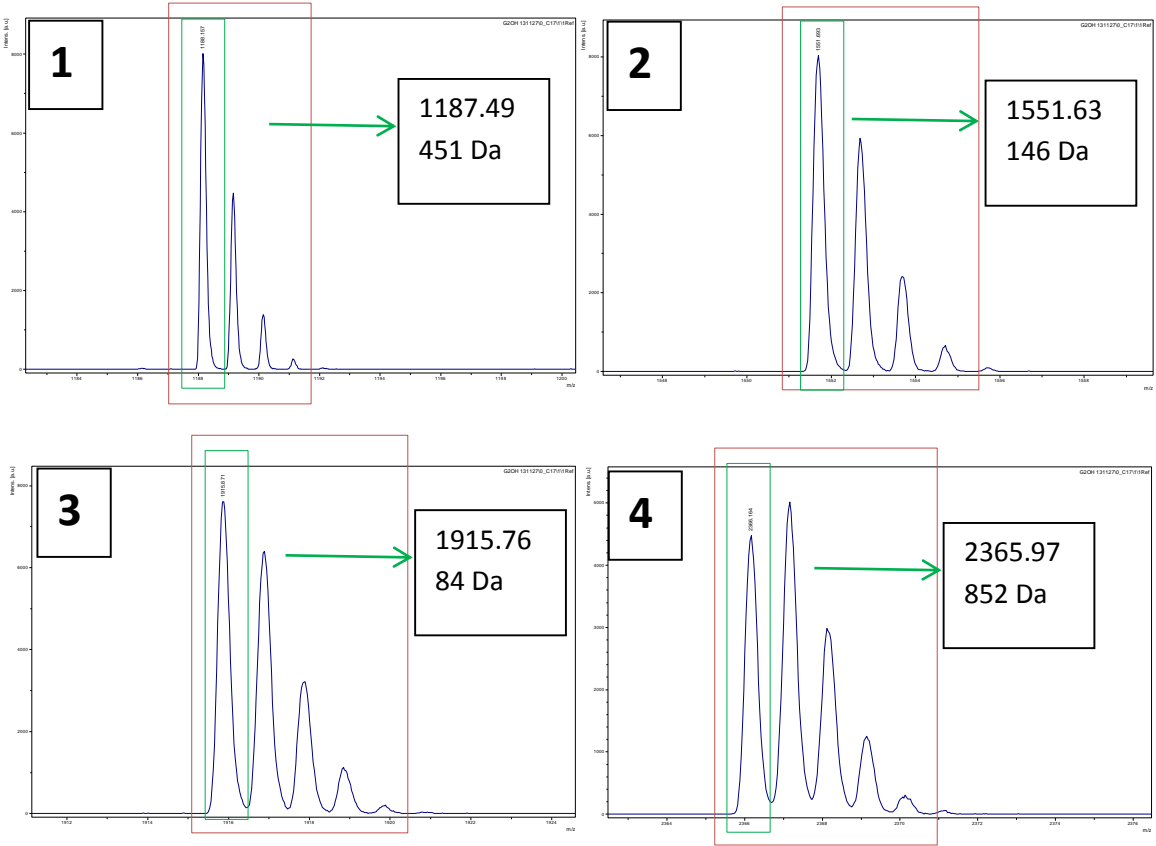
