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Supporting Information

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Discrimination of Human Astrocytoma Subtypes by Lipid Analysis using Desorption Electrospray Ionization Imaging Mass Spectrometry**

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Abstract

This supporting information includes experimental descriptions covering tissue preparation and DESI mass spectrometry. Comments on sample selection and experimental design and on data acquisition and interpretation are included. Also presented are additional positive and negative ion DESI spectra including replicates to demonstrate reproducibility and an MS/MS spectrum as well as tables showing standard mass spectrometry operating conditions and listing the lipids detected in the negative and positive ion modes with their masses and characteristic fragment ions.

Experimental Section

Comments on Sample Selection and Experimental Design

Our study sought to address the potential utility of DESI-MS to discriminate malignant diffuse gliomas subtypes, which are the most frequent primary brain tumors in adults, and for which the validated method would be the most beneficial. These types of tumors occur in three histologic grades, WHO Grade II, III, and IV and their distinction represents a challenge in the intraoperative and diagnostic pathology setting. The WHO Grade I designation generally is reserved for rare slow growing pediatric astrocytomas. Future studies will include examining a larger set of tissue samples of all types and infiltration patterns, but the presented study addresses those tumors of most concern.

Normal samples are generally not available in brain studies but in future work samples obtained from multiple sites with varying tumor cell density will be used as a means of minimizing the effect of the lack of true non-tumor controls. White matter versus grey matter location is essentially irrelevant for the selected specimens, since the tumor has replaced those normal tissues. The visualization of infiltrative tissue in brain tumor surgery is the very problem driving the research presented. Contrary to cancer surgery of other organs, surgical margins cannot be extrapolated to what might be infiltrated tissue. However, if mass spectrometry proves to be more sensitive and specific than current approaches and the initial results on grade differentiation presented here are validated, then DESI-imaging followed by tumor cell density assessment might result in reevaluation of tumor margins criteria.

Tissue Preparation

All tissue samples were cerebrum tumor tissue obtained from the BWH Neurooncology Program Biorepository collection, and analyzed under approved Institutional Review Board (IRB) protocol, with informed written consent obtained by licensed neurosurgeons at BWH. Samples were flash frozen and stored in liquid nitrogen until sectioned at 14 µm thickness using a Microm HM550 cryostat (Mikron Instruments Inc., San Marcos, CA), and thaw mounted onto glass slides. The slides were stored in closed containers at -80° C; prior to analysis they were allowed to come to room temperature, and dried under nitrogen in a dessicator for approximately 20 minutes.

DESI Imaging

- *Aston Laboratories (Purdue University):*

The DESI ion source used in our experiments was a lab-built prototype, configured as described previously.[1] The spray solvent used for MS acquisition was methanol:water (50:50) with a 5 kV spray voltage applied. methanol was purchased from Sigma-Alrich (St. Louis, MO, USA) and water (18.2 MΩ-cm) was from a PureLab ultra system by Elga LabWater (High Wycombe, UK). The nitrogen gas pressure was 150 psi and the solvent flow rate was 1.5 µL/min. The tissues were

scanned using a 2D moving stage in horizontal rows separated by a 200 μm vertical step until the entire sample surface had been assayed. This was done by recording 2D images consisted of arrays of pixels, each pixel covering an area of 200 x 200 μm^2. The samples were analyzed in both positive (*m/z* 150 – 1400) and negative (*m/z* 150 – 1000) ion modes. The horizontal rows were scanned at a constant speed of 167 μm/s (positive ion mode) and 175 μm/s (negative ion mode) for all samples analyzed. The number of rows and the number of pixels per row varried with the sample size, therefore the analysis time for each sample also varied. An average of 30 minutes was typical. The surface moving stage included an XYZ integrated linear stage (Newport, Richmond, CA) and a rotary stage (Parker Automation, Irwin, PA). All experiments were carried out using a LTQ linear ion trap mass spectrometer controlled by XCalibur 2.0 software (Thermo Fisher Scientific, San Jose, CA, USA) operating using the same instrument parameters. An in-house program allowed the conversion of the XCalibur 2.0 mass spectra files (.raw) into a format compatible with the Biomap software (freeware, htto://www.maldo-msi.org). Spatially accurate images were assembled using the BioMap software.

