

Analysis of human gliomas by swab touch spray - mass spectrometry: applications to intraoperative assessment of surgical margins and presence of oncometabolites

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SUPPLEMENTARY INFORMATION

Table S1. Pathological Evaluation and Chemical Assessments

				Pathological Evaluation				Chemical Assessment				
Case	# Touches	Tissue Weight (mg)	# Samples in Main Text	Diagnosis	Comments ^a	TCP	IDH ^b	Base Peak Lipid Profile (m/z)	S/N	Diagnosis	NAA ^c	2HG ^d
1	1	1.9	1	IT	Mostly WM	Low	0	888.6	58	IT (WM)	623	0.18
	2	3.1	2	IT	Mixture of GM and WM	Low		834.4	63	IT (GM)	344	
2	1	4.5	3	G	Presence of necrosis	High	0	n.d. ^e	n.d.	-	2.4	n.d.
3	1	4.9	4	G	-	High	1	788.4	27	G	8.2	1.34
	2	n/a	5	G	-	High		794.4	17	G	6.9	
4	1	3.5	6	G	Mostly necrotic and hemorrhagic tissue	High	0	885.5	36	G	3.1	n.d.
5	1	5.4	7	IT	Infiltrated GM	Medium	1	834.4	62	IT (GM)	52.5	10.51
6	1	1.9	8	IT	Mixture of GM and WM	Low	0	888.6	71	IT (WM)	236	0.14
	2	1.5	9	IT	GM	Low		834.5	45	IT (GM)	179	
7	1	1.2	10	G	Presence of calcification	High	1	885.5	18	G	10.1	18.22
	2	1.1	11	G	Presence of calcification	High		885.5	42	G	19.0	
8	1	1.8	12	IT	Mostly GM	Low	0	834.4	50	IT (GM)	531	0.19

	2	1.8	13	IT	Mostly WM	Low		888.6	226	IT (WM)	232	
9	1	3.3	14	G	-	High	0	885.5	45	G	7.8	0.09
	2	3.7	15	G	-	High		885.5	52	G	1.6	
10	1	3.2	16	IT	Mixture of GM (90%) and WM (10%)	Low	0	834.5	36	IT (GM)	131	0.63
	2	1.0	17	IT (NOS)	-	Low		888.5	130	IT (WM)	1063	
11	1	1.7	18	IT	Mostly WM	Low	0	888.6	88	IT (WM)	81.0	0.40
	2	1.9	19	IT (NOS)	-	Low		888.6	157	IT (WM)	171	
12	1	2.5	20	G	-	High	0	794.4	10	G	1.9	0.08
13	1	1.6	21	G	Mostly necrotic tissue	High	0	885.5	11	G	n.d.	n.d.
14	1	1.8	22	IT	Mixture of WM (50%) and GM (50%)	Low	0	834.4	83	IT (GM)	150	0.17
	2	2.5	23	IT	Mixture of WM (50%) and GM (50%)	Low		888.6	38	IT (WM)	89.2	
15	1	1.8	24	IT	Mostly GM	Low	1	834.4	22	IT (GM)	99.6	1.63
16	1	8.6	25	IT (NOS)	-	Medium	1	794.4	31	G	10.1	25.33
	2	4.7	26	IT (NOS)	-	Medium		794.4	28	G	63.0	
17	1	3.1	27	IT	Mostly WM	Low	0	888.6	57	IT (WM)	23.6	0.14

