Technical Note PFTN001:

Sample preparation for SpheriCal® 10-Point with CHCA matrix

This technical note details the preparation of SpheriCal® 10-Point MALDI-TOF calibration spots with CHCA matrix, commonly used for the analysis of proteins, peptide and other biological molecules. For optimal results, matrix and solvents of the highest purity available should be used.

1.1 Chemicals required

- CHCA (α-cyano-4-hydroxycinnamic acid) matrix, ≥ 99 %
- Sodium trifluoroacetate (NaTFA), HPLC grade, ≥ 99 %
- Acetonitrile (ACN) solvent, chromatography grade, 99.9 %
- Ultrapure water (Milli-Q or similar)

1.2 Preparation of solutions

1. Dissolve the contents of the SpheriCal® 10-Point vial in 50 µl acetonitrile.
2. Prepare a solution of sodium trifluoroacetate at a concentration of 2 µg / µl in acetonitrile.
3. Prepare a saturated solution of CHCA in acetonitrile (approximately 10 µg / µl can be added). The solution should be thoroughly mixed and sonicated to aid dissolution for at least 5 minutes. The resulting solution should be centrifuged for 5 minutes at 6,000 rpm and the transparent phase collected.

1.3 Preparation of calibration sample on target plate

1. Combine the above solutions in a volume ratio [calibrant]:[NaTFA]:[CHCA] of [1]:[2]:[8] and mix thoroughly with a vortex mixer.
2. Using the dried droplet method, apply 1 µl of this solution to your target and allow to dry at room temperature.
3. **OPTIONAL:** the sample spot can be washed with 3 µl Milli-Q water to remove salts and matrix clusters from the surface, which can contribute to increased noise in the resulting mass spectra. Note that washing may reduce the homogeneity of the crystalline deposit, and may not be suitable for laser shot raster methods with a smaller focus.

2.1 Calibration mass list

Unlike protein or peptide calibration standards, SpheriCal® favors ionization with alkali metal cations. Therefore, the reference masses for calibration will typically be sodium adducts. The chemical formula, monoisotopic exact mass and average mass for each standard included in the SpheriCal® 10-Point product are given below.

		[M + Na]$^+$	
Standard	**Formula**	**Exact mass***	**Average Mass**
PFS-50A (#1)	$C_{92}H_{108}O_{30}$	1715.68231	1716.82
PFS-50B (#2)	$C_{121}H1_{40}O_{40}$	2255.88186	2257.38
PFS-50C (#3)	$C_{150}H_{172}O_{50}$	2796.08141	2797.94
PFS-50D (#4)	$C_{184}H_{214}O_{61}$	3422.35412	3424.63
PFS-50E (#5)	$C_{257}H_{300}O_{88}$	4816.88977	4820.08
PFS-50F (#6)	$C_{320}H_{372}O_{110}$	5997.34129*	6001.31
PFS-50G (#7)	$C_{398}H_{468}O_{138}$	7477.95010*	7482.89
PFS-50H (#8)	$C_{529}H_{620}O_{184}$	9938.90558*	9945.47
PFS-50I (#9)	$C_{660}H_{772}O_{230}$	12399.8611*	12408,1
PFS-50J (#10)	$C_{796}H_{934}O_{277}$	14946.8897*	14956,8

***The first monoisotopic peak may not be of sufficient abundance to calibrate from. Please see the SpheriCal® 10-Point calibration guide additional isotopic mass lists or use the average mass to calibrate.*

2.2 Example MALDI-TOF Mass Spectra

Spectra were recorded with a Bruker Daltonics UltraFlex, operated in linear mode with real-time smoothing function applied. No further processing was performed. SpheriCal® 10-point combined with CHCA matrix will provide spectra with sufficient intensity of all peaks for accurate calibration in linear mode. For more detailed information, please refer to the calibration guide.