- *Prosolia Inc. (Indianapolis):*

All experiments conducted at Prosolia were performed using a commercial DESI ion source (Omni Spray® 2D) coupled to a Thermo Scientific LTQ linear ion trap mass spectrometer in both positive and negative ionization modes. The spray solvent and flow rate were identical to that used in the Purdue laboratory. The pixel size was set to 250μm x 250μm using the Omni Spray motion control software. Data were recorded using Xcalibur 2.0.7 on a row by row basis and subsequently converted to Analyze 7.5 format using FireFly 1.3.0.0 (Prosolia, Inc, Indianapolis, IN). Spatially accurate images were assembled using the BioMap software.

Comments on Data and Interpretation

The tumor grades examined are highly relevant clinically. The same tumor can contain regions of different grades. Even when this is not the case, differentiating these grades can be difficult, even with microscopic evaluation, making the DESI results clinically relevant. Limitations of the data include the fact that multiple lipids might be present at particular *m/z* values; attempts to map lipids in the single reaction monitoring (SRM) mode in other tissues has met with limited success, but was not used in these studies to avoid wasting valuable tissue. We also note that while it is important to have an understanding of the underlying molecular species contributing to the change between tumor grades, it is not essential for establishing molecular profiles of disease state. The authors plan future studies using high resolution mass spectrometry, which would complement SRM studies and sometimes allow for isobaric lipid distinction. For the study presented here, the assignments made for particular *m/z* ratios are based on the independently known concentrations of lipids of the appropriate molecular weights.