	2	2.6	28	IT	Mostly GM	Low		834.4	58	IT (GM)	249	
18	1	3.9	29	IT	Mostly GM	Medium	0	834.4	113	IT (GM)	71.8	0.63
	2	4.3	30	IT	Mixture of GM and WM	Medium		794.4	28	G	88.4	
19	1	2.4	31	G	-	High	1	794.5	29	G	22.3	6.54
	2	2.7	32	G	-	high		788.4	15	G	7.91	
	3	3.5	33	G	Infiltrated GM	Medium		834.5	84	IT (GM)	29.6	
20	1	2.4	34	IT	Mostly GM	Low	0	834.5	30	IT (GM)	117	n.d.
21	1	3.5	35	IT	Mostly GM	Low	1	794.4	28	IT (GM)	101	23.51
	2	2.2	36	IT	Mixture of GM (65%) and WM (35%)	Low		788.4	3	IT (WM)	165	
22	1	4.0	37	IT	Mostly GM with small pockets of WM	Low	0	834.5	58	IT (GM)	277	0.17
	2	4.1	38	IT	Mostly GM	Low		834.5	136	IT (GM)	227	
23	1	4.7	39	IT	GM	Low	0	834.5	24	IT (GM)	419	0.78
24	1	2.4	40	IT	Mostly GM	Low	0	834.4	45	IT (GM)	312	0.04
	2	1.3	41	IT	Mostly GM	Low		834.4	113	IT (GM)	207	
25	1	8.6	42	IT (NOS)	Presence of edema	Medium	0	788.4	43	IT (WM)	1.67	0.04

26	1	2.4	43	IT	Mixture of GM and WM	Medium	0	888.6	55	IT (WM)	31.7	0.22
27	1	1.3	44	IT	Mostly GM	Low	0	834.5	77	IT (GM)	441	0.52
28	1	1.0	45	IT	Mostly GM	Low	0	834.5	42	IT (GM)	383	0.02
	2	1.1	46	IT	Mixture of GM and WM	Low		888.6	91	IT (WM)	191	
29	1	3.5	47	G	-	High	1	885.5	74	G	3.5	3.62

^aGM, grey matter; WM, white matter; G, glioma; IT, infiltrated tissue; IT (NOS), infiltrated tissue not otherwise specified

^b0 = non-immunoreactive; 1 = immunoreactive

^cNAA signal intensity of the transition m/z 174→114 is normalized to the signal intensity of the internal standard NAA-d₃ using the transition m/z 177→116.

^d2HG signal intensity of the sequential product ion m/z 147→129→101 is normalized to the signal intensity of the internal standard NAA-d₃ using the transition m/z 177→116.

^en.d. = not detected

Table S2. MS Instrumental Settings

<i>Full Scan MS</i>	Lipid Profile		Metabolite Profile
Mass range	<i>m/z</i> 700-1000		<i>m/z</i> 80-200
Tuned mass	<i>m/z</i> 786		<i>m/z</i> 174
Tube lenses potential	−80 V		−20 V
Capillary voltage	0 V		−8 V
Microscans	2		
Injection time	25 ms		
Capillary temperature	275 °C		
Capillary voltage	−6.5 kV		
<i>MSⁿ CID* fragmentation</i>	NAA	NAA-d ₃	2-HG
MS ⁿ transitions	<i>m/z</i> 174 → o	<i>m/z</i> 177 → o	<i>m/z</i> 147 → 129 → o
Tube lenses potential	−20 V		
Capillary voltage	−8 V		
Microscans	2		
Injection time	25 ms		
Collision Energy (a.u.)	28		
Q value	0.3		

Table S3. Concentrations of NAA and 2HG determined by ESI-MS³⁰. Data reproduced with permission, copyright from Clinical Chemistry 2017.

Case	NAA concentration (ng/mg)	2HG concentration (ng/mg)
1	15.9	13.8
2	33.0	13.0
3	81.0	104.6
4	12.1	10.4
5	223.3	254.5
6	1182.5	12.3
7	43.7	506.4
8	827.3	10.3
9	76.4	8.15
10	821.0	10.8
11	313.0	5.4
12	43.0	2.3
13	3.3	9.2
14	604.6	14.3
15	610.8	87.9
16	332.0	289.7
17	679.4	14.9
18	718.1	24.0
19	270.6	385.8
20	27.9	24.2
21	144.2	124.7
22	1113.2	16.1
23	1358.0	24.2
24	1160.7	10.4
25	0.4	0.2
26	984.9	13.4
27	1424.9	14.4
28	1186.7	12.2
29	n.d.	n.d.

