SpheriCal® 10-Point MALDI-TOF Calibration Guide

Thank you for choosing SpheriCal® 10-point as your calibrant. The following is a short and simple explanation of how to set up the necessary files for calibration and prepare your SpheriCal® sample on the target plate. It is written to comply with Bruker instruments.

Mass Control List

To calibrate your MALDI instrument with the SpheriCal® standards you will need the mass control list (.mcl), which is available for download at: http://www.polymerfactory.com/spherical/instrument-files

Alternatively, you can use the mass list below to create your own mass control list, or use the chemical structures to calculate your own mass list with your software of choice. If you download the file from our homepage, place it in the relevant folder D:\Methods\MassControlLists.

The SpheriCal® 10-point mass control list has the mass of each standard as average values, which should be used if the resolution of your instrument and acquisition parameters are insufficient to observe the isotopic distribution. Also listed are the monoisotopic exact masses for the highest accuracy if the isotopic distribution can be resolved. For users working in reflector mode we typically recommend using the exact mass for calibration points 1 - 6 and the average mass for calibration points 7 - 10. Users working in linear mode are recommended to use average masses.

Product Masses (M_w)

The masses of each standard in the SpheriCal® 10-Point product are listed below with no counter-ion and as sodium adducts**.** The molecular formula of each standard is also provided.

Standard	Formula	No counter-ion		$[M + Na]^+$	
		Exact mass	Average mass	Exact mass	Average Mass
PFS-50A (#1)	$C_{92}H_{108}O_{30}$	1692.69254	1693.85	1715.68231	1716.82
PFS-50B (#2)	$C_{121}H1_{40}O_{40}$	2232.89209	2234.41	2255.88186	2257.38
PFS-50C (#3)	$C_{150}H_{172}O_{50}$	2773.09164	2774.98	2796.08141	2797.94
PFS-50D (#4)	$C_{184}H_{214}O_{61}$	3399.36435	3401.68	3422.35412	3424.63
PFS-50E (#5)	$C_{257}H_{300}O_{88}$	4794.90000	4797.14	4816.88977	4820.08
PFS-50F (#6)	$C_{320}H_{372}O_{110}$	5974.35153*	5978.39	5997.34129*	6001.31
PFS-50G (#7)	$C_{398}H_{468}O_{138}$	7454.96033*	7459.89	7477.95010*	7482.89
PFS-50H (#8)	$C_{529}H_{620}O_{184}$	9915.91581*	9922.49	9938.90558*	9945.47
PFS-50I (#9)	$C_{660}H_{772}O_{230}$	12376.8713*	12385.1	12399.8611*	12408,1
PFS-50J (#10)	$C_{796}H_{934}O_{277}$	14923.8999*	14933.8	14946.8897*	14956,8

**The first monoisotopic peak may not be of sufficient abundance to calibrate from. See exhibit 3 in the appendix for additional isotopic mass lists or use the average mass to calibrate.*

Acquisition Parameters

SpheriCal® 10-point will provide spectra with sufficient intensity of all peaks for accurate calibration, in both reflector and linear modes, using conditions suited to a large range of analytes. However, exact settings required will change from instrument to instrument. Below are acquisition parameters optimized for a Bruker UltraFlex instrument, but users are advised to optimize their own instrument for the best results.

Reflector mode:	**IS1:** 25.0	**IS2:** 21.5	**Lens:** 11	**PIE:** 80 ns
Linear mode:	**IS1:** 25.0	**IS2:** 23.0	**Lens:** 9	**PIE:** 40 ns

