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

Valentina Pirro[a,b*], Raquel Sero Llor[a#], Alan K. Jarmusch[a], Clint M. Alfaro[a], Aaron A. Cohen-Gadol[c], Eyas M. Hattab[d], and R. Graham Cooks[a,b*]

[a]Chemistry Department, Purdue University, West Lafayette, Indiana, United States of America
[b]Center for Analytical Instrumentation Development, West Lafayette, Indiana, United States of America
[c]Department of Neurological Surgery, Indiana University School of Medicine, Indianapolis, Indiana, United States of America
[d]Department of Pathology and Laboratory Medicine, University of Louisville, Louisville, Kentycky, United States of America

#Permanent address: Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain

**Corresponding author**
Prof. R.G. Cooks
Dr. Valentina Pirro
Chemistry Department, Purdue University
560 Oval Drive
West Lafayette, IN, 47907, USA
Email: cooks@purdue.edu; vpirro@purdue.edu
Phone: +1 765-494-5263

**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 |

[a]GM, grey matter; WM, white matter; G, glioma; IT, infiltrated tissue; IT (NOS), infiltrated tissue not otherwise specified

[b]0 = non-immunoreactive; 1 = immunoreactive

[c]NAA signal intensity of the transition *m/z* 174→114 is normalized to the signal intensity of the internal standard NAA-$d_3$ using the transition *m/z* 177→116.

[d]2HG signal intensity of the sequential product ion *m/z* 147→129→101 is normalized to the signal intensity of the internal standard NAA-$d_3$ using the transition *m/z* 177→116.

[e]n.d. = not detected

**Table S2.** MS Instrumental Settings

| *Full Scan MS* | **Lipid Profile** | | **Metabolite Profile** |
|---|---|---|---|
| **Mass range** | *m/z* 700-1000 | | *m/z* 80-200 |
| **Tuned mass** | *m/z* 786 | | *m/z* 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 | | |
| *$MS^n$ CID* fragmentation* | **NAA** | **NAA-$d_3$** | **2-HG** |
| **$MS^n$ transitions** | *m/z* 174 → ○ | *m/z* 177 → ○ | *m/z* 147 → 129 → ○ |
| **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-MS[30]. 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

| | Chemical Evaluation of Disease State | | |
|---|---|---|---|
| **Pathological Evaluation** | **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.