Table S4. Association between chemical predictions of disease state vs. pathology assessment

Pathological Evaluation	Chemical Evaluation of Disease State		
	Grey Matter	White Matter	Glioma
Infiltrated tissue	18	12	3
Glioma	1	0	12



Figure S1. Photograph of medical swabs, model 160C, from Copan Diagnostics Inc. with and without the plastic sealing tube.

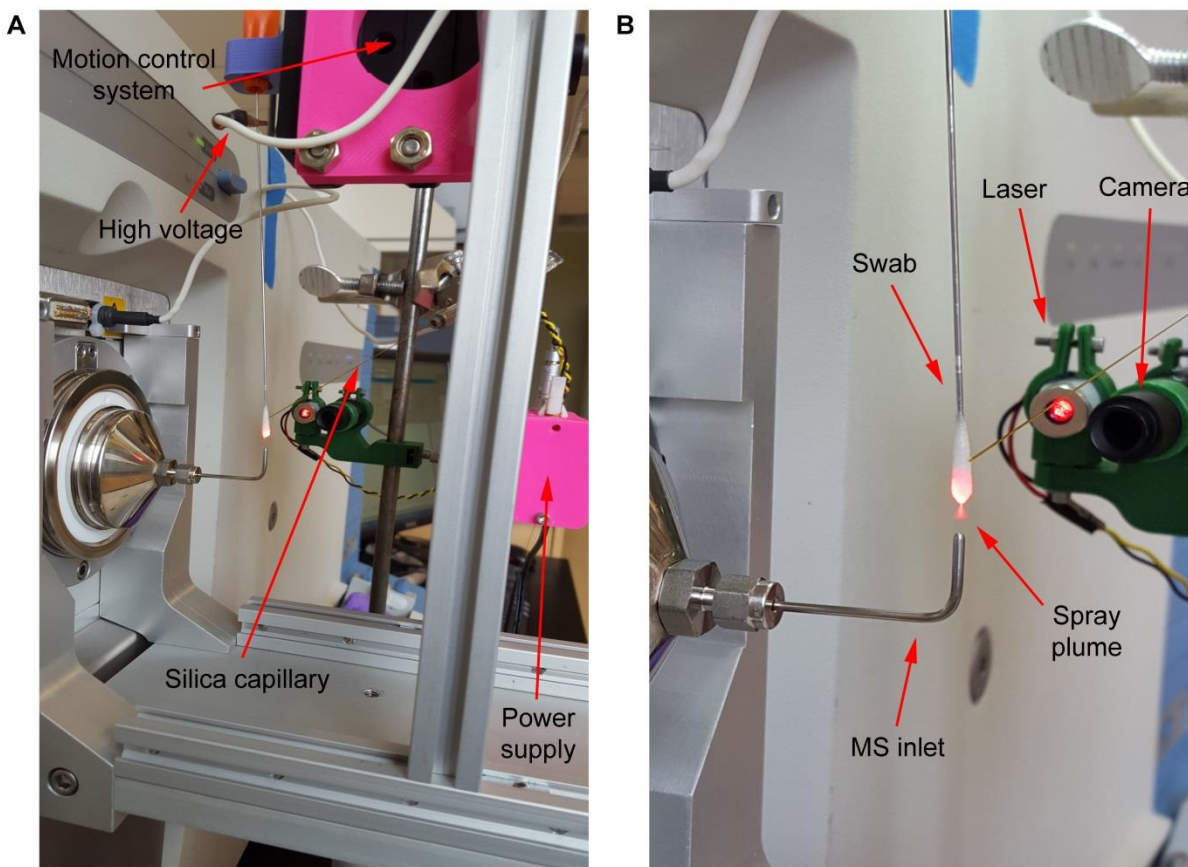


Figure S2. (A) Image of the custom-build ion source for TS-MS with medical swabs. **(B)** Photograph of electrospray generated from the swab, red laser pointer was used to illuminate the spray plume.