Figures S1 – S5

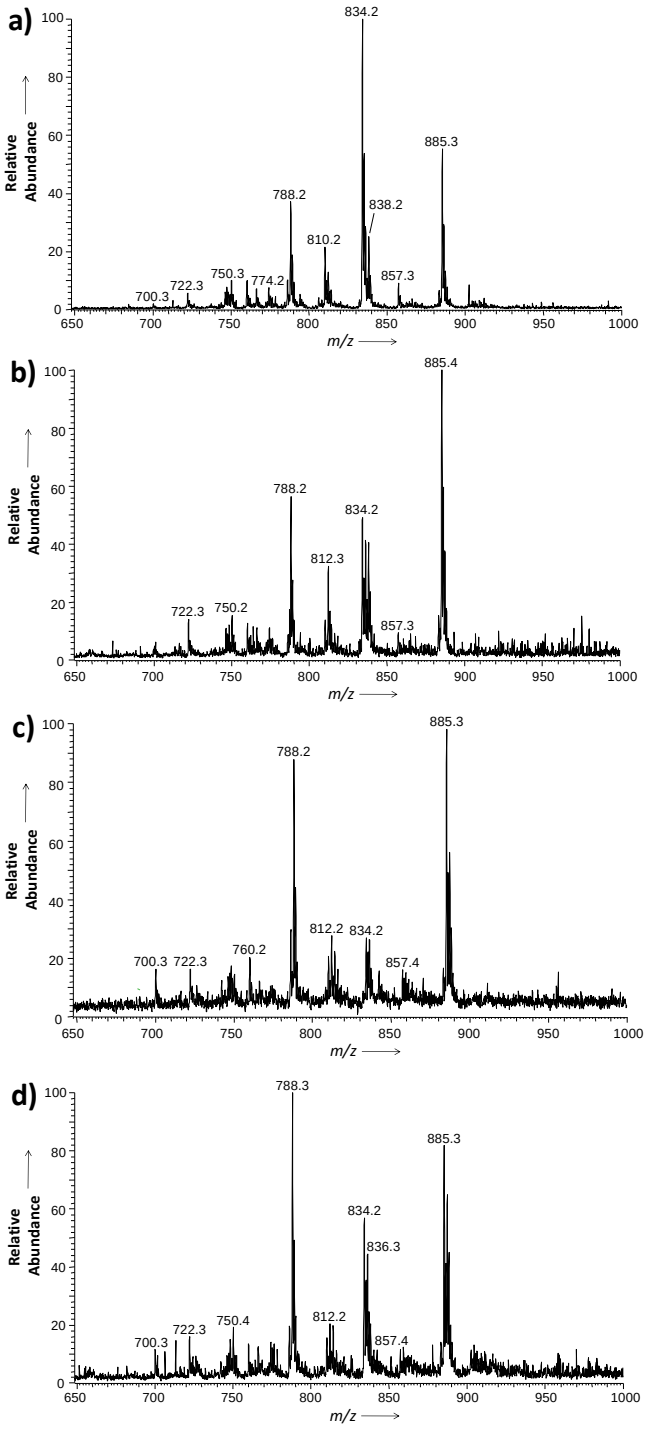

Figure S1. Representative negative ion mode DESI spectra in the range of *m/z* 650 to 1000 of a) sample G5, human glioblastoma tumor tissue (WHO grade IV), b) sample G6, human glioblastoma tumor tissue (WHO grade IV), c) sample G7, human glioblastoma tumor tissue (WHO grade IV) and d) sample G8, human glioblastoma tumor tissue (WHO grade IV).