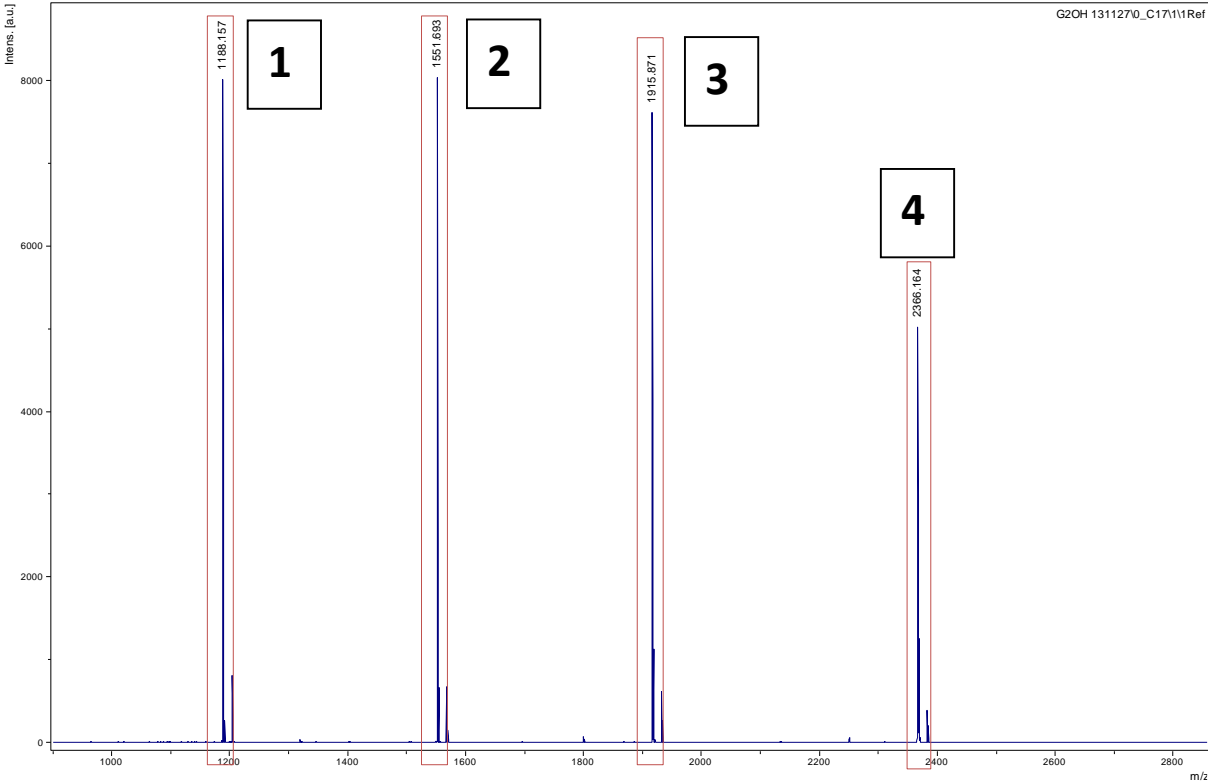


