

Application News

MALDI-TOF Mass Spectrometry Analysis

SpheriCal Calibrants on the MALDI-8020. High Purity, Monodisperse Calibrants Suitable for a Range of Applications

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User Benefits

- ◆ High purity, monodisperse calibrants that provide the flexibility to use as either an internal calibrant or external calibrant
- ◆ Lower residual mass errors when compared to standard protein and peptide calibrants
- ◆ Clearly defined calibrant masses applicable to a wide range of applications e.g. polymers, proteins etc.

Introduction

The MALDI-8020 linear benchtop MALDI-TOF mass spectrometer (Figure 1) is a versatile instrument that has proven itself an analytical workhorse with a diverse array of analytes. One challenge users often face is finding mass calibrants to suit their analyte. In MALDI laboratories, a large selection of peptides, proteins, oligonucleotides, polymers, lipids and other inorganic molecules are used for instrument calibration.

An alternative approach for MALDI-TOF calibration is using SpheriCal standards (Polymer Factory, Sweden). These dendrimer polymer calibrants are available as SpheriCal 'Neat' and 'Aqua' forms (Figure 2, Figure 3). With improved stability relative to traditional peptide or protein calibrants¹, these compounds produce highly resolved peaks which can provide better mass accuracy, particularly at higher masses. SpheriCal calibrants exhibit long dry shelf-lives at room temperature, even when exposed to ambient light and Oxygen (no visible sign of degradation even after 4 years and 5 months¹).

Synthetic dendrimer calibrants are offered to cover the mass range m/z 200 through to m/z 30,000 and work with a range of commonly used MALDI matrices.

In this application note, we demonstrate the utility of these calibrants in some common workflows.

Calibrant	Mass range
SpheriCal Peptide Low + Small Molecule	250 - 1,600 Da
SpheriCal Peptide Low	700 - 1,600 Da
SpheriCal Peptide Medium	1,600 - 3,500 Da
SpheriCal Peptide High	3,500 - 7,500 Da
SpheriCal Protein Low	7,500 - 15,000 Da
SpheriCal Protein Medium	15,000 - 30,000 Da
SpheriCal Aqua Peptide Low	500 - 1,100 Da
SpheriCal Aqua Peptide Medium	1,000 - 2,500 Da
SpheriCal Aqua Peptide High	2,500 - 5,200 Da
SpheriCal Aqua Protein Low	5,200 - 11,000 Da

Figure 2. SpheriCal calibrant range



Figure 1. MALDI-8020 mass spectrometer

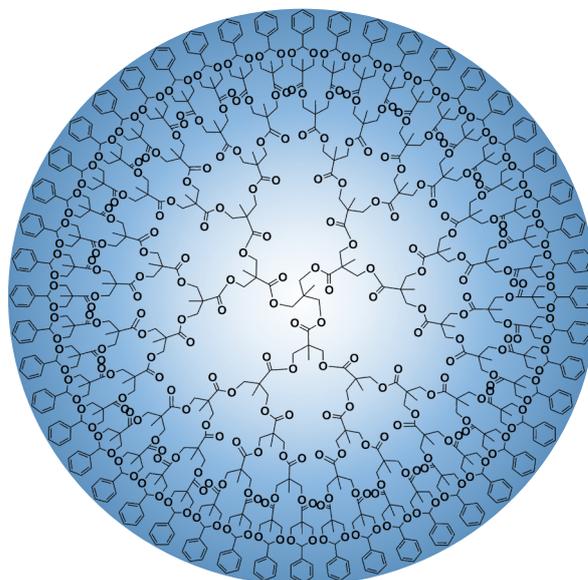


Figure 3. Representative structure of the SpheriCal calibrant¹

■ Measurement Conditions and Samples

SpheriCal Neat calibrants were evaluated in comparison to standard MALDI-TOF calibrants. In addition, SpheriCal Aqua was used as an internal calibrant to determine its suitability in a meat typing project.

SpheriCal Neat

SpheriCal Neat samples were reconstituted in 50 μ L THF. DCTB matrix was prepared at 10 mg/mL in THF. NaTFA was prepared at 2 mg/mL in THF. The calibrant was mixed with the matrix solution and salt solution as 5 μ L calibrant, 5 μ L DCTB matrix and 5 μ L NaTFA. 0.5 μ L of the mixed sample was spotted on a FlexiMass-SR48 stainless steel slide and allowed to dry.