Sample preparation

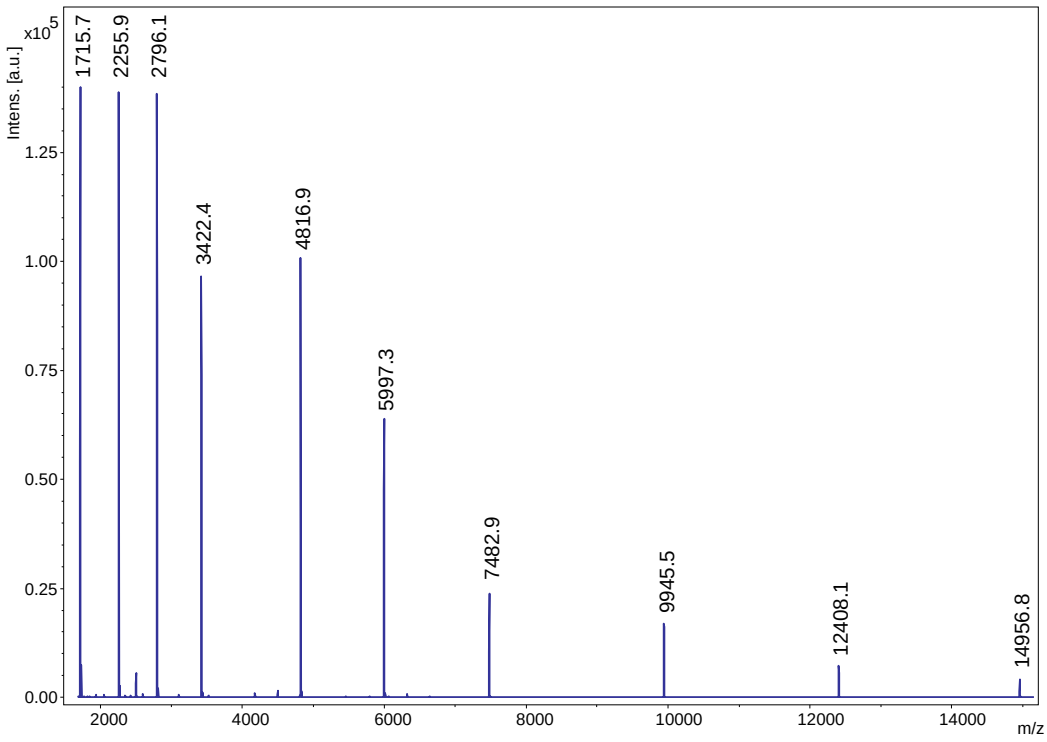
The SpheriCal® standards are efficiently ionized with alkali metal cations and we recommend preparing the sample for calibration using sodium trifluoroacetate (NaTFA) as counter-ion source. The standards display a somewhat "universal" nature with respect to the matrix compound used, but we recommend both DCTB (*trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile, CAS 300364-84-5*) and DHB (*2,5-Dihydroxybenzoic acid, CAS 490-79-9*) for rapid calibration with minimal optimization. The standard can be applied to the target plate using the sandwich or dried droplet methods.

Recommended procedure

1. SpheriCal® 10-Point is provided with 50 µg of standards per vial. Dissolve in 50 µl THF (other solvents may be used, for more information contact our team for further support) – 1 µl of this solution will provide sufficient standard for calibration. The remaining solvent should be allowed to evaporate prior to storage, and portions may be aliquoted to additional vials for subsequent use. It is recommended to store the materials in sealed containers below 0 °C.
2. Prepare a solution of your matrix at a concentration of 10 mg/ml in THF.
3. Prepare a solution of NaTFA counter-ion source at a concentration of 10 mg/ml in THF.
4. Mix your solutions of SpheriCal® standard, counter-ion and matrix in a ratio ([v]:[v]) 1 : 1 : 20.
5. Apply ca. 1 µl of the mixed solution to your target plate and allow to dry. This solution can be stored below 0 °C for short time periods for additional calibration spots, if required. Long term storage in solution is not recommended.

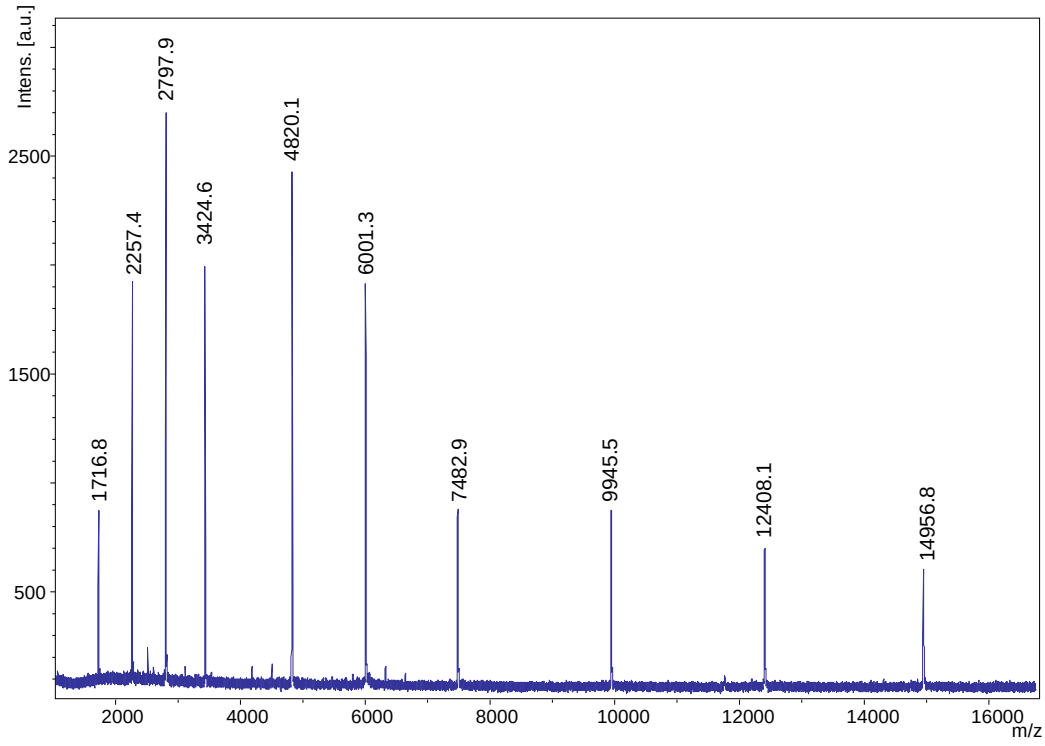
APPENDIX: Examples of calibration spectra and pick peaking