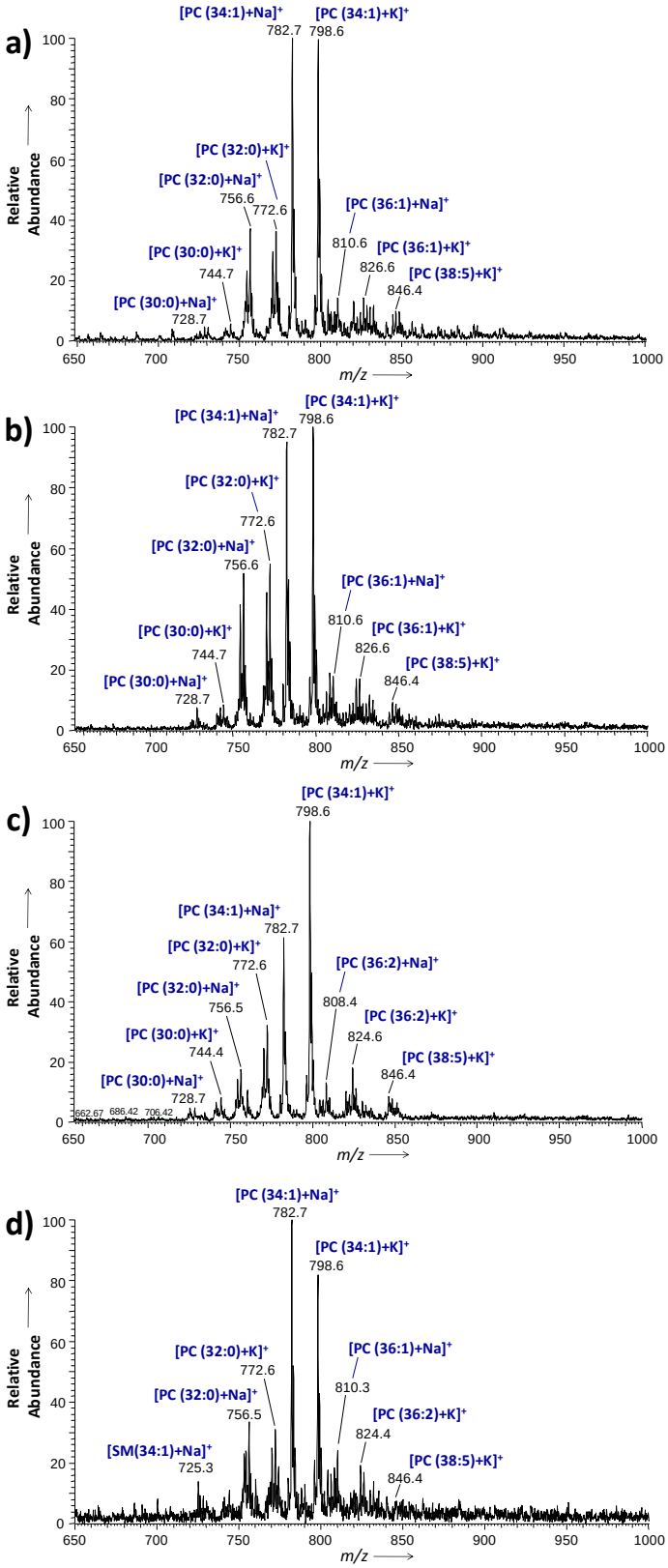

Figure S2. Representative positive ion mode DESI spectra in the range of *m/z* 650 to 1000 of a) sample G5, human glioblastoma tumor tissue (WHO grade IV), b) sample G6, human glioblastoma tumor tissue (WHO grade IV), c) sample G7, human glioblastoma tumor tissue (WHO grade IV) and d) sample G8, human glioblastoma tumor tissue (WHO grade IV).

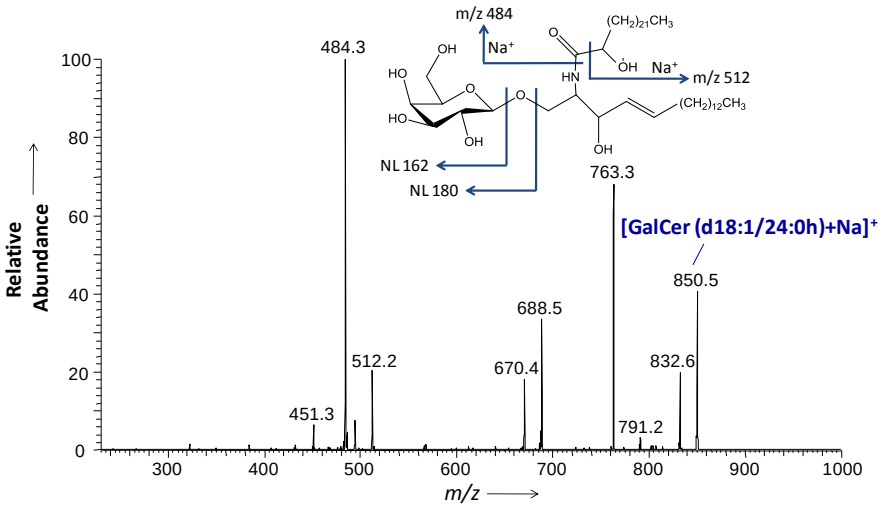

Figure S3. Tandem mass spectrum of *m/z* 850.5, positively charged sodium adduct of GalCer(d18:1/24:0h), from sample G2. Neutral loss (NL) of 162, forming *m/z* 688.3, and NL of 180, forming *m/z* 670.3, are both highly characteristic patterns in the first-generation product ion spectra for precursor ions of galactoceramides. The main fragment peak *m/z* 484.3, which corresponds to the sodiated C_{18} sphingosine long-chain base, and *m/z* 512.2, which corresponds to the sodiated aldehyde form of the C_{18} sphingosine long-chain base, represent significant mass fragment ions for galactoceramides, further confirming the lipid assigned structure.