PEG 1,500 was prepared as per our in-house standard PEG sample preparation method. 2 mg of PEG standard was weighed out and 1 mL of THF was added. DHB matrix was prepared at 10 mg/mL in THF. AgTFA was prepared at 2 mg/mL in THF. The sample spot was precoated with 0.5 μ L AgTFA and allowed to dry. 0.5 μ L of the PEG1500 solution was spotted and overlaid with 0.5 μ L matrix on a FlexiMass-SR48 stainless steel slide and allowed to dry (Figure 4).

0.5 μ L of peptide mix was spotted with 0.5 μ L CHCA (5 mg/mL in 50:50 ACN:0.1 % TFA). 0.5 μ L of Cytochrome C (5 pmol/ μ L) was spotted with 0.5 μ L sinapinic acid (20 mg/mL in 50:50 ACN:0.1% TFA (Figure 5).

SpheriCal Aqua

SpheriCal Aqua samples were reconstituted in 50 μ L 50:50 ACN:H₂O. CHCA was prepared as 5 mg/mL in 50:50 ACN:H₂O and the NaTFA was prepared as 2 mg/mL in 50:50 ACN:H₂O. The calibrant was mixed with the matrix solution and salt solution as 5 μ L calibrant, 5 μ L matrix and 5 μ L salt. 0.5 μ L of the mixed solution was spotted on a FlexiMass-SR48 stainless steel slide and allowed to dry. SpheriCal Aqua samples were retested after 36 days in solution with fresh matrix and salt solution and the samples continued to perform well.

SpheriCal Aqua as an internal calibrant

SpheriCal Aqua samples were reconstituted in 50 μ L 50:50 ACN:H₂O. Meat homogenate samples (Figure 6) were prepared by taking 20 mg of meat (avoiding fatty sections where possible), adding 500 μ L of deionised H₂O and homogenising for 1 minute. The homogenates were centrifuged at 13,000 RPM for 5 minutes. The supernatant was transferred to a fresh tube, 1 μ L 10% TFA was added until the solution was acidified. NaTFA was prepared at 2mg/mL in 50:50 ACN:H₂O. CHCA was prepared at 40 mg/mL in 33:33:33 ACN:EtOH:H₂O. The SpheriCal Aqua was mixed with the salt solution in the ratio 5 μ L calibrant: 5 μ L salt solution. 1 μ L of the internal calibrant solution was then added to 9 μ L of sample. 0.5 μ L of spiked sample was then spotted with 0.5 μ L of matrix on a FlexiMass-SR48 stainless steel slide and allowed to dry. Linear calibration was used.