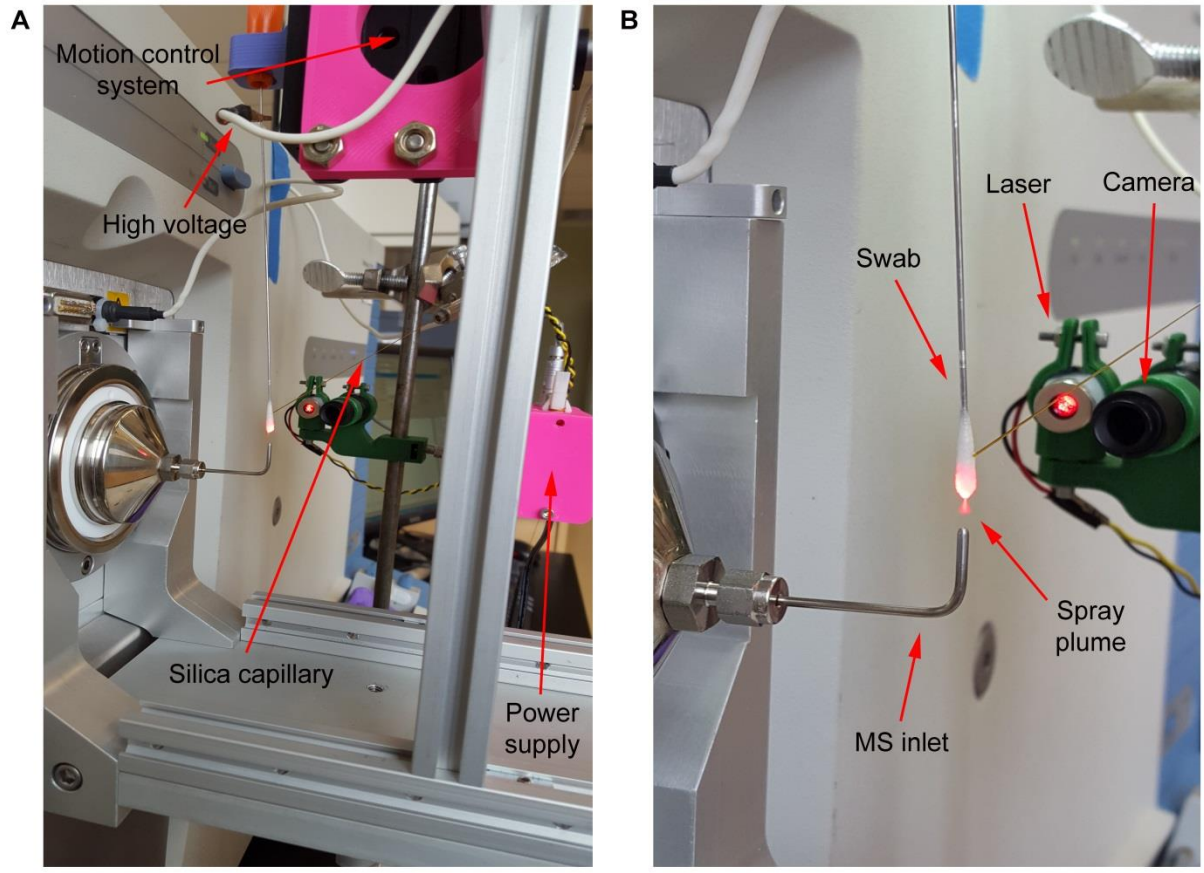


**Figure S2. (A)** Image of the custom-build ion source for TS-MS with medical swabs. **(B)** Photograph of electrospray generated from the swab, red laser pointer was used to illuminate the spray plume.

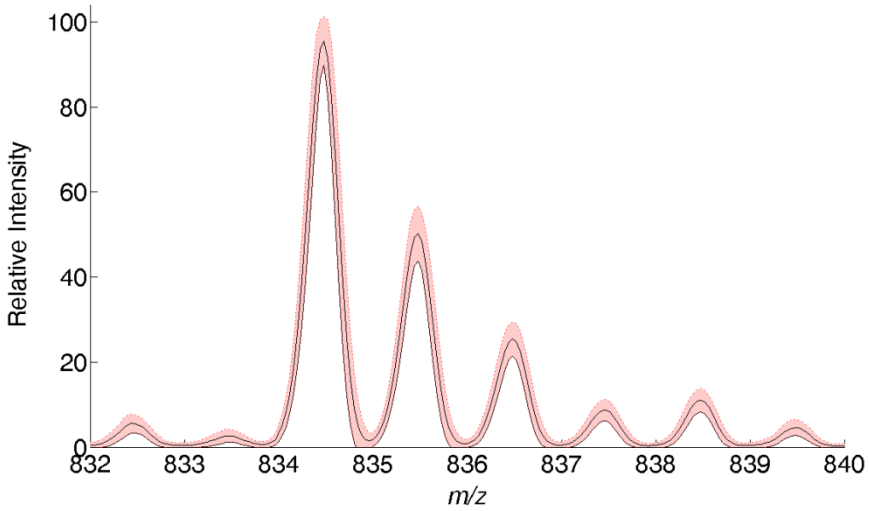


**Figure S3.** Isotope distribution of PS 18:0_22:6, *m/z* 834.5. Mean ion intensity denoted by solid line with ± standard deviation illustrated by the shaded area between the dotted lines. Number of scans averaged = 1026 over 10 min of data acquisition. Ion Intensities are normalized to the base peak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

