

## SpheriCal® for tuning and calibration of IM-MS equipment

Thank you for purchasing SpheriCal® for IM-MS.

SpheriCal® IM-MS Medium Range is a mixture of 9 different dendritic components that ionize both with MALDI and ESI sources. In MALDI, singly charged  $[M+Na]^+$  are expected and work in positive mode only. In ESI, both negative and positive mode are working and yield multiple charge states depending on the molecular mass of the dendrimer.

This mixture covers the following ranges within  $m/z$ , CCS, and charge states:

Mode	Molecular masses [Da]	$m/z^*$		CCS <sub>N2</sub> range* [ $\text{\AA}^2$ ]	Charge states*
ESI positive	600 – 7,103	$[M+nNa]^{n+}$	623 – 2,390	220 – 975	+1 → +4
		$[M+nH]^{n+}$	601 – 2,368	218 - tbd	+1 → +4
MALDI positive	600 – 7,103	$[M+Na]^+$	623 – 7,126	220 - tbd	+1
ESI negative	600 – 7,103	$[M-H]^{n-}$	599 – 2,366	tbd	-1 → -4
		$[M+FA-nH]^{n-}$	645 – 2,412	tbd	-1 → -4
		$[M+TFA-nH]^{n-}$	713 – 2,480	tbd	-1 → -4

\* Observable ions and charge states depend on instrumentation, sample preparation and ionization conditions.

## 1. Product Description and Storage Information

SpheriCal® IM-MS Medium Range is a mixture of nine monodisperse dendrimers which are intended for  $m/z$  and CCS calibration and tuning of IM-MS instrumentation.

### 1.1. Storage and shelf life

SpheriCal® is shipped without temperature control. Once received, please store the vials in the freezer at  $-20^\circ\text{C}$  to ensure a shelf life of 3 years post production. The expiring 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 prior to use. Once aqueous aliquots are prepared, it is crucial to store them in the freezer.

## 2. Instructions for Usage

### 2.1. Preparation of ESI spraying solution

The product is soluble in polar solvents, such as methanol, ethanol, THF and aqueous mixtures of these, as well as aqueous acetonitrile. For aqueous acetonitrile, we recommend mixing the solvents first before adding the mixture to the product vial. The products have been developed based on 50/50 ACN/H<sub>2</sub>O v/v. For negative mode, add small amounts of formic acid, acetic acid or tri-fluoro acetic acid (0.1% of the aqueous portion).

Simply add 1.00 mL of spraying solution the product vials and shake well or use a vortex mixer.

Prepare aliquots that match your intended/expected use in suitable vials and store in the freezer. Prevent repeated thawing/freezing of the aliquots.

## 2.2. Preparation of MALDI spots

- Pre-spot the desired position with 0.5  $\mu$ L of NaTFA solution at 2 mg/mL.
- Add 20  $\mu$ L solvent to prepare the SpheriCal solution at 0.5 mg/mL. Add 0.5  $\mu$ L onto the desired spot and let dry.
- Prepare the matrix solution at 20 mg/mL and add 0.5  $\mu$ L on top of the NaTFA/SpheriCal and let dry.
- Suitable matrices are HCCA, DHB, graphite.

## 3. Observable ions and their parameters

### 3.1. Composition

SpheriCal<sup>®</sup> IM-MS consists of nine dendritic components that cover a molecular mass between 600 and 7,099 Da, see Table 1.

Table 1. Components of SpheriCal<sup>®</sup> IM-MS Medium Range

Component ID	Chemical Formula	M <sub>mono</sub> [Da]
PFS-1042	C <sub>25</sub> H <sub>44</sub> O <sub>16</sub>	600.2629
PFS-3012	C <sub>37</sub> H <sub>62</sub> O <sub>22</sub>	858.3733
PFS-2032	C <sub>50</sub> H <sub>84</sub> O <sub>30</sub>	1164.5047
PFS-2042	C <sub>65</sub> H <sub>108</sub> O <sub>40</sub>	1528.6417
PFS-2052	C <sub>80</sub> H <sub>132</sub> O <sub>50</sub>	1892.7786
PFS-2062	C <sub>100</sub> H <sub>166</sub> O <sub>61</sub>	2342.9887
PFS-3042	C <sub>145</sub> H <sub>236</sub> O <sub>88</sub>	3385.3992
PFS-3052	C <sub>180</sub> H <sub>292</sub> O <sub>110</sub>	4213.7255
PFS-4042	C <sub>305</sub> H <sub>492</sub> O <sub>184</sub>	7098.9142

### 3.2. Positive mode

In general, the components in the mixture form mostly  $[M+nNa]n+$  ions in positive mode.  $[M+nH]n+$  might also be observable in ESI. In ESI, intensity ratios between sodiated and protonated ions are related to the availability of sodium ions during the ionization process. Observable ions are listed in Table 2. Figure 1 shows an ESI-MS spectrum, Figure 2 shows the MALDI-MS spectrum, and Figure 3 shows the mobility traces of this product.