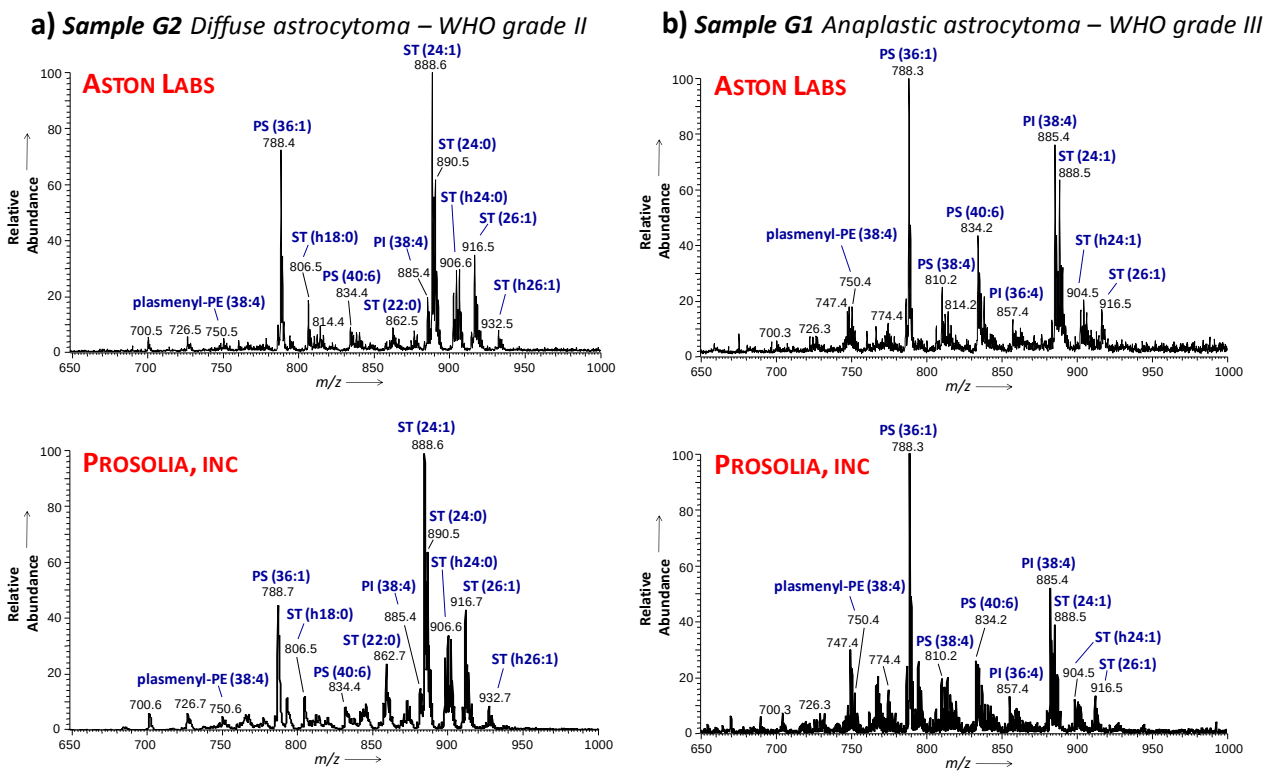

Figure S4. Representative negative ion mode DESI mass spectra in the range of *m/z* 650 to 1000 of different sections of a) sample G2, human diffuse astrocytoma tumor tissue (WHO grade II) and b) sample G1, human anaplastic astrocytoma (WHO grade III), analyzed at Aston labs, Purdue University (top) and Prosolia, Inc, Indianapolis (bottom).

