

## Supporting Information

### **Separation and Sensitive Determination of Sphingolipids at Low Femtomole Level by Using HPLC-PIESI-MS/MS**

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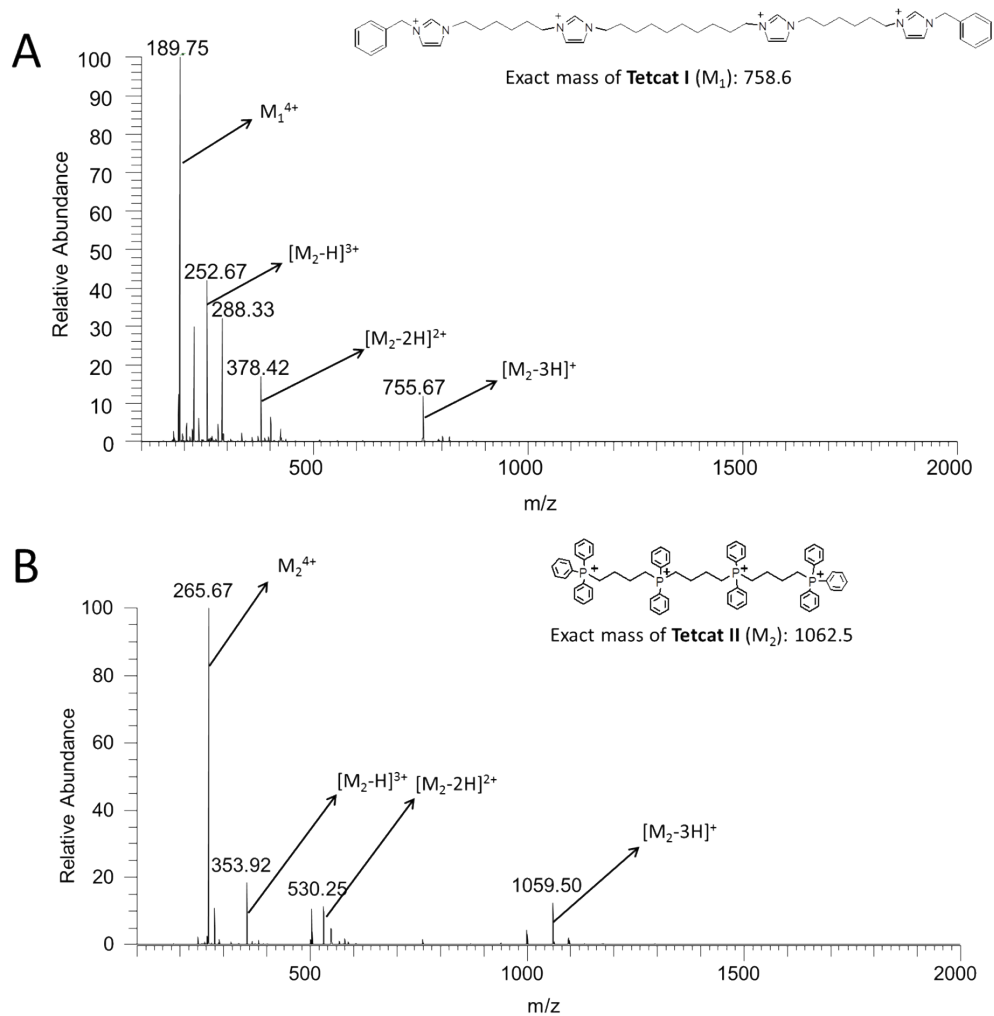
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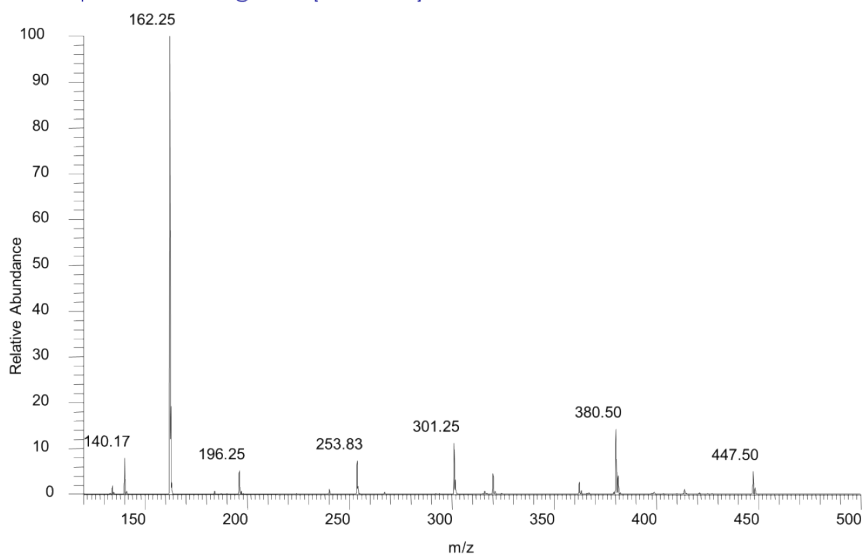
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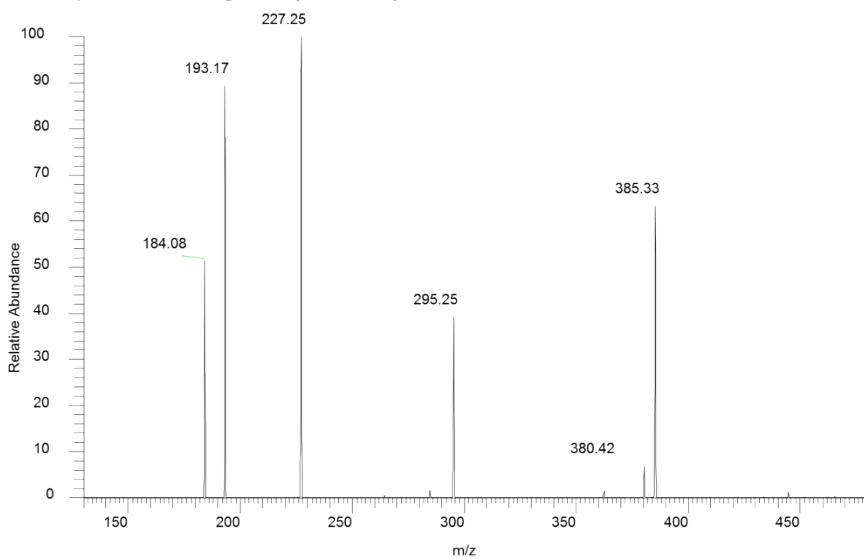
**Fig. S1** Mass spectra of (A) **Tetcat I** and (B) **Tetcat II** obtained in full scan mode. Notice that even the base peak is  $M^{4+}$ , the other deprotonated forms of the tetracation ( $[M-H]^{3+}$ ,  $[M-2H]^{2+}$  and  $[M-3H]^+$ ) which are generated in the ion source were also observed as major peaks. It is proposed that this deprotonation of tetracations could result in the different charge states of the complexes.