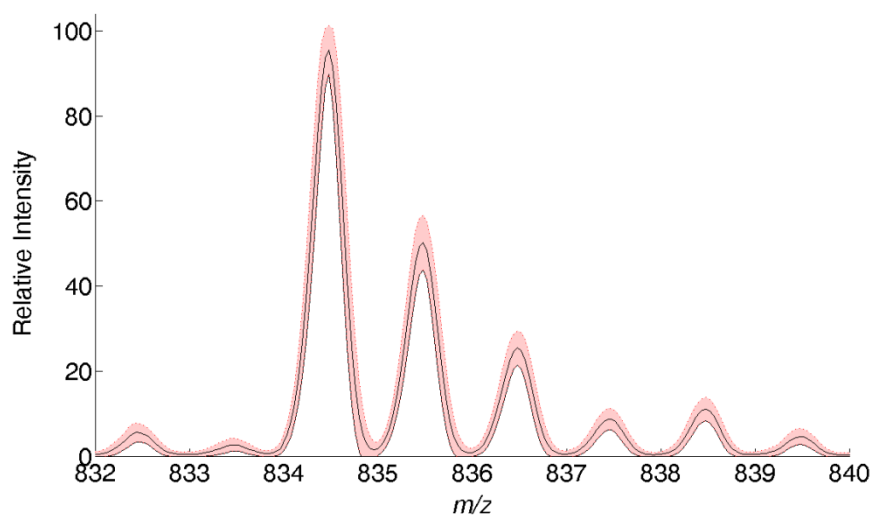


Figure S3. Isotope distribution of PS 18:0_22:6, m/z 834.5. Mean ion intensity denoted by solid line with \pm standard deviation illustrated by the shaded area between the dotted lines. Number of scans averaged = 1026 over 10 min of data acquisition. Ion Intensities are normalized to the base peak (m/z 834.5) over the mass range m/z 760-920.