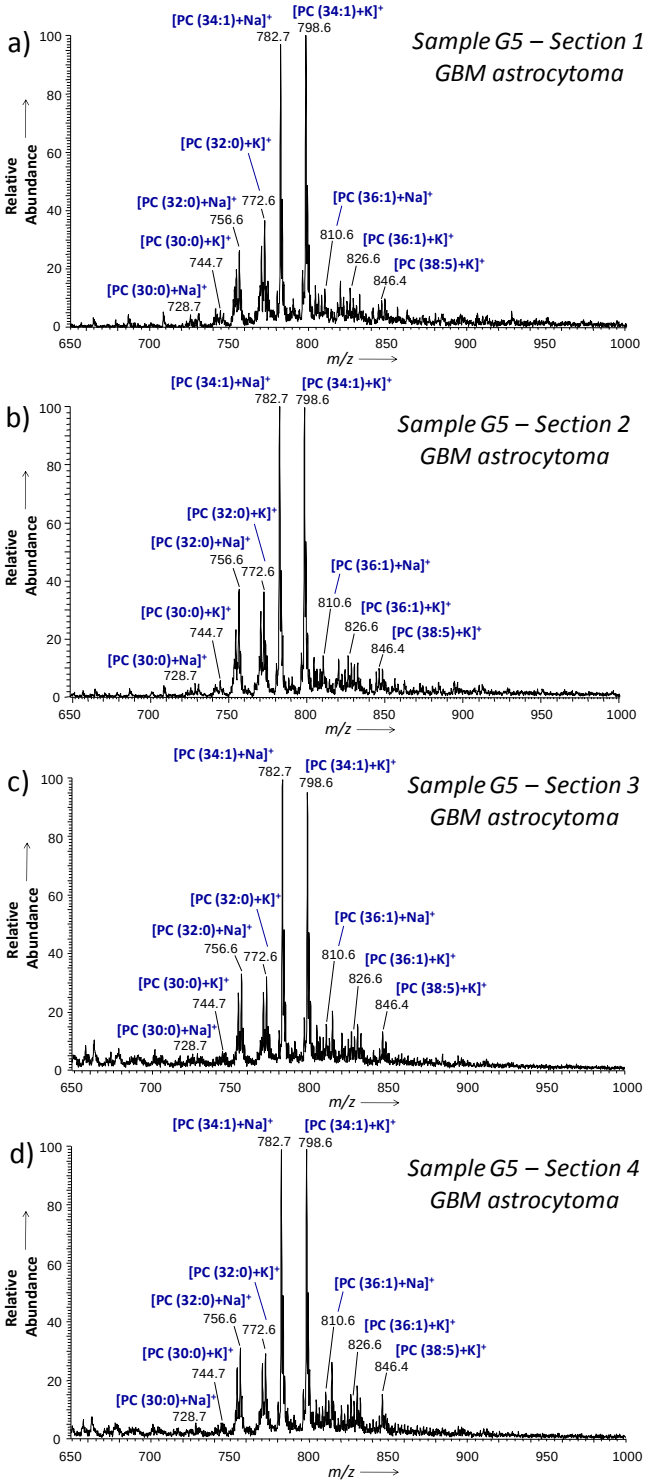

Figure S5. Representative positive ion mode DESI spectra in the range of *m/z* 650 to 1000 of different sections of sample G5, human glioblastoma tumor tissue (WHO grade IV); a) section 1, analyzed on December 1st 2009 at 10 AM, b) section 2, analyzed on December 1st 2009 at 5 PM, c) section 3, analyzed on December 3rd 2009 at 5 PM, and d) section 4, analyzed on December 8th 2009 at 9 AM.

Tables S1 – S3

Table S1. Lipid molecular species detected from human glioma tissue in negative ion mode.

Mass-to-charge ratio (m/z)	Molecular Species[a]	Main fragment ions (m/z)
700.5	Plasmenyl-PE (34:1)	255.2, 281.3. 418.3, 436.3
722.3	Plasmenyl-PE (16:0/20:4)	303.2, 436.3
726.5	Plasmenyl-PE (36:2)	281.2, 307.3, 436.2, 462.3
750.5	Plasmenyl-PE (38:4)	303.2, 331.3, 436.3, 464.3
760.2	PS (34:1)	255.3, 283.3, 419.2, 673.3
774.3	Plasmenyl-PE (40:6)	281.2, 327.3, 464.3, 687.3
788.3	PS (18:0/18:1)	281.3, 283.3, 419.2, 701.3
806.5	ST (h18:0)	522.3, 566.2, 788.4
810.2	PS (18:0/20:4)	281.2, 283.0, 419.1, 723.3
814.4	PS (38:2)	283.3, 419.2, 437.3, 727.3
834.2	PS(18:0/22:6)	283.3, 419.2, 437.2, 747.3
838.3	PS (18:0/22:4)	283.2, 419.3, 751.3
857.3	PI (16:0/20:4)	255.3, 303.3, 391.2, 553.2
862.5	ST (22:0)	281.2, 522.3, 844.5
885.4	PI (18:0/20:4)	283.3, 303.3, 419.2, 581.2, 599.2
888.8	ST (24:1)	390.3, 522.3, 650.3, 870.5
890.4	ST (24:0)	390.3, 522.3, 650.3, 872.5
904.6	ST (h24:1)	522.2, 540.3, 568.2, 886.5
906.6	ST (h24:0)	522.2, 540.3, 568.2, 888.5
916.6	ST (26:1)	522.2, 540.3, 678.3, 898.4
932.5	ST (h26:1)	522.2, 540.2, 568.3, 914.5