Exhibit 3: SpheriCal® Aqua Peptide Medium, “for the first ‘monoisotopic’ peak”

SpheriCal® Aqua 2,500 Da – 5,200 Da (Na⁺ adduct): 2580.06264 Da, 3408.38896 Da, 4236.71529 Da, 5151.11478 Da

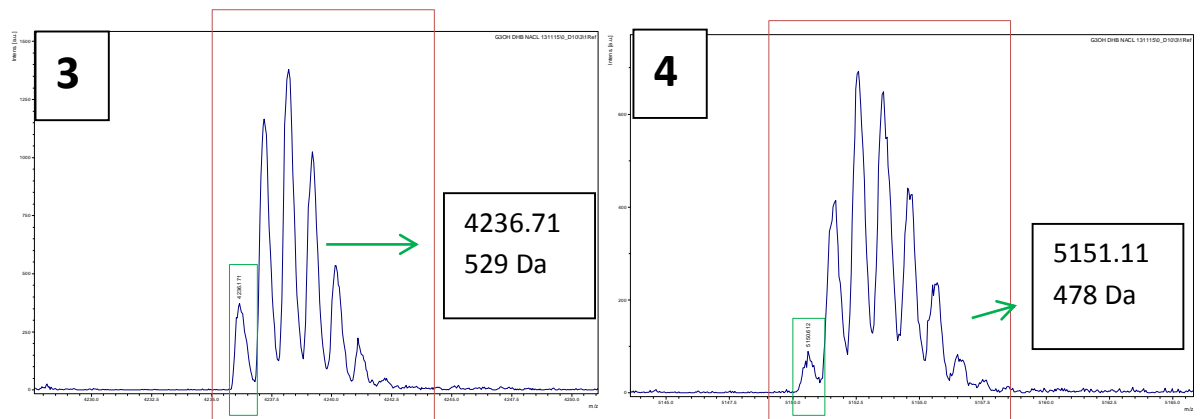
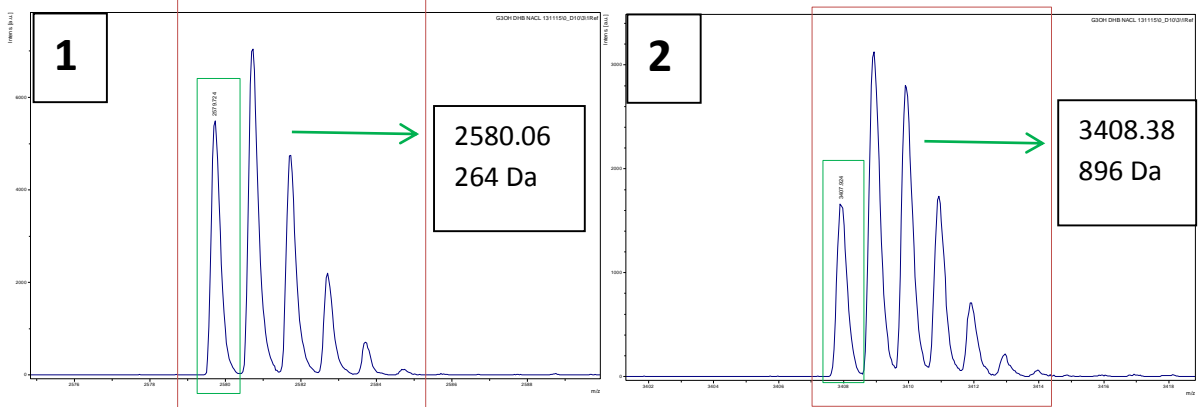
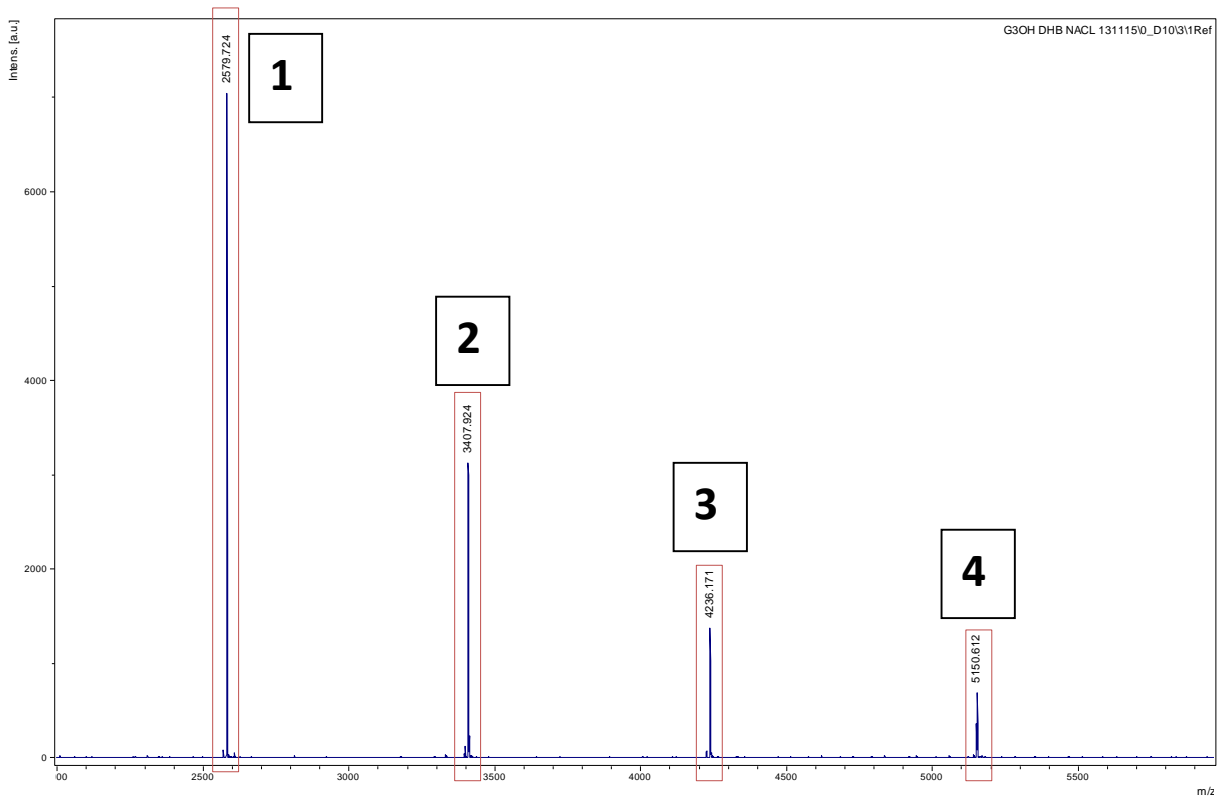
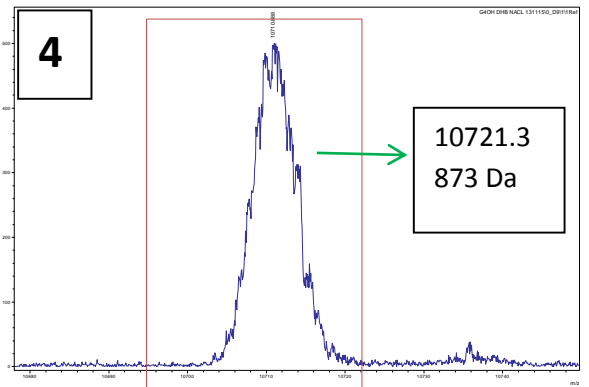
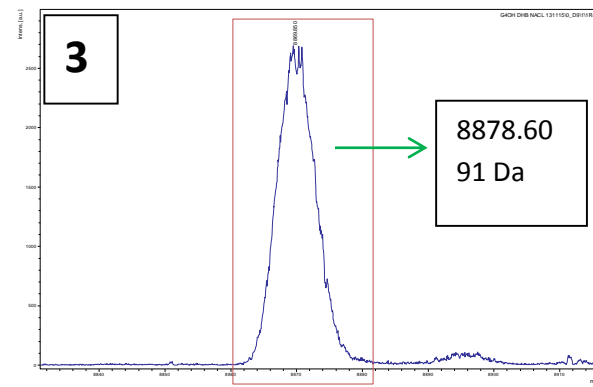