Exhibit 1: SpheriCal® 10-point spectrum with CHCA matrix. Sample spot washed with water prior to analysis

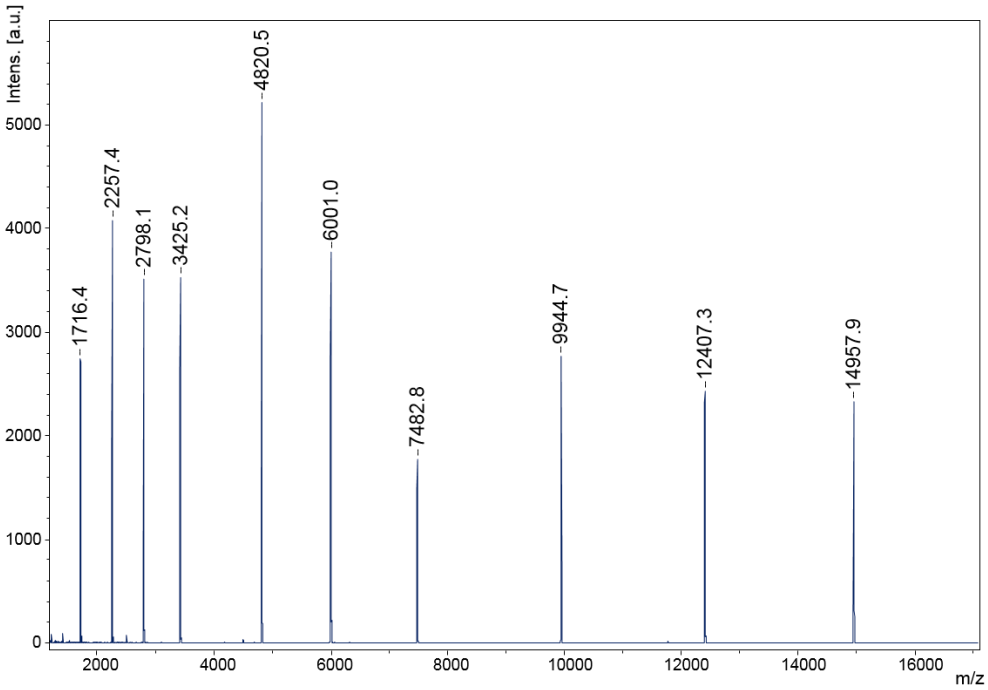
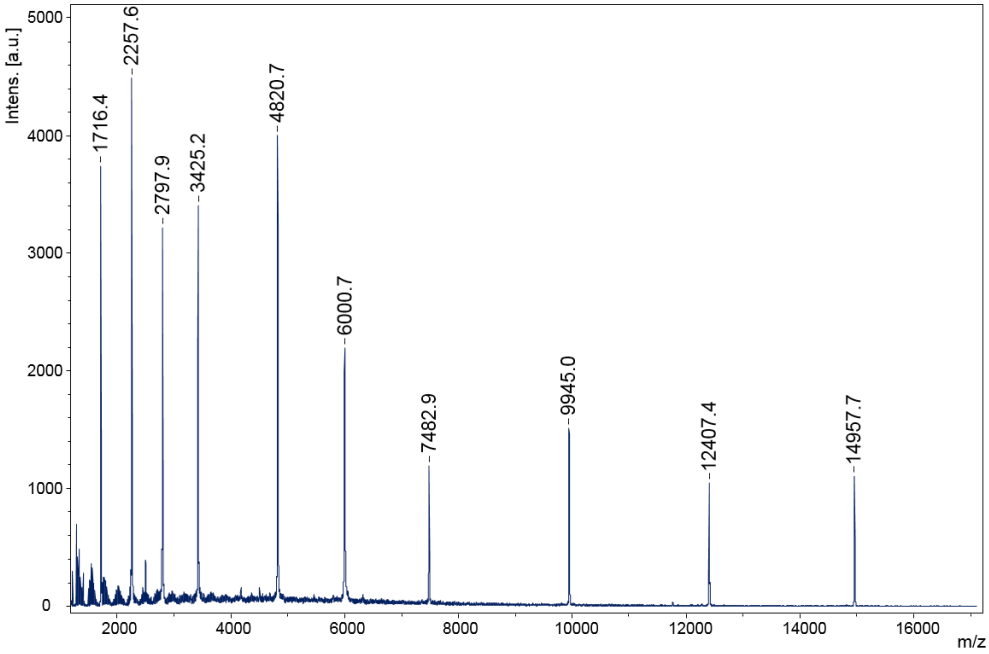


Exhibit 2: SpheriCal® 10-point spectrum with CHCA matrix



Contact

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