T: ITMS + p ESI Full ms2 443.38@cid30.00 [120.00-500.00]

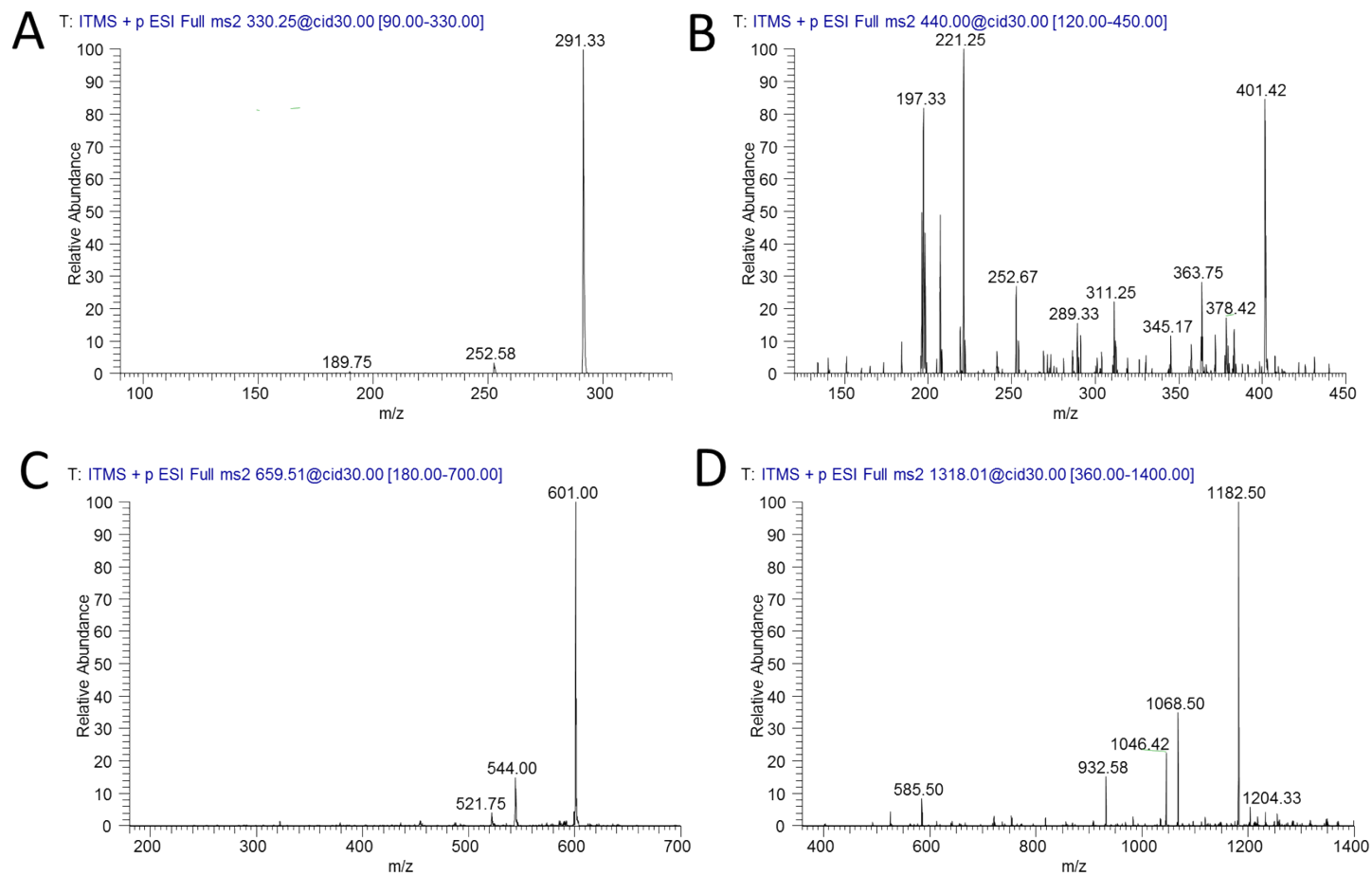


**Fig. S2** Product ion spectrum of the complex [SM(d18:1/6:0) + Dicat I]<sup>2+</sup> m/z: 443.4 obtained in positive SRM mode.