Exhibit 1A: SpheriCal® 10-point spectrum in reflector mode



**Instrument: Bruker UltraFlex, Matrix: DCTB*

Exhibit 1B: SpheriCal® 10-point spectrum in linear mode



**Instrument: Bruker UltraFlex, Matrix: DCTB*

Exhibit 2A: Picking of monoisotopic peaks for points 1 - 6

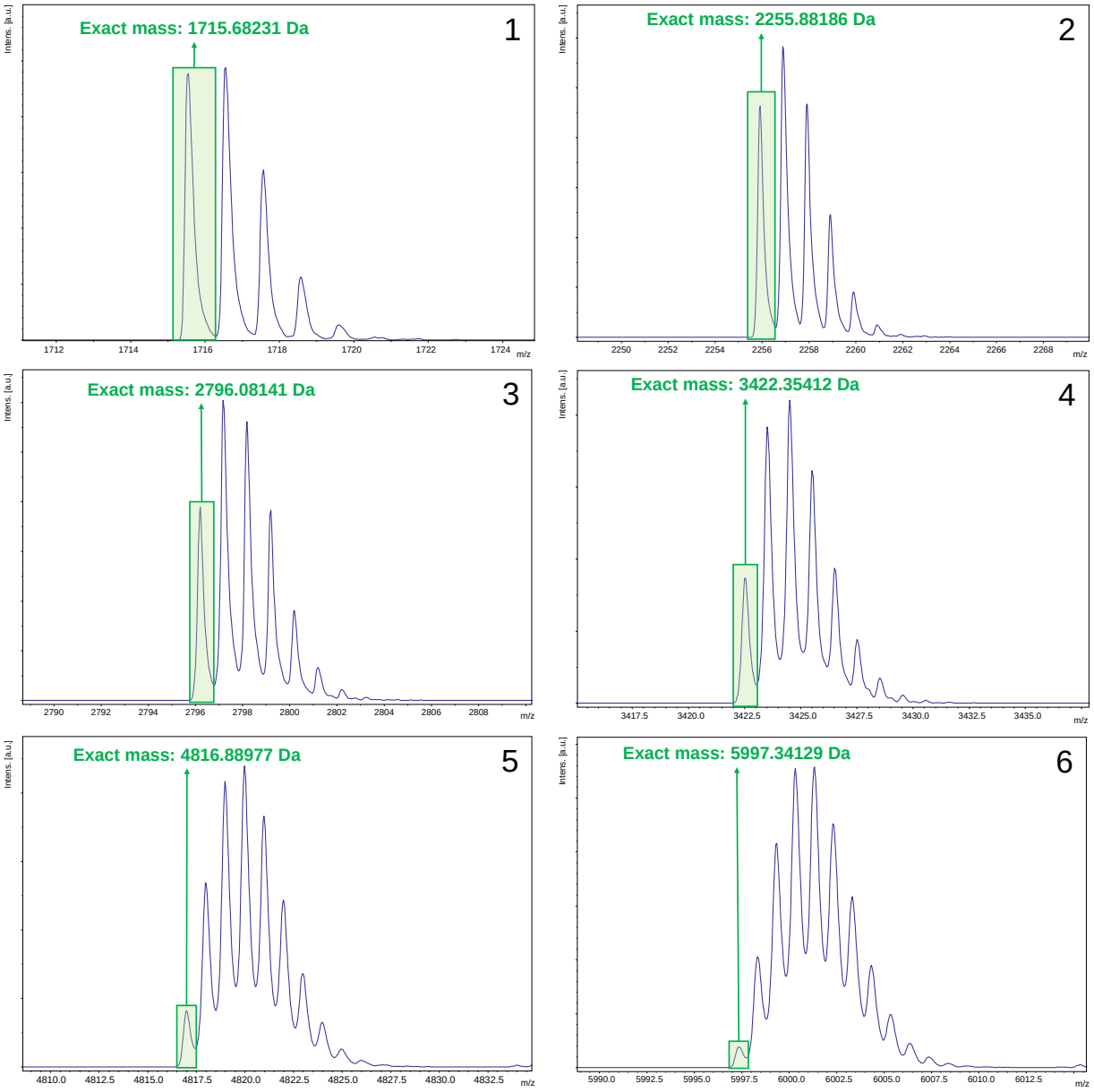


Exhibit 2B: Picking of average mass peaks for points 7 - 10

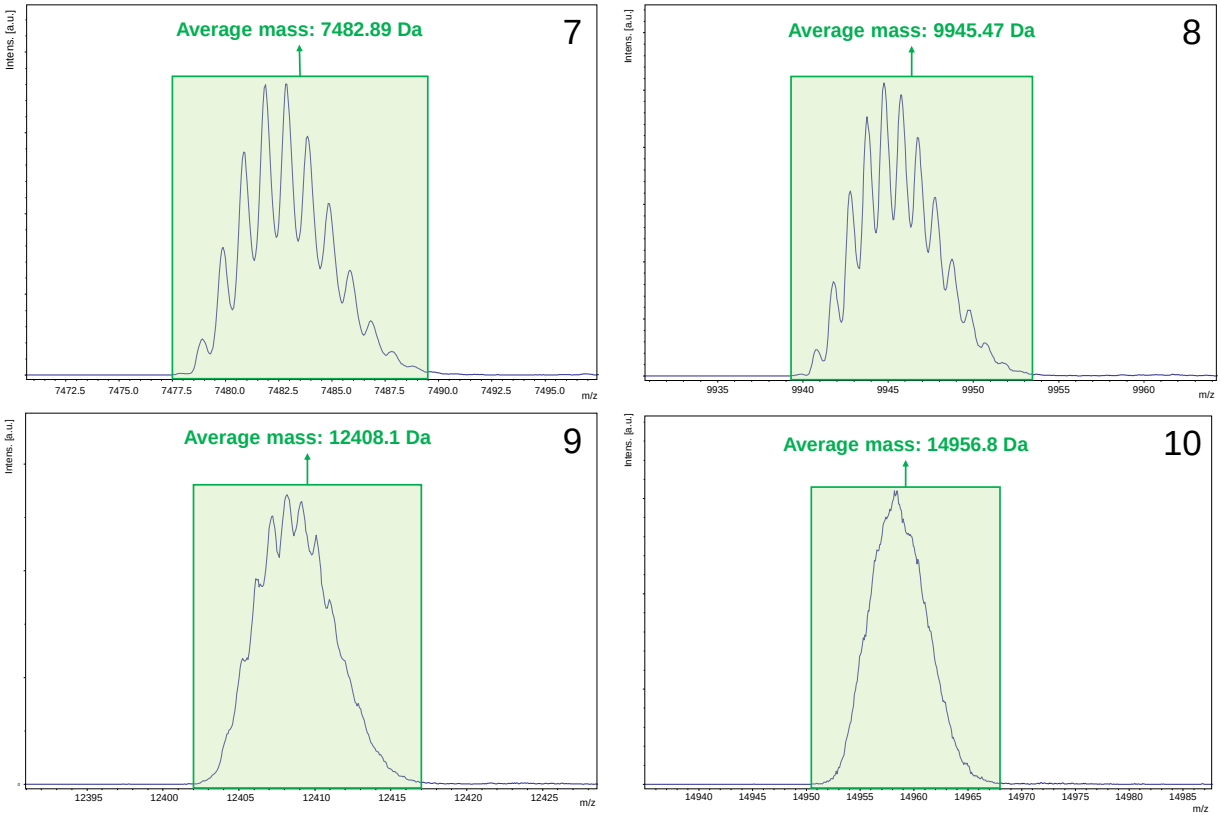
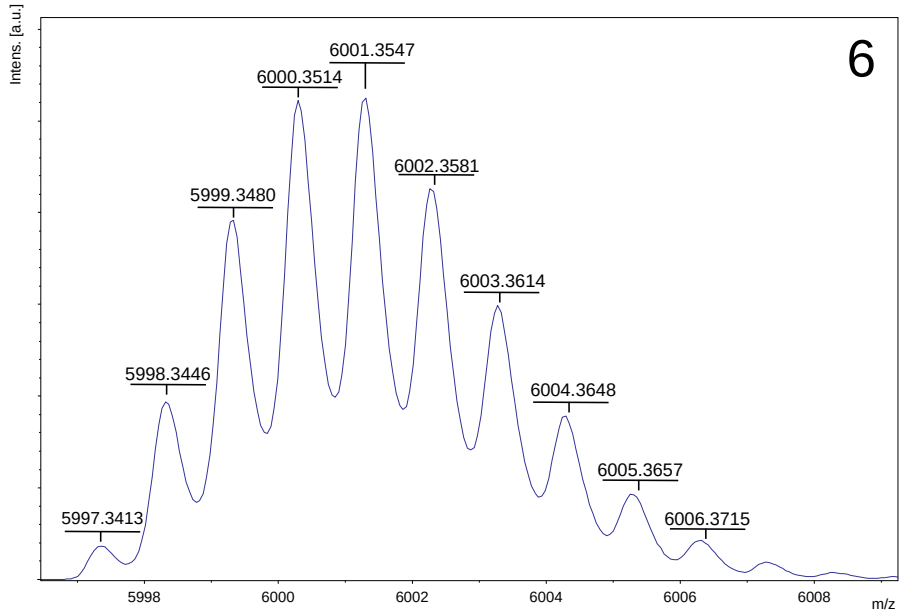