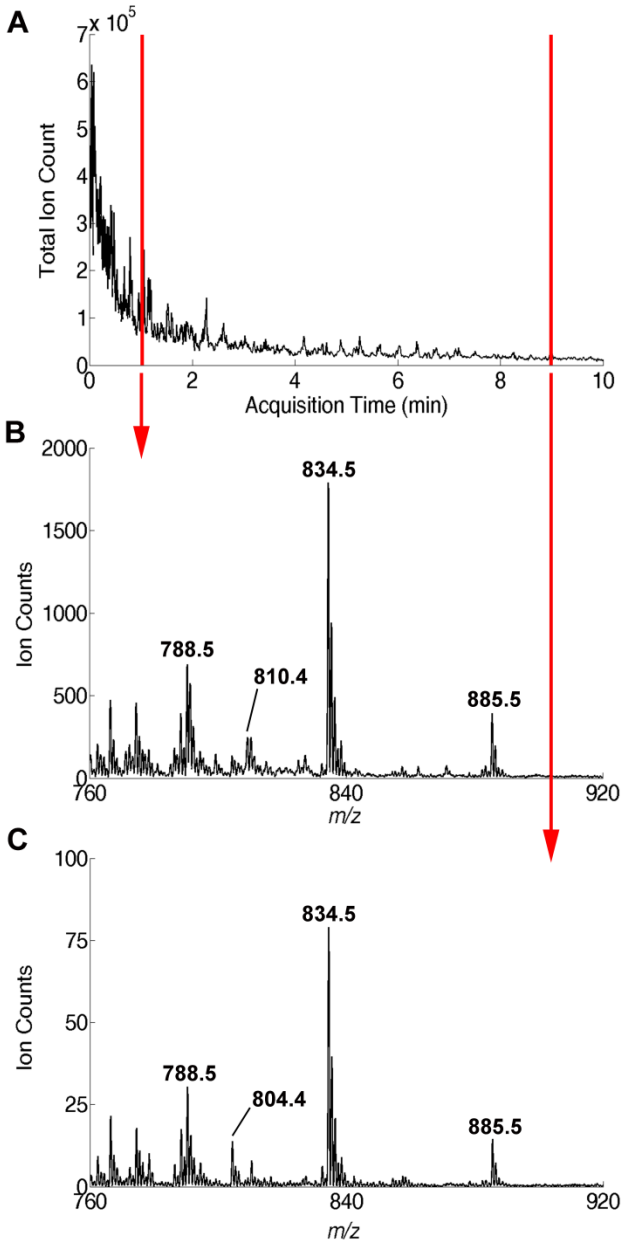


**Figure S4.** Case #17; pathological diagnosis: grey matter infiltrated with low TCP. **(A)** Total ion count (TIC) over a 10-minute window of data acquisition. Vertical red lines are drawn at minutes 1 and 9, respectively; the red arrows point at the full-scan mass spectrum acquired at those two-time 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.

