MALDI Calibration with SpheriCal® Peptide Medium

The following is a short and simple explanation on how to set up the necessary files as well as a suggestion for sample preparation for the best possible calibration of your MALDI-ToF. It is written to comply with Bruker instruments, but is applicable to other instruments – please adapt to your software and hardware.

1. Product Description and Storage information

SpheriCal® Peptide Medium is a mixture of six monodisperse dendrimers that is intended for m/z calibration of MALDI-TOF mass spectrometers. This product is intended for sample preparation with organic solvents and allows for m/z calibration in the range between 500 Da and 3,500 Da.

1.1. Storage and shelf life

SpheriCal® is shipped without temperature control. Once received, please store the vials in the freezer at -20°C to ensure a shelf life of 3 years post production. The expiry date is stated on each vial.

Chemically, the SpheriCal® technology can be considered a polyester. As such, the molecules are very stable over a long period of time. However, hydrolysis can occur in aqueous environments at highly acidic or highly basic conditions. Accordingly, we advise you to store SpheriCal® in dry conditions in the freezer.

1.2. Order Information

This product (product number PFS-0002) can be re-ordered at https://bit.ly/PFS-0002

2. Instructions for calibration

2.1. Software - Mass Control List

To calibrate your instrument you will need a Mass Control List. You can either generate your own or, if compatible with your software, use the lists supplied by us on our homepage. You may download a compressed folder with all our products mass control lists at:

https://bit.ly/SpheriCal_MCL

Once downloaded, decompress and place the files in the relevant folder on your instrument computer (e.g. *Mass Control Lists* at *D:\Methods\MassControlLists*). Make sure it has the correct file extension (.mcl). If your software requires another file format, you should be able to create your own mass control list with the values provided in Table *1*.

You will find “monoisotopic” and “average” lists. We recommend choosing according to your instrument’s resolving power in the intended m/z range and your method (e.g. linear or reflector mode). Alternatively, the chemical formula of each component is listed below for you to calculate your own masses if necessary.

Once you have placed the supplied mass list file in the correct folder, or generated your own, the masses will be visible in your calibrant list in the 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 Na-adducts as these usually are the dominating species for our calibration molecules.

Table 1. Masses and formulas for SpheriCal® Peptide Medium.

Component	Formula	$[M+Na]^+$ (monoisotopic)
PFS2011	$C_{31}H_{38}O_{10}$	593.236 Da
PFS2021	$C_{60}H_{70}O_{20}$	1 133.436 Da
PFS2031	$C_{92}H_{108}O_{30}$	1 715.682 Da
PFS2041	$C_{121}H_{140}O_{40}$	2 255.882 Da
PFS2051	$C_{150}H_{172}O_{50}$	2 796.081 Da
PFS2061	$C_{184}H_{214}O_{61}$	3 422.354 Da

2.2. Calibration - general

The following is a guide on how to calibrate the MALDI-ToF MS after the previous step is 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.

To ensure the highest possible accuracy, we recommend preparing a calibration spot in the direct vicinity of your analyte spots. By generating a pattern as shown in Figure *1*, you can use one calibration spot for 8 analyte spots.

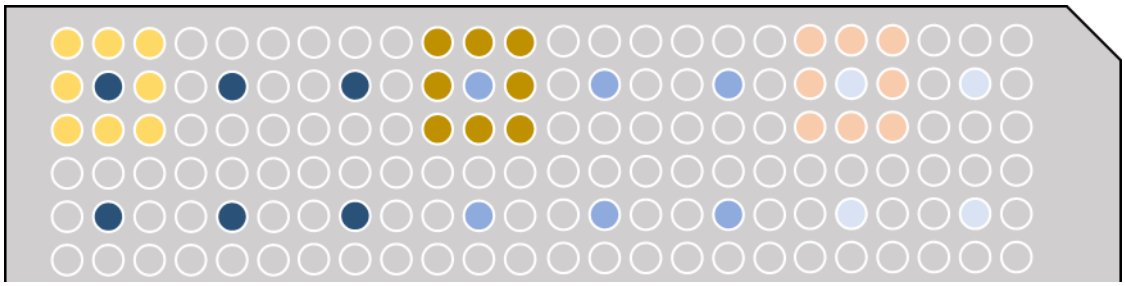

Figure 1. Suggested calibration spot pattern. Blue represents calibration spots. Yellow, brown, and pink represent analyte spots.

2.3. Calibration - preparation of spots by pre-mixing, dried droplet method

For the best results, we suggest using a dry organic solvent. THF, acetonitrile, chloroform and dichloromethane are suitable solvents. Sodium trifluoroacetate (NaTFA) is recommended as the cation-source, and trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) or 2,5-Dihydroxybenzoic acid (DHB) as the matrix. Other suitable matrices for this product are HCCA, Sinapic acid, 9NA, HABA, Ditranol, NALDI, graphite, etc.

To achieve calibration spots with good signal intensity and distribution, we recommend to pre-mix calibrant, counter ion, and matrix and store in aliquots.

