

Method: To prove whether the lipids in fresh samples can be fully recovered with this procedure in the study, an experiment of sample preparation for sphingolipids extraction was repeated to compare the procedure in this study with the control procedure of $35,000 \times g$ centrifugation for 30 min. For each extraction condition, 3 replicate hippocampus and cortex samples (10 mg each) were extracted after adding ice-cold cell lysis buffer (including compound protease inhibitors), homogenizing and standing for 30 min in the ice box, contamination precipitation was removed by centrifuging at $6,000 \times g$ for 10 min at 4°C , supernatant was centrifuged at $15,000 \times g$ ($35,000 \times g$ for 30 min in control group) for 30 min at 4°C , the resulting pellet was resuspended with a modified MTBE extraction procedure using methanol-MTBE- H_2O (10/3/2.5, v/v) to build the two-phase system. The upper phase (consisting of sphingolipids) was combined and then was reconstituted in suitable solvents for the analysis of LC-MS/MS. The quantification of Cer, CBS, ST, and SM in samples by NPLC-MS/MS was conducted using the method in 2.7.3 section in this article.

Table Comparison between two sphingolipid extraction methods (15,000 × g vs 35,000 × g)

m/z	Molecular Species	Area							Ratio Method1/Method2 (%)	
		Method 1 (15000 g for 30 min)				Method 2 (35000 g for 30min)				
		Replicate 1	Replicate 2	Replicate 3	Average	Replicate 1	Replicate 2	Replicate 3	Average	
538.5	Cer(d18:1/16:0)	1318027	1905996	1604897	1609640	1719493	1721386	1739678	1827353	88.09%
552.5	Cer(d18:1/17:0)	165930	260699	233620	220083	260230	283662	429704	300708	73.19%
564.5	Cer(d18:1/18:1)	5329968	3785416	3752145	4289176	5014543	4378769	3154263	4288814	100.01%
566.6	Cer(d18:1/18:0)	64135388	65681615	59423759	63080254	59253856	79205158	67923890	75239134	83.84%
582.5	Cer(d18:1/18:0h)	952417	767684	774180	831427	917398	790059	446984	809816	102.67%
592.6	Cer(d18:1/20:1)	959690	1010422	748409	906174	1154500	1211581	693957	1371097	66.09%
594.6	Cer(d18:1/20:0)	2795482	3255614	2986355	3012484	4041035	4732009	3953951	4567820	65.95%
620.6	Cer(d18:1/22:1)	10844596	9501387	7365632	9237205	11978524	12211202	7891693	13409313	68.89%
622.6	Cer(d18:1/22:0)	731324	1038770	1281606	1017233	916402	1176546	1339821	1196730	85.00%
634.6	Cer(d18:1/23:1)	2761941	2790906	2175276	2576041	2934191	3090512	2126368	3369882	76.44%
636.6	Cer(d18:1/23:0)	367618	741382	711350	606783	502944	782777	815438	696836	87.08%
648.6	Cer(d18:1/24:1)	3781533	4029921	3953253	3921569	2835746	3158745	2572213	3644375	107.61%
664.6	Cer(d18:1/24:1h)	1332035	1201237	811617	1114963	1385667	1337604	906420	1499236	74.37%
650.6	Cer(d18:1/24:0)	1456205	2223779	2825545	2168510	14266136	5223331	11708249	3254856	68.70%
	Total Cer	96932154	98194827	88647644	94591542	107180665	119303341	105702629	119775971	78.97%
700.6	GalCer(d18:1/16:0)	1381187	1615784	1238165	1411712	1713231	1799727	2099730	1870896	75.46%
726.6	GalCer(d18:1/18:1)	33110670	25789694	29156176	29352180	33832207	24419317	24192747	27481424	106.81%
728.6	GalCer(d18:1/18:0)	10208321	10555630	9153402	9972451	11833683	11778832	13638545	12417020	80.31%
754.6	GalCer(d18:1/20:1)	6892488	7974308	5430448	6765748	8571203	8574552	10997832	9381196	72.12%
782.7	GalCer(d18:1/22:1)	69153705	65868182	54738419	63253435	77601958	75260319	98573706	83811994	75.47%
784.7	GalCer(d18:1/22:0)	7006680	8737298	6697150	7480376	8061046	9100930	11021895	9394624	79.62%
796.7	GalCer(d18:1/23:1)	18428209	19495812	16643939	18189320	17680528	19133841	24863601	20559323	88.47%
798.7	GalCer(d18:1/23:0)	2540047	3882660	2646074	3022927	3157304	3181242	4374541	3571029	84.65%
810.7	GalCer(d18:1/24:1)	128466219	123228106	108403286	120032537	152060339	157125461	184607121	164597640	72.92%
812.7	GalCer(d18:1/24:0)	25286744	20306852	32232187	25941928	23341524	20252914	25337226	22977221	112.90%
824.7	GalCer(d18:1/25:1)	1542868	1838024	1995288	1792060	1743487	2219023	2049564	2004025	89.42%
826.7	GalCer(d18:1/25:0)	839097	670474	1119314	876295	865816	651638	735692	751049	116.68%
838.7	GalCer(d18:1/26:0)	1172882	1302176	1459240	1311433	1759605	1592572	2021251	1791143	73.22%
	Total GalCer	306029117	291265000	270913088	289402402	342221931	335090368	404513451	360608583	80.25%
778.5	ST(d18:1-C16:0)	368055	336706	279252	328004	400338	353495	344375	366069	89.60%
806.5	ST(d18:1-C18:0)	8452543	6985928	6896005	7444825	8595888	9256862	7564352	8472367	87.87%
822.5	ST(d18:1-C18:0h)	2492695	2056864	2208034	2252531	2689818	2650092	2102210	2480707	90.80%
834.6	ST(d18:1-C20:0)	954901	673248	764837	797662	1062846	956578	1084971	1034798	77.08%
862.6	ST(d18:1-C22:0)	5878993	4089344	4624337	4864224	6012890	6072534	6935124	6340183	76.72%
878.6	ST(d18:1-C22:0h)	9441818	6289411	7735528	7822252	9156177	9239194	9556009	9317127	83.96%
888.6	ST(d18:1-C24:1)	32777592	24258370	26994735	28010232	33104858	33428935	38881849	35138547	79.71%
904.6	ST(d18:1-C24:1h)	4541735	3244416	3238313	3674821	3964542	3934516	4602856	4167305	88.18%
890.6	ST(d18:1-C24:0)	12376390	8575453	9239066	10063636	12934354	13353334	16779335	14355674	70.10%
906.6	ST(d18:1-C24:0h)	7572765	5668958	6429225	6556983	7694504	8186349	8494181	8125011	80.70%
916.7	ST(d18:1-C26:1)	272780	220467	260996	251414	335385	347867	390920	358057	70.22%