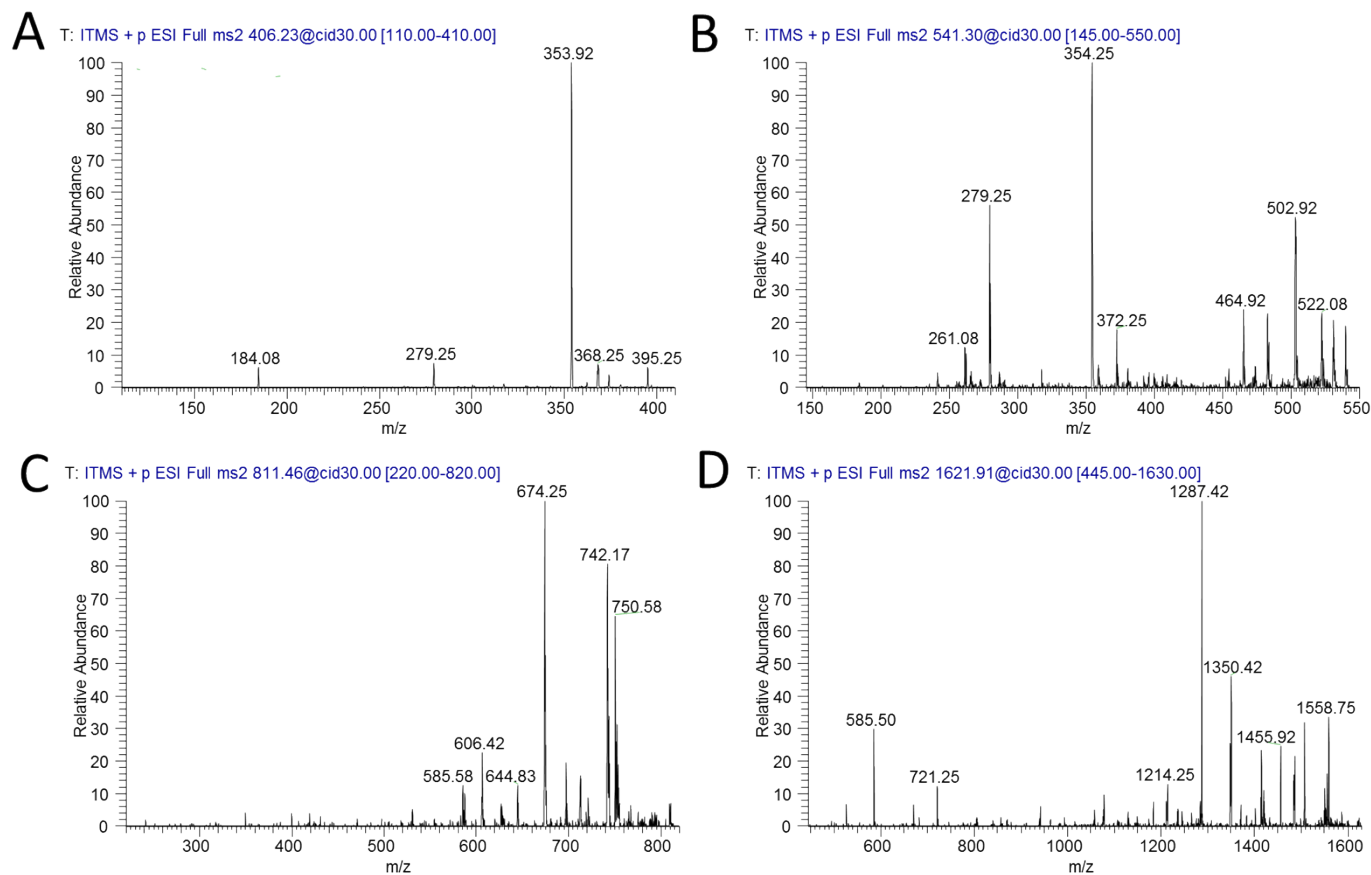
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**Fig. S3** Product ion spectrum of the complex [SM(d18:1/6:0) + Dicat II]<sup>2+</sup> m/z: 474.3 obtained in positive SRM mode.



**Fig. S4** Product ion spectrum of (A) complex [SM(d18:1/6:0) + **Tetcat I**]<sup>4+</sup> m/z: 330.3, (B) complex [SM(d18:1/6:0) + **Tetcat I - H**]<sup>3+</sup> m/z: 440.0, (C) complex [SM(d18:1/6:0) + **Tetcat I - 2H**]<sup>2+</sup> m/z: 659.5, and (D) complex [SM(d18:1/6:0) + **Tetcat I - 3H**]<sup>+</sup> m/z: 1318.0.



**Fig. S5** Product ion spectrum of (A) complex [SM(d18:1/6:0) + **Tetcat II**]<sup>4+</sup> m/z: 406.2, (B) complex [SM(d18:1/6:0) + **Tetcat II - H**]<sup>3+</sup> m/z: 541.3, (C) complex [SM(d18:1/6:0) + **Tetcat II - 2H**]<sup>2+</sup> m/z: 811.5, and (D) complex [SM(d18:1/6:0) + **Tetcat II - 3H**]<sup>+</sup> m/z: 1621.9.