1) Each new vial contains 50 µg of SpheriCal®. Add 50µL of solvent into the vial to prepare a SpheriCal® solution at a concentration of 1 mg/mL (**solution A**).
2) Prepare a NaTFA solution in the same solvent at a concentration of 2 mg/mL (**solution B**).
3) Prepare a matrix solution in the same solvent at a concentration of 20 mg/mL (**solution C**).
4) Add 5 µL of solution A, 5µL of solution B and 20 µL of solution C to a suitable vial (e.g. an Eppendorf vial or their lid). Mix well to generate **solution D**. This solution contains the calibrant at 0.166 mg/mL and the calibrant:counterion:matrix ratio (by mass) is 1:2:80.
5) Pipette up to 0.5 µL of solution D onto the desired spots on the target plate and leave to dry under gentle air flow.
6) Allow the remaining solution A to dry in the vial, preferably under a gentle air/nitrogen flow. Close the lid once dried and note the remaining SpheriCal® amount.
7) Once the prepared calibration spots have dried, you are ready to start your measurements.
8) Measure the MALDI spectrum of a calibration spot, ensure good signal to noise spectra. Compare your collected spectrum with *Figure 2*. Intensities and intensity ratios of the 6 components will vary, depending on the matrix used and the tuning of your instrument.
9) Within the calibration tab of your software, choose the *SpheriCal® Peptide Medium* mass list and click “automatic assignment”. The software should automatically assign the peaks. Click through the assignments and ensure that the software has chosen a good peak positon (cf. *Figure 2*, bottom). With the available 6 calibration points, you can use advanced algorithms such as *cubic enhanced*. Click *calibrate* to perform your calibration.
10) You are now ready to measure your analyte spots in direct vicinity with a well-calibrated MALDI.

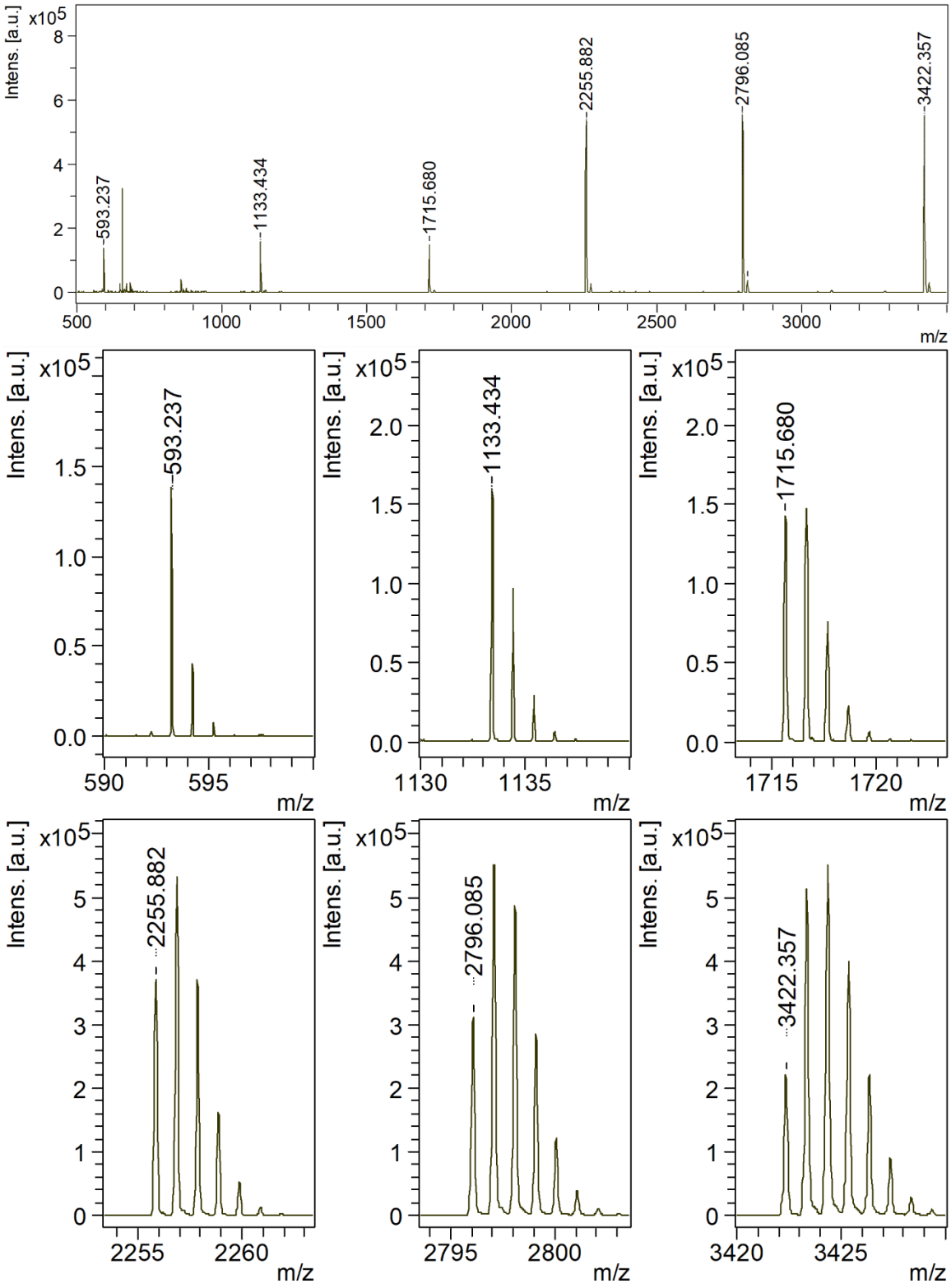

Figure 2. MALDI spectrum of PFS-0002 obtained with HCCA as matrix.

Notes:

- Changing acquisition parameters will invalidate the calibration. Re-calibrate once you have changed parameters.
- If you prefer to simplify the process of preparing calibration spots, you might consider purchasing our SpheriCal® Mix products. The SpheriCal® Mix product range is available for many common matrices and enables an even quicker and easier calibration. These products are available at https://bit.ly/SpheriCal-MIX

Contact

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