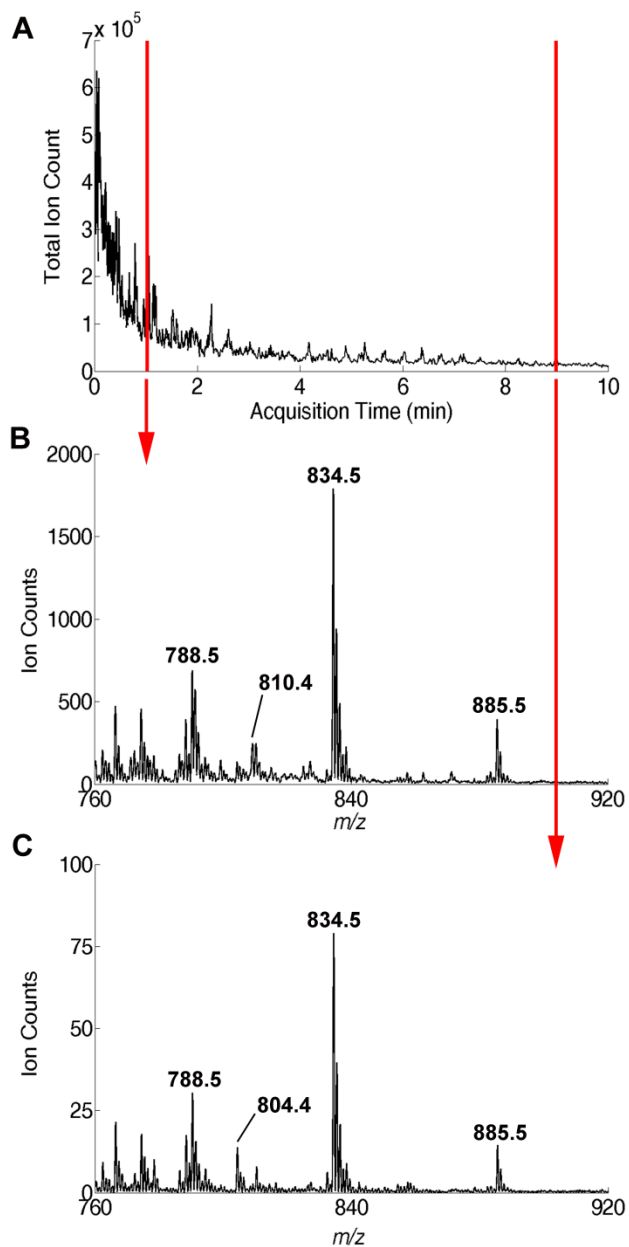


Figure S4. Case #17; pathological diagnosis: grey matter infiltrated with low TCP. **(A)** Total ion count (TIC) over a 10-minute window of data acquisition. Vertical red lines are drawn at minutes 1 and 9, respectively; the red arrows point at the full-scan mass spectrum acquired at those two-time points. **(B)** Full-scan mass spectrum in negative ion mode after 1 minutes of data acquisition; TIC = $1.67 \cdot 10^5$ **(C)** Full-scan mass spectrum in negative ion mode after 9 minutes of data acquisition; TIC = $0.26 \cdot 10^5$. Absolute ion counts are shown on the Y-axes.

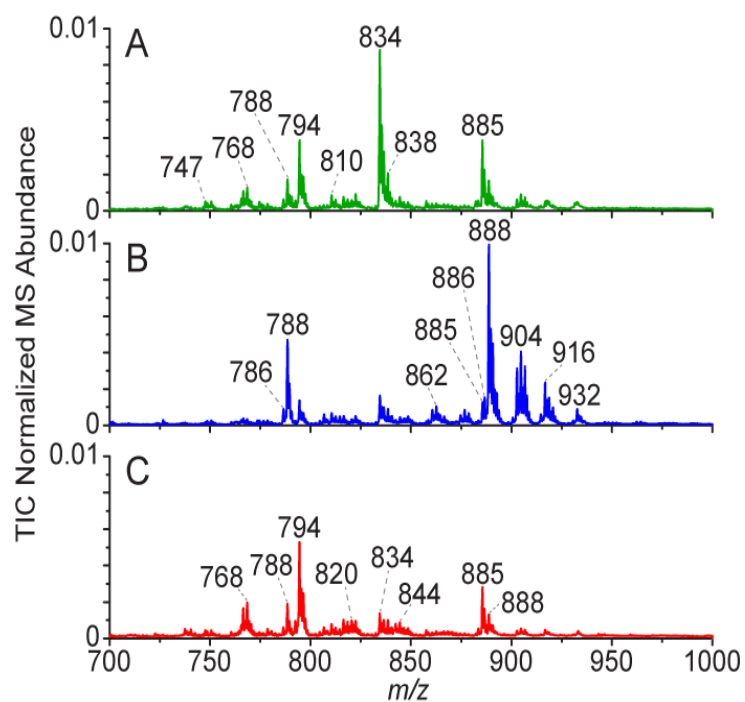


Figure S5. Average DESI-MS lipid (m/z 700–1000) MS profiles for **(A)** grey matter (N=223), **(B)** white matter (N=66), and **(C)** glioma (N=158). Ion intensities are normalized to the total ion count (TIC). Figure is reproduced with permission of the National Academy of Sciences (2017).