Exhibit 3A: Monoisotopic peak distribution for peak 6

NOTE: Because of the low abundance of the first monoisotopic peak, it may be easier to identify one of the alternative peaks in this distribution. Below is the mass list for the isotopic peak distribution and theoretical relative abundance.

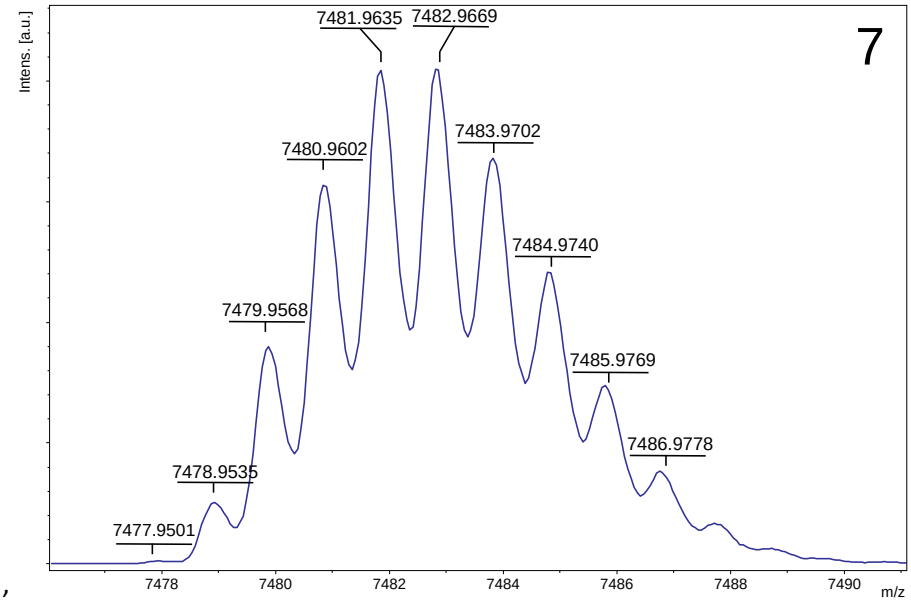


Isotope 1 (Na+ Adduct)	Isotope 2 (Na+ Adduct)	Isotope 3 (Na+ Adduct)	Isotope 4 (Na+ Adduct)	Isotope 5 (Na+ Adduct)
12.2 % 5997.3413 Da	43.4 % 5998.3446 Da	79.5 % 5999.3480 Da	100 % 6000.3514 Da	96.8 % 6001.3547 Da

Isotope 6 (Na+ Adduct)	Isotope 7 (Na+ Adduct)	Isotope 8 (Na+ Adduct)	Isotope 9 (Na+ Adduct)	Isotope 10 (Na+ Adduct)
77.0 % 6002.3581 Da	52.2 % 6003.3614 Da	31.0 % 6004.3648 Da	16.4 % 6005.3657 Da	7.9 % 6006.3715 Da

Exhibit 3B: Monoisotopic peak distribution of peak 7

NOTE: Because of the low abundance of the first monoisotopic peak, it may be easier to identify one of the alternative peaks in this distribution. Below is the mass list for the isotopic peak distribution and theoretical relative abundance.