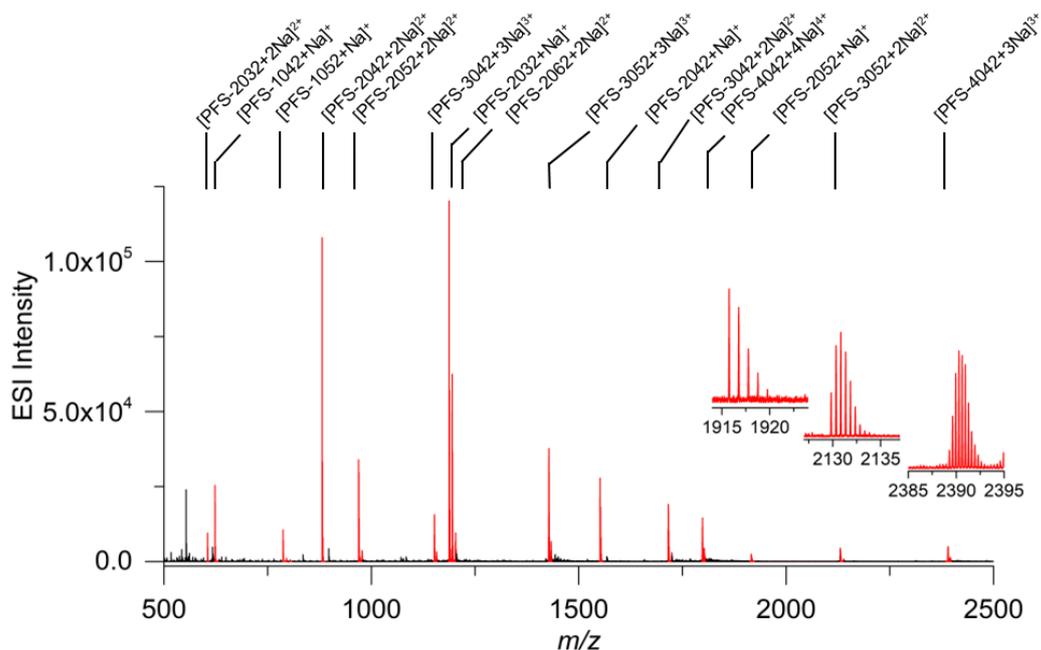


Figure 1: ESI spectrum of SpheriCal® IM-MS Medium Range in positive mode as measured on a Waters Cyclic IMS from ACN/H<sub>2</sub>O/FA 50/49.95/0.05.