918.7	ST(d18:1-C26:0)	86518	62045	82798	77120	107604	114589	130942	117712	65.52%
934.7	ST(d18:1-C26:0h)	94427	62887	74390	77235	124265	105701	117754	115907	66.64%
	Total ST	85311211	62524097	68827516	72220941	86183469	88000046	96984878	90389464	79.90%
747.6	SM(d18:1/16:0)	2473761.6	4190612	2667763	5554466	3038668	2821209	3228457	3029445	102.68%
775.6	SM(d18:1/18:0)	75260431.8	84166724	75451572	125190349	100819598	97805193	90584369	96403053	81.21%
803.6	SM(d18:1/20:0)	4461269.4	3081767	3671816	8286727	6168176	6136096	6023939	6109404	61.19%
831.7	SM(d18:1/22:0)	1060080.6	671567	931560	2451329	1478283	1208325	1444688	1377099	64.46%
857.7	SM(d18:1/24:1)	3058286.4	2031405	2572036	9428999	3596021	3567611	4093945	3752526	68.06%
	Total SM	86313829	94142075	85294747	150911870	115100746	111538434	105375398	110671526	80.04%
	Total sphingolipid (Cer, GalCer, ST, SM)	574586312	546125999	513682994	544798435	650686811	653932189	739717633	681445544	79.95%

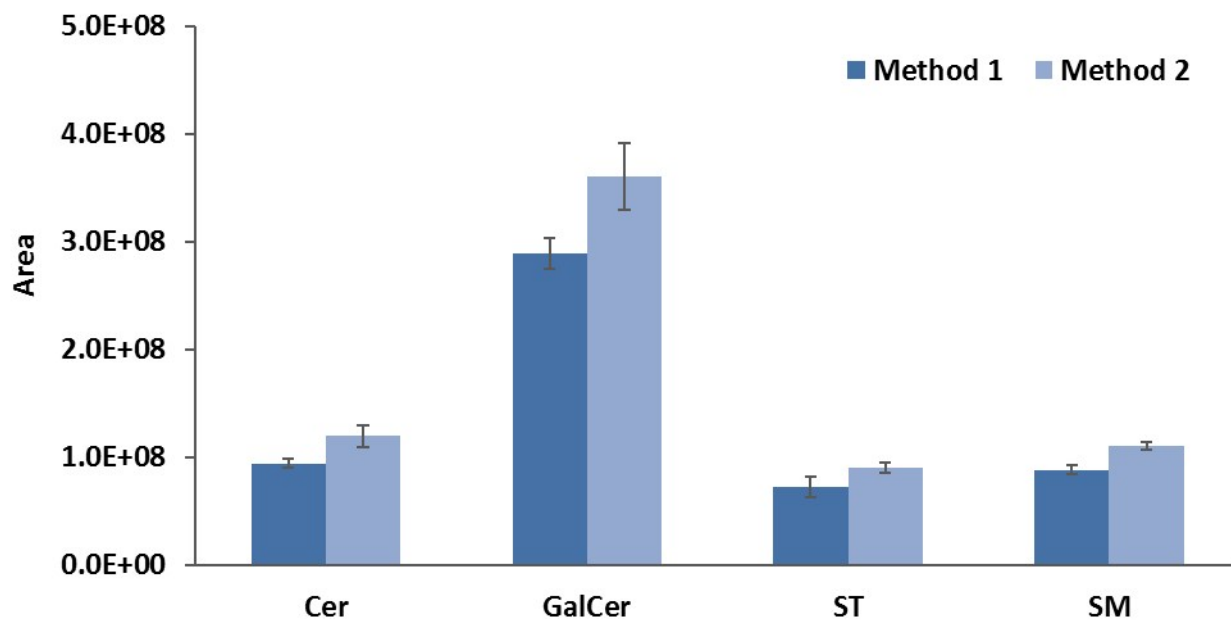


Figure The relative contents of sphingolipids (Cer, GalCer, ST, SM) in hippocampus extracted using the two method. Method 1: centrifugation at $15,000 \times g$ for 30 min; Method 2: centrifugation at $35,000 \times g$ for 30 min. The relative contents of four sphingolipid classes were represented by peak area detected in mass spectrometry as means \pm SD from three

hippocampus tissues of normal mice.