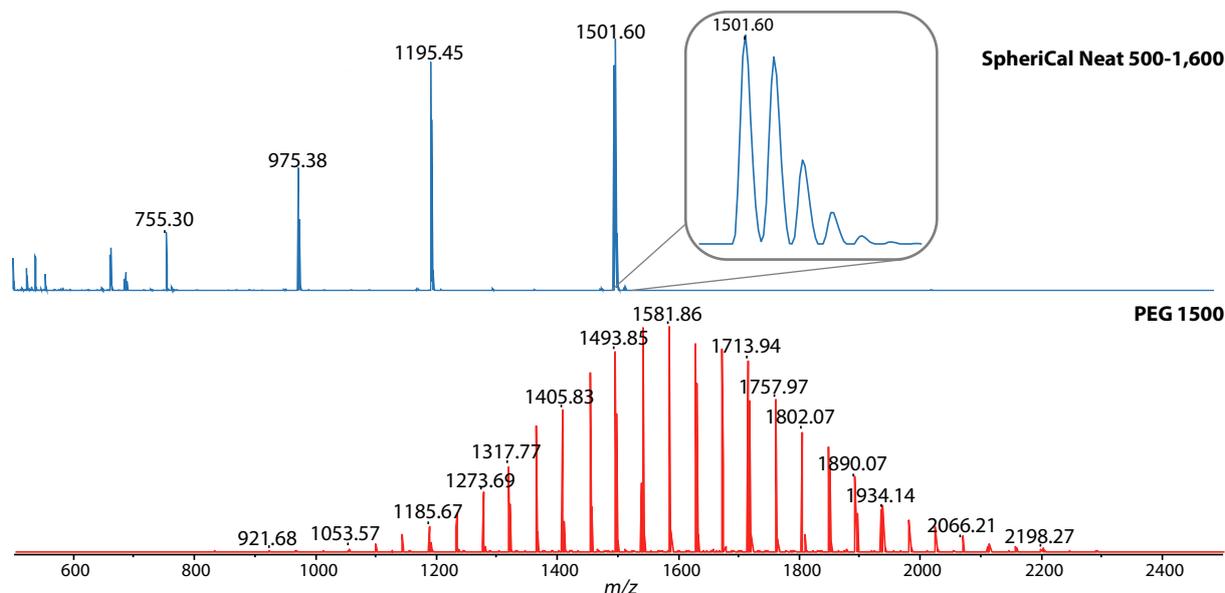


Figure 4. Top: SpheriCal Neat 500-1,600 (NaTFA + DCTB) and Bottom: PEG 1500 (AgTFA + DHB). SpheriCal calibration peaks at m/z 755.30, m/z 975.38, m/z 1195.45 and m/z 1501.60.

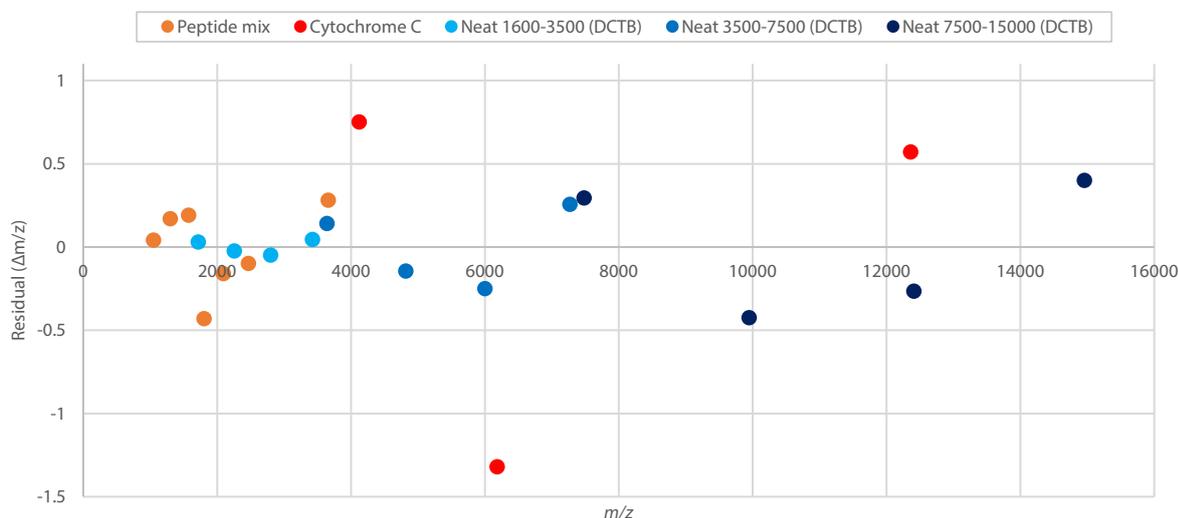


Figure 5. The calibration residual mass errors for SpheriCal neat (light blue, blue and dark blue) plotted against residual errors for a peptide mix (orange) and Cytochrome C (red, average mass).

■ Results

There are two forms of SpheriCal calibrant for both polar and aqueous compounds (Neat and Aqua) and they cover a useful mass range of m/z 250-30,000 for SpheriCal Neat and m/z 500-11,000 for SpheriCal Aqua.

When calibrating an instrument with a new pulsed extraction delay for analysis in a different mass range, calibrations can sometimes be quite far out from where they should be. Also, when calibrating using polymer calibrants, it can sometimes be confusing which monomer unit to assign to a given calibrant m/z , which could result in an incorrect calibration.

Using the SpheriCal Neat samples, a minimum of 4 clearly defined calibration peaks (Figure 4) make assigning the correct peak simple. Both DCTB and Sinapinic acid have been found to be effective with SpheriCal Neat samples when prepared with NaTFA as a cation and using THF as a solvent. SpheriCal Aqua works well with CHCA and solvents commonly used in peptide and protein analysis (namely 50:50 ACN:H₂O with the addition of NaTFA).