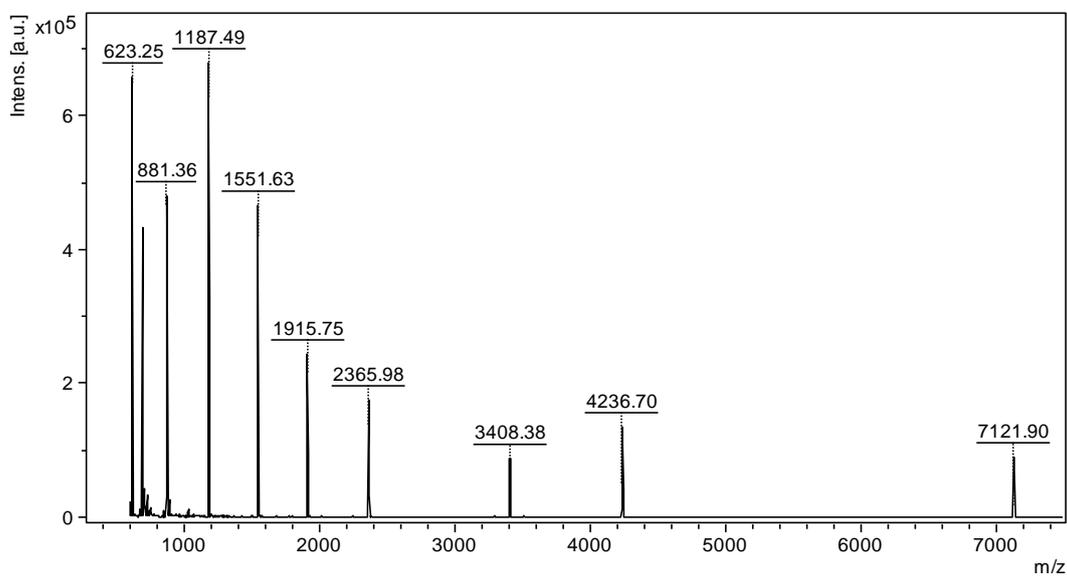


Figure 2: MALDI-MS spectrum of SpheriCal IM-MS Medium Range in positive mode, using DHB as matrix.

Table 2. Observable ions of SpheriCal® IM-MS Medium Range in positive mode.

Ion	$m/z$	$^{DT}CCS_{N_2}$ [ $\text{Å}^2$ ]
[PFS-2032+2H] <sup>2+</sup>	583.2596	346.21
[PFS-1042+H] <sup>+</sup>	601.2702	218.61
[PFS-2032+2Na] <sup>2+</sup>	605.2416	351.19
[PFS-1042+Na] <sup>+</sup>	623.2522	220.65
[PFS-2042+2H] <sup>2+</sup>	765.3281	386.62
[PFS-2062+3Na] <sup>3+</sup>	782.338	tbd
[PFS-2042+2Na] <sup>2+</sup>	787.3101	388.89
[PFS-2062+3Na] <sup>3+</sup>	804.3199	519.29
[PFS-3012+H] <sup>+</sup>	859.3806	tbd
[PFS-3012+Na] <sup>+</sup>	881.3625	287.23
[PFS-2052+2H] <sup>2+</sup>	947.3966	433.39
[PFS-2052+2Na] <sup>2+</sup>	969.3785	438.51
[PFS-3042+3Na] <sup>3+</sup>	1151.79	625.48
[PFS-2032+H] <sup>+</sup>	1165.512	315.77
[PFS-2062+2H] <sup>2+</sup>	1173.003	485.59
[PFS-2032+Na] <sup>+</sup>	1187.494	317.41
[PFS-2062+2Na] <sup>2+</sup>	1194.985	494.35
[PFS-3052+3Na] <sup>3+</sup>	1428.233	703.57
[PFS-2042+H] <sup>+</sup>	1529.649	363.02
[PFS-2042+Na] <sup>+</sup>	1551.631	366.74
[PFS-3042+2Na] <sup>2+</sup>	1716.191	600.23
[PFS-4042+4Na] <sup>4+</sup>	1798.47	975.23
[PFS-2052+H] <sup>+</sup>	1893.786	408.23
[PFS-2052+Na] <sup>+</sup>	1915.768	411.72
[PFS-3052+2Na] <sup>2+</sup>	2130.855	673.77
[PFS-4042+3Na] <sup>3+</sup>	2390.297	950.54

Emily R. Sekera, Árpád Somogyi, Zoltan Takáts, Florian Stappert, Cedric Thom, Oliver J. Schmitz, Claudia Moeckel, Béla Paizs, Jens Sommertune: Utilization of bis-MPA Dendrimers for the Calibration of Ion Mobility Collision Cross Section Calculations; *J. Am. Soc. Mass Spectrom.* 2024, 35, 6, 1101–1109