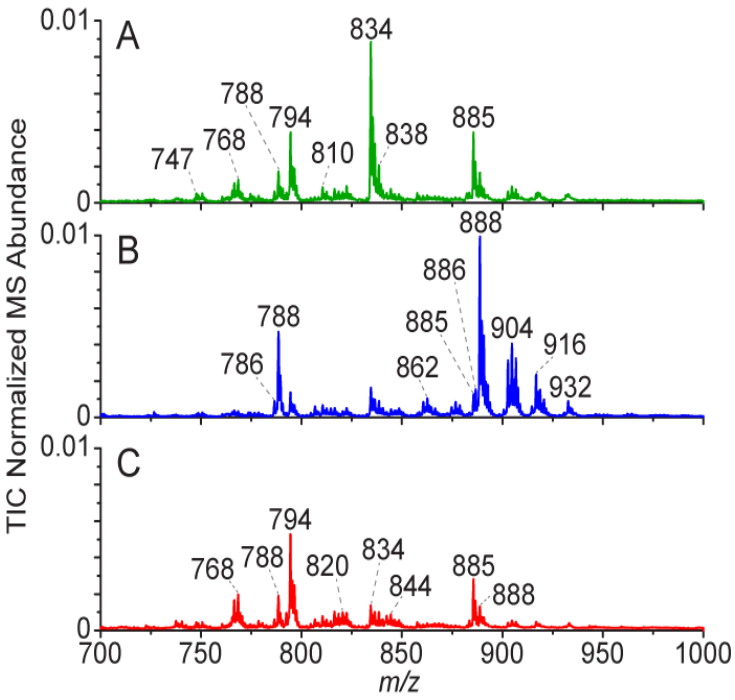


**Figure S5.** Average DESI-MS lipid (*m/z* 700–1000) MS profiles for **(A)** grey matter (N=223), **(B)** white matter (N=66), and **(C)** glioma (N=158). Ion intensities are normalized to the total ion count (TIC). Figure is reproduced with permission of the National Academy of Sciences (2017).

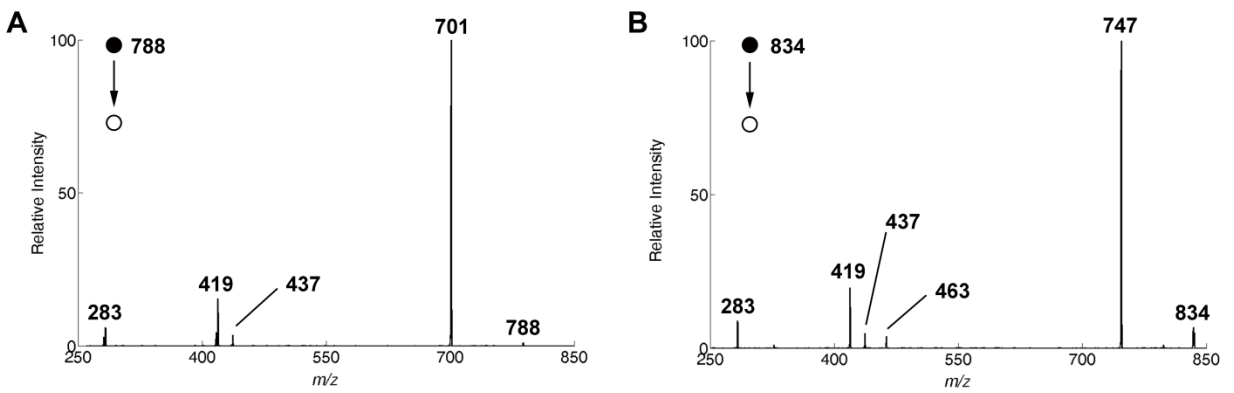


**Figure S6.** MS/MS product ion spectra for *m/z* 788 **(A)** and *m/z* 834 **(B)** detected in the negative ionization mode from Sample #8. Characteristic losses used in determining the lipid class; e.g., −87 (*m/z* 788→701, head group loss of phosphatidylserines). Furthermore, acyl chain could be 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.

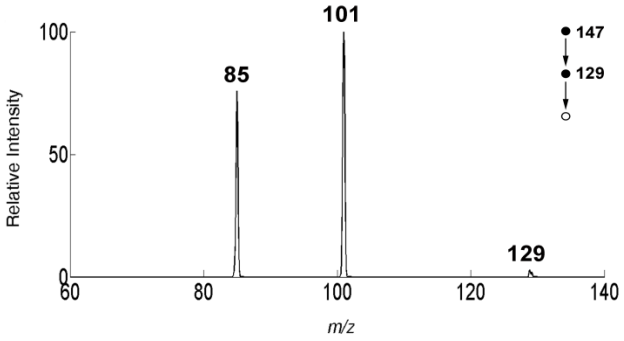


**Fig. S7.** Sequential