[a] (X:Y) represents the different number of carbon atoms and the different number of double bonds in the fatty acid chains. The notation (hX:Y) denotes a hydroxylated sulfatide species; ST sulfatide; PI phosphatidylinositol; PE phosphatidylethanolamine; PS phosphatidylserines.

Table S2. Lipid molecular species detected from human glioma tissue in positive ion mode.

Mass-to-charge ratio (m/z)	Molecular Species[a]	Main fragment ions (m/z)
725.7	SM(18:1/16:0)+Na^+	666.3, 682.3, 725.3
728.4	PC(30:0)+Na^+	545.3, 669.3
744.3	PC(30:0)+K^+	685.2
750.8	GalCer (d18:1/18:0)+Na^+	412.3, 570.6, 588.4, 707.3
754.7	PC(32:1)+Na^+	571.4, 695.2
756.7	PC(32:0)+Na^+	573.3, 697.2
770.7	PC(32:1)+K^+	587.0, 711.2, 727.3
772.7	PC(32:0)+K^+	589.0, 713.1, 729.4
782.7	PC (34:1)+Na^+	599.3, 723.3
796.4	PC(34:0)+K^+	613.2, 737.3, 753.3
798.7	PC(34:1)+K^+	615.2, 739.3, 755.3
808.4	PC(36:2)+Na^+	625.3, 749.3, 765.2
810.7	PC(36:1)+Na^+	627.3, 751.3
824.4	PC(36:2)+K^+	765.1
826.6	PC(36:1)+K^+	767.1
832.8	GalCer(d18:1/24:1)+Na^+	652.4, 670.5, 814.5
846.3	PC(38:5)+K^+	562.0, 787.1
848.7	GalCer(d18:1/24:1h)+Na^+	484.3, 668.4, 686.5, 830.4
850.7	GalCer(d18:1/24:0h)+Na^+	484.3, 512.2, 670.4, 688.5, 763.3
864.7	GalCer (d18:1/25:0h)+Na^+	484.2, 702.7, 846.4
876.7	GalCer (d18:1/26:1h)+Na^+	484.2, 512.2, 696.6, 714.4, 789.3

[a] (X:Y) represents the different number of carbon atoms and the different number of double bonds in the fatty acid chains. The notation (dX:Y) for denotes a 1,3 dihydroxy long-chain bases for galactoceramides; PC phosphatidylcholine; SM sphingomyelin; GalCer galactoceramide.

Table S3. Tune file values of the optimized LTQ parameters used in the negative and positive ion modes for all samples analysis.

Parameters	NEGATIVE POLARITY	POSITIVE POLARITY
Capillary Temp (^{o}C):	275	275
Source Voltage (kV):	5	5
Source Current (uA):	100	100
Capillary Voltage (V):	-50	5
Tube Lens (V):	-100	110
Skimmer Offset (V):	0	0
Multipole RF Amplifier (Vp-p):	400	400
Multipole 00 Offset (V):	4	-4
Lens 0 Voltage (V):	3.5	-4.5
Multipole 0 Offset (V):	4.5	-4.25
Lens 1 Voltage (V):	9	-12
Gate Lens Offset (V):	32	-56
Multipole 1 Offset (V):	13	-20
Front Lens (V):	5	-4
Zoom Micro Scans:	1	1
Zoom Max Ion Time (ms):	50	50
Full Micro Scans:	2	2
Full Max Ion Time (ms):	500	500

Supporting References

(1) D.R. Ifa, J.M. Wiseman, Q.Y. Song, R.G. Cooks, *Int. J. Mass Spectrom.* **2007**, *259*, 8-15.