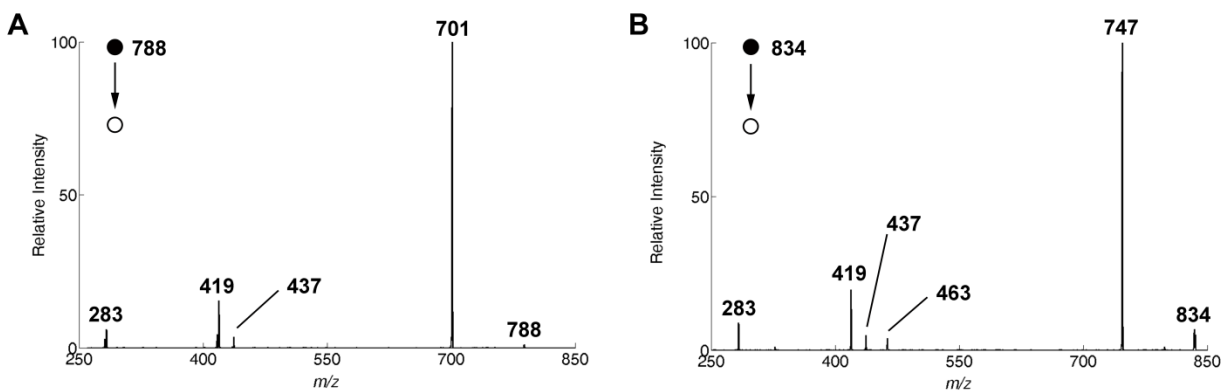


Figure S6. MS/MS product ion spectra for m/z 788 (**A**) and m/z 834 (**B**) detected in the negative ionization mode from Sample #8. Characteristic losses used in determining the lipid class; e.g., -87 (m/z 788 \rightarrow 701, head group loss of phosphatidylserines). Furthermore, acyl chain could be determined based on fatty acid product ions; e.g., m/z 283, stearic acid. Ion intensities are normalized to the base peak over the product mass range m/z 250-850.

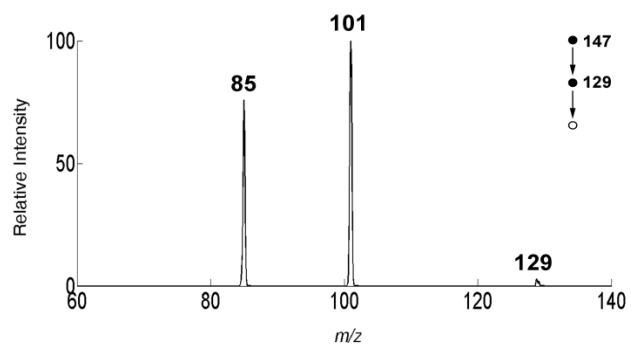


Fig. S7. Sequential product ion scan for 2HG from Case #16, IDH-mutant glioma. Fragmentation of 2HG matches previously reported pattern detected by DESI-MS (20, 22). The fragmentation pattern was matched also against a certified analytical standard. Ion intensities are normalized to the base peak over the product mass range m/z 60-140.

Video S1. Swab electrospray process. The video shows side-by-side the electrospray plume formation and the mass spectral data acquired simultaneously from Sample #12 (Case #8, Table S1). The swabs are unconventional probes for electrospray because of their fused shape and large tip (>1 mm). Swab TS differs from regular electrospray in that the solvent is pumped on a rough and porous surface; it is initially absorbed and then becomes suspended in a droplet at the apex of the swab tip once the porous material is completely saturated. When high voltage is applied to the handle of the swab, the voltage is transferred to the solvent at the swab tip. The suspended droplet becomes elongated. The elongation reduces droplet diameter, which in turn increases the electric field strength such that it exceeds the solvent surface tension resulting in the electrospray generation. The Taylor cone is formed at the tip of the swab. The plume of microdroplets generated by the electrospray process is visible through illumination with a laser. Microdroplets undergo cycles of solvent evaporation and Coulomb fission and are vacuumed into the mass spectrometer and mass analyzed.