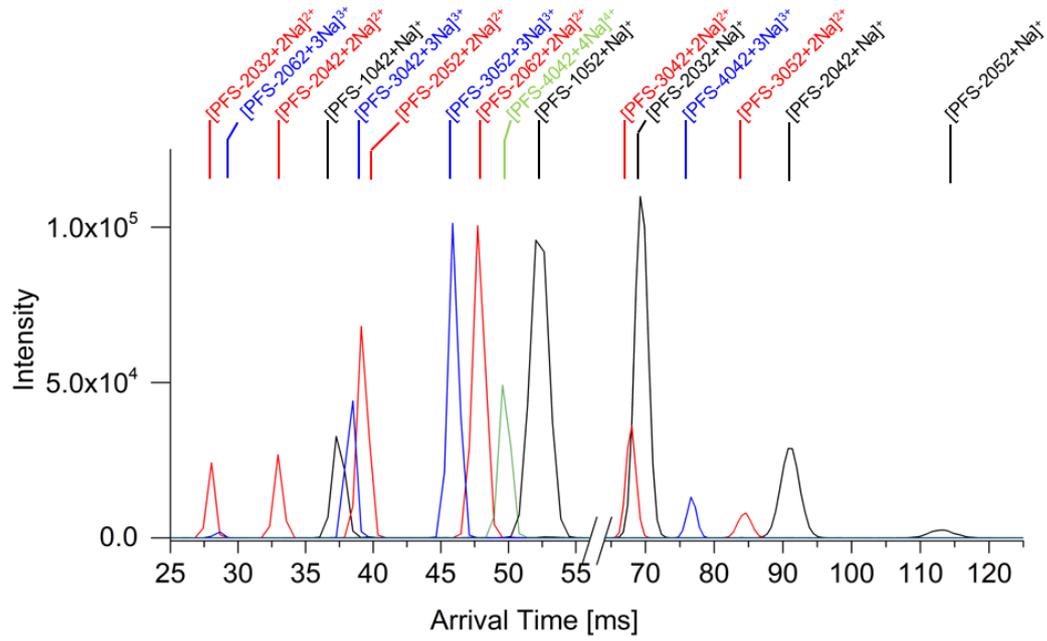


Figure 3. Arrival times for sodiated ions of SpheriCal<sup>®</sup> IM-MS Medium Range as measured on the Waters Cyclic IMS, one cycle.

### 3.3. Negative mode

Please observe: Negative mode works in ESI, but not in MALDI. No CCS values for the negative ions have been reported yet.

In ESI-MS, the components will form  $[M-nH]^{n-}$  and adducts with anions of available acids, such as  $[M+nCOOH-nH]^{n-}$  for formic acid. If several acids are available, the components will form various adducts. The combination TFA and FA yield well-spaced signals.

Figure 4 shows an ESI-MS spectrum in negative mode and Table 4 lists observable ions with their  $m/z$ .

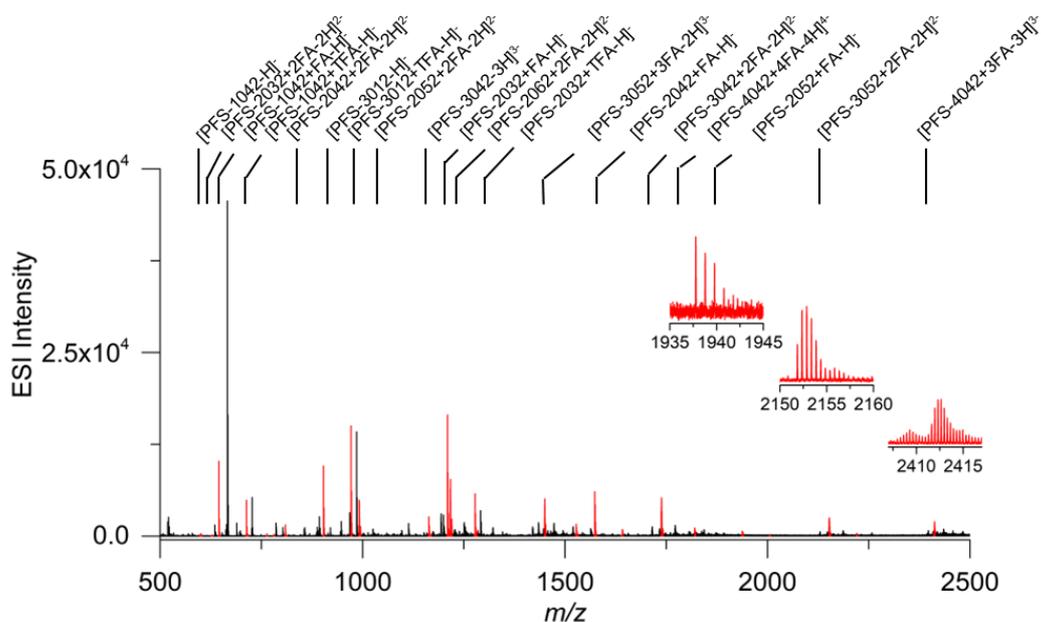


Figure 4: Mass spectrum of SpheriCal® IM-MS Medium Range in negative mode as measured on a Waters Cyclic IMS from ACN/H<sub>2</sub>O/FA 50/49.95/0.05. Remaining traces of TFA in the used hardware result in additional signals.