**Table S1.** Mass-to-charge ratio of sphingolipids monitored in the positive SIM mode using PIESI-MS.

Analyte	Dicat I		Dicat II		Tetcat I		Tetcat II		Without IPR	
	m/z monitored	Charge state	m/z monitored	Charge state	m/z monitored	Charge state	m/z monitored	Charge state	m/z monitored	Charge state
d18:1, C18:0 SPM	527.5	2+	558.4	2+	496.4	3+	448.3	4+	731.6	1+
d18:1, C16:0 SPM	513.5	2+	544.4	2+	365.3	4+	441.3	4+	703.6	1+
d18:1, C23:0 SPM	562.5	2+	593.5	2+	519.4	3+	465.8	4+	801.7	1+
d18:1 SPM	394.4	2+	425.3	2+	610.5	2+	508.6	3+	465.3	1+
d18:1, C2:0 SPM	415.4	2+	446.3	2+	421.3	3+	522.6	3+	507.4	1+
d18:1, C6:0 SPM	443.4	2+	474.3	2+	330.3	4+	541.3	3+	563.4	1+
d18:1, C12:0 SPM	485.4	2+	516.4	2+	351.3	4+	569.3	3+	647.5	1+
d18:1 SPPE	373.3	2+	404.3	2+	589.5	2+	1481.8	1+	423.3	1+
d17:1, C12:0 SPPE	457.4	2+	488.4	2+	673.5	2+	825.5	2+	591.4	1+
GM1	1869.2	1+	1931.1	1+	767.8	3+	869.1	3+	1544.9	1-
GM3	1574.2	1+	1636.1	1+	669.5	3+	1155.7	2+	1249.8	1-
GD3	1865.3	1+	1927.2	1+	766.5	3+	867.8	3+	769.9	2-
d18:1, C24:0 GalCer	1130.9	1+	1192.8	1+	782.6	2+	934.5	2+	806.6	1-
d18:1, C12:0 GalCer	1046.8	1+	1108.7	1+	740.0	2+	892.0	2+	722.5	1-

**Table S2.** Mass-to-charge ratio monitored during collision induced dissociation (CID) in the positive SRM mode.

Analyte	Dicat I		Dicat II		Tecat I		Tecat II	
	m/z precursor ion	m/z monitored	m/z precursor ion	m/z monitored	m/z precursor ion	m/z monitored	m/z precursor ion	m/z monitored
d18:1, C18:0 SPM	528.48	162.2	558.43	227.3	496.39	252.6	448.54	279.3
d18:1, C16:0 SPM	513.46	162.2	544.41	227.3	365.29	252.6	441.27	184.1
d18:1, C23:0 SPM	562.52	436.4	593.47	227.3	519.43	252.6	465.8	184.1
d18:1 SPM	394.35	162.2	425.3	227.3	610.47	385.7	508.61	406.3
d18:1, C2:0 SPM	415.35	162.3	446.3	227.3	421.32	252.6	522.62	353.9
d18:1, C6:0 SPM	443.38	162.3	474.33	193.3	330.25	252.6	541.3	353.9
d18:1, C12:0 SPM	485.43	162.2	516.38	193.3	351.28	252.6	569.33	353.9
d18:1 SPPE	373.32	162.2	404.27	193.3	589.45	378.3	1481.79	557.3
d17:1, C12:0 SPPE	457.4	162.3	488.35	193.3	673.52	594.4	825.47	694.5
GM1	1869.22	1742.1	— <sup>a</sup>	— <sup>a</sup>	767.81	671.2	869.12	503.3
GM3	1574.17	1447	— <sup>a</sup>	— <sup>a</sup>	669.47	252.3	1155.66	1025.1
GD3	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>	766.5	669.5	867.8	821.9
d18:1, C24:0 GalCer	1130.9	923.9	— <sup>a</sup>	— <sup>a</sup>	782.56	427.3	934.61	803.7
d18:1, C12:0 GalCer	— <sup>a</sup>	— <sup>a</sup>	1108.7	385.3	— <sup>a</sup>	— <sup>a</sup>	891.98	761.5

<sup>a</sup> Not detected.