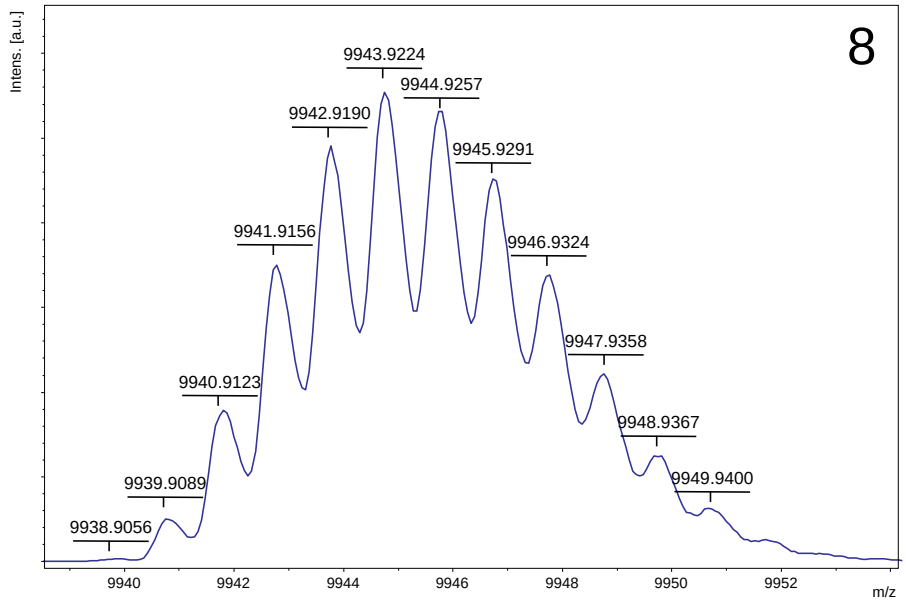
,

Isotope 1 (Na+ Adduct)	Isotope 2 (Na+ Adduct)	Isotope 3 (Na+ Adduct)	Isotope 4 (Na+ Adduct)	Isotope 5 (Na+ Adduct)
7.1 %	30.6 %	65.6 %	93.6 %	100 %
7477.9501 Da	7478.9535 Da	7479.9568 Da	7480.9602 Da	7481.9635 Da

Isotope 6 (Na+ Adduct)	Isotope 7 (Na+ Adduct)	Isotope 8 (Na+ Adduct)	Isotope 9 (Na+ Adduct)	Isotope 10 (Na+ Adduct)
85.2 %	60.4 %	36.6 %	19.3 %	10.4 %
7482.9669 Da	7483.9702 Da	7484.9736 Da	7485.9769 Da	7486.9778 Da

Exhibit 3C: Monoisotopic peak distribution of peak 8

NOTE: Because of the low abundance of the first monoisotopic peak, it may be easier to identify one of the alternative peaks in this distribution. Below is the mass list for the isotopic peak distribution and theoretical relative abundance.



Isotope 1 (Na+ Adduct)	Isotope 2 (Na+ Adduct)	Isotope 3 (Na+ Adduct)	Isotope 4 (Na+ Adduct)	Isotope 5 (Na+ Adduct)	Isotope 6 (Na+ Adduct)
2.0 %	11.4 %	32.6 %	61.9 %	88.1 %	100 %
9938.9056 Da	9939.9089 Da	9940.9123 Da	9941.9156 Da	9942.9190 Da	9943.9224

Isotope 7 (Na+ Adduct)	Isotope 8 (Na+ Adduct)	Isotope 9 (Na+ Adduct)	Isotope 10 (Na+ Adduct)	Isotope 11 (Na+ Adduct)	Isotope 12 (Na+ Adduct)
94.5 %	76.3 %	53.9 %	33.7 %	20.4 %	12.8 %
9944.9257 Da	9945.9291 Da	9946.9324 Da	9947.9358 Da	9948.9367 Da	9949.9400

Contact

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