Table 4. Observable ions of SpheriCal® IM-MS Medium Range in negative mode.

Ion	m/z
[PFS-2032-2H] <sup>2-</sup>	581.244
[PFS-1042-H] <sup>-</sup>	599.2546
[PFS-2032+2FA-2H] <sup>2-</sup>	627.2495
[PFS-1042+FA-H] <sup>-</sup>	645.26
[PFS-2032+2TFA-2H] <sup>2-</sup>	695.2369
[PFS-1042+TFA-H] <sup>-</sup>	713.2474
[PFS-2042-2H] <sup>2-</sup>	763.3125
[PFS-2062-3H] <sup>3-</sup>	780.3223
[PFS-2042+2FA-2H] <sup>2-</sup>	809.318
[PFS-2062+3FA-3H] <sup>3-</sup>	826.3278
[PFS-3012-H] <sup>-</sup>	857.3649
[PFS-2042+2TFA-2H] <sup>2-</sup>	877.3053
[PFS-2062+3TFA-3H] <sup>3-</sup>	894.3152
[PFS-3012+FA-H] <sup>-</sup>	903.3704
[PFS-2052-2H] <sup>2-</sup>	945.3809
[PFS-3012+TFA-H] <sup>-</sup>	971.3578
[PFS-2052+2FA-2H] <sup>2-</sup>	991.3864
[PFS-2052+2TFA-2H] <sup>2-</sup>	1059.374
[PFS-3042-3H] <sup>3-</sup>	1127.792
[PFS-2032-H] <sup>-</sup>	1163.496
[PFS-2062-2H] <sup>2-</sup>	1170.988
[PFS-3042+3FA-3H] <sup>3-</sup>	1173.798
[PFS-2032+FA-H] <sup>-</sup>	1209.502
[PFS-2062+2FA-2H] <sup>2-</sup>	1216.993
[PFS-3042+3TFA-3H] <sup>3-</sup>	1241.785
[PFS-2032+TFA-H] <sup>-</sup>	1277.489
[PFS-2062+2TFA-2H] <sup>2-</sup>	1284.981
[PFS-3052-3H] <sup>3-</sup>	1404.236
[PFS-3052+3FA-3H] <sup>3-</sup>	1450.241
[PFS-3052+3TFA-3H] <sup>3-</sup>	1518.229
[PFS-2042-H] <sup>-</sup>	1527.633
[PFS-2042+FA-H] <sup>-</sup>	1573.639
[PFS-2042+TFA-H] <sup>-</sup>	1641.626
[PFS-3042-2H] <sup>2-</sup>	1692.193
[PFS-3042+2FA-2H] <sup>2-</sup>	1738.198
[PFS-4042-4H] <sup>4-</sup>	1774.473
[PFS-3042+2TFA-2H] <sup>2-</sup>	1806.186
[PFS-4042+4FA-4H] <sup>4-</sup>	1820.478
[PFS-4042+4TFA-4H] <sup>4-</sup>	1888.466
[PFS-2052-H] <sup>-</sup>	1891.77
[PFS-2052+FA-H] <sup>-</sup>	1937.776
[PFS-2052+TFA-H] <sup>-</sup>	2005.763
[PFS-3052-2H] <sup>2-</sup>	2106.858
[PFS-3052+2FA-2H] <sup>2-</sup>	2152.863
[PFS-3052+2TFA-2H] <sup>2-</sup>	2220.851
[PFS-4042-3H] <sup>3-</sup>	2366.3
[PFS-4042+3FA-3H] <sup>3-</sup>	2412.305
[PFS-4042+3TFA-3H] <sup>3-</sup>	2480.293

## 4. Feedback

We hope you are satisfied with the product. We are eager to hear your thoughts on our products and reward shared data and constructive feedback with a discount on your next order. Please reach out to [jens.sommertune@polymerfactory.com](mailto:jens.sommertune@polymerfactory.com) for more information.

## **Contact**

Please feel free to email any inquiries about SpheriCal® to [jens.sommertune@polymerfactory.com](mailto:jens.sommertune@polymerfactory.com).

Patent, licensing and trademark information

SpheriCal® is a trademark owned by Polymer Factory Sweden AB and registered in the EU, USA, Japan, South Korea and Australia. The technology used in SpheriCal® calibrants is covered by IPR protection held by Polymer Factory. Polymer Factory Sweden AB has the exclusive worldwide right to commercialize SpheriCal® calibrant technology.