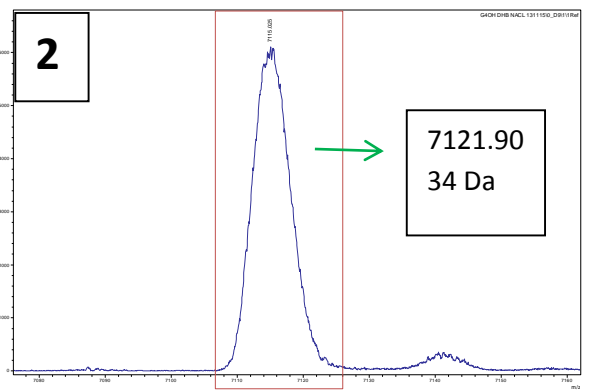
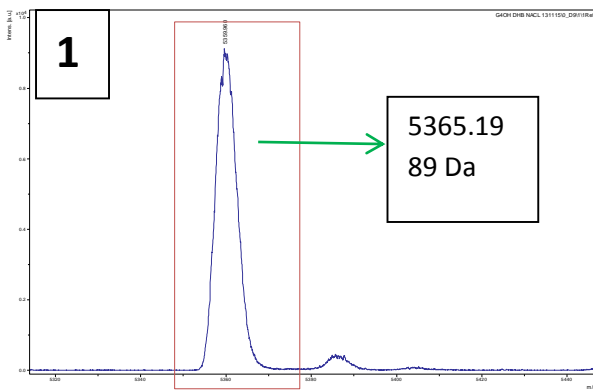
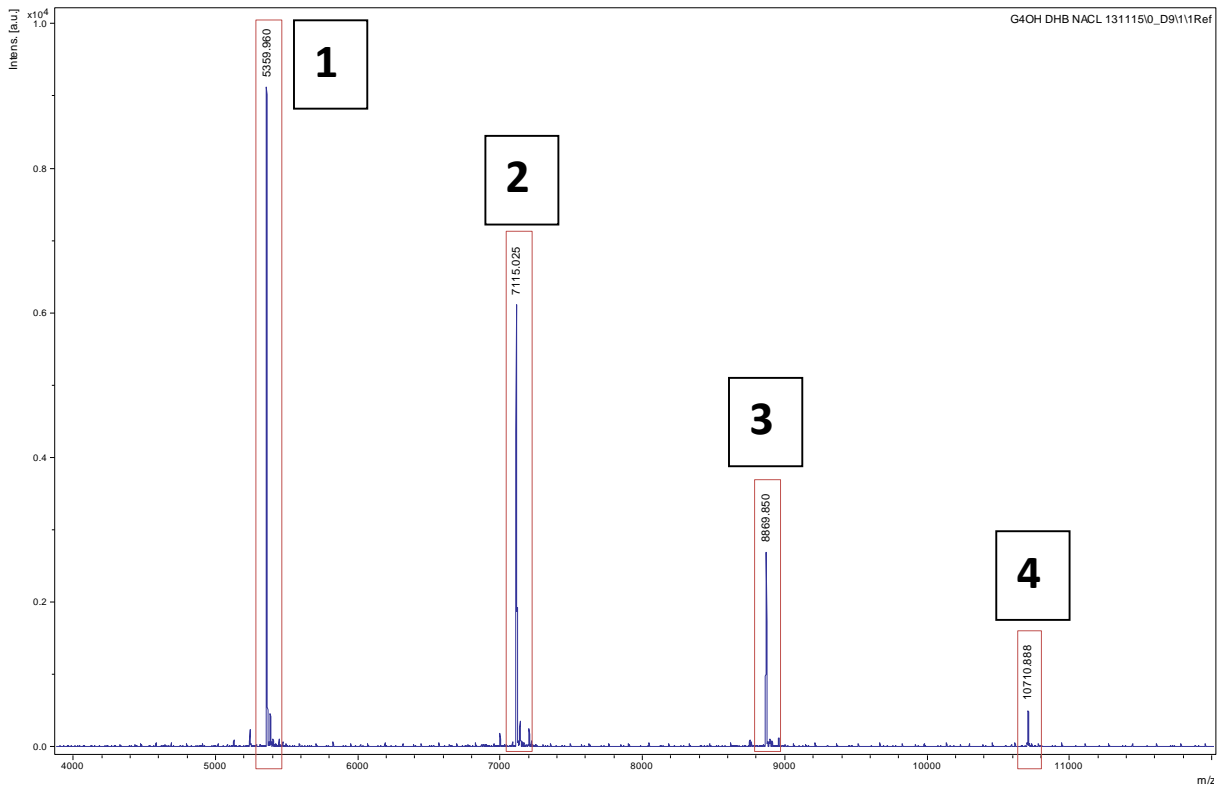


Exhibit 4: SpheriCal® Aqua Protein Low, 'average mass peaks'

SpheriCal® Aqua 5,200-11,000 (Na⁺ adduct): 5365.1989 Da, 7121.9034 Da, 8878.6091 Da, 10721.3873 Da



Contact

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

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

Patent, licensing and trademark information

The technology used in SpheriCal® calibrants is covered by IPR protection held by Tulane University, New Orleans, USA, and Perstorp AB, Sweden. Polymer Factory Sweden AB holds the exclusive worldwide right and license to commercialize SpheriCal® calibrant technology. SpheriCal® is a trademark owned by Polymer Factory Sweden AB and registered within the EU.