In addition to providing low mass error calibration peaks (Figure 5), in-vial stability of some SpheriCal Aqua calibrants has been shown to exceed 36 days when stored in solution under standard lab conditions (stored in the dark at 20°C (+/- 4°C, (data not shown)).

■ Conclusion

- SpheriCal calibrants are a versatile range of calibrants that can be very useful when performing MALDI-TOF analysis. Available in both hydrophilic (Aqua) and hydrophobic (Neat) varieties, there is a calibration option for both hydrophilicities.
- SpheriCal calibrants can produce instrument calibrations with residual errors lower than those achieved with common peptide and protein standards.
- The SpheriCal calibrants provide a helpful option for internal calibration in complex samples like extracts from biological samples.
- When working with polymers using novel matrices, SpheriCal calibrants can provide absolute confidence that the right peaks are selected unlike when using other polymer standards.
- SpheriCal calibrants make a great addition to the MALDI calibrant line up for use on the MALDI-8020 and other MALDI instruments.

<References>

- 1) S. M. Grayson, B. K. Myers, J. Bengtsson, and M. Malkoch, "Advantages of Monodisperse and Chemically Robust "SpheriCal" Polyester Dendrimers as a "Universal" MS Calibrant", *J. Am. Soc. Mass Spectrom.* (2014) 25, 3, 303–309.

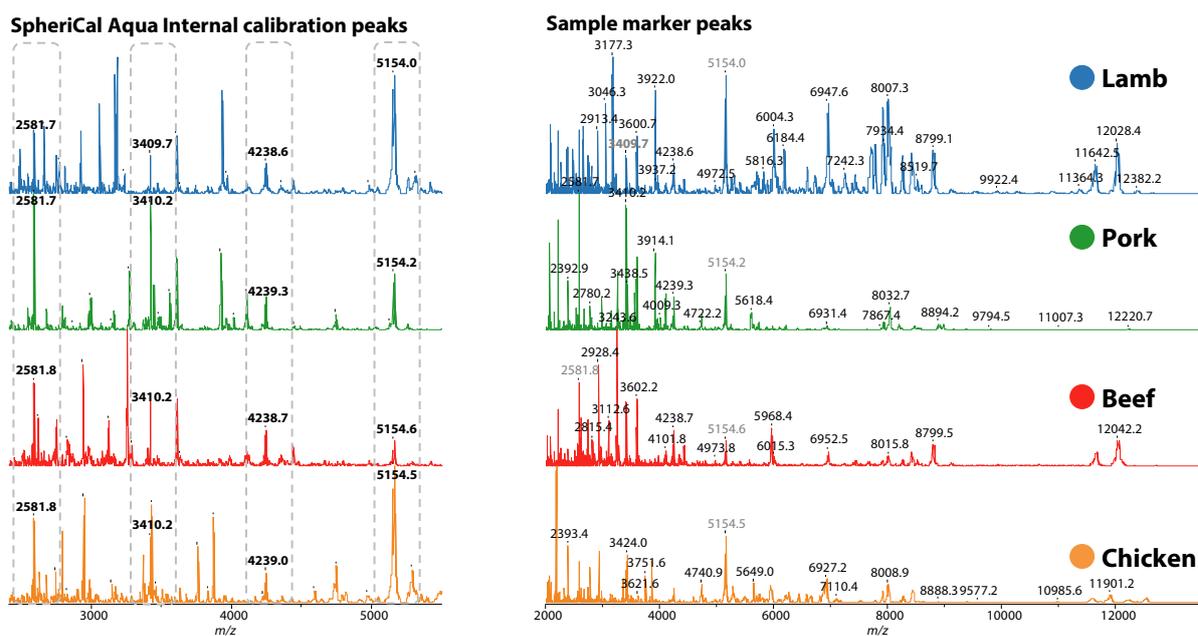


Figure 6. Meat typing spectra containing SpheriCal Aqua Peptide High internal calibration peaks at m/z 2581.6, m/z 3410.4, m/z 4239.2 and m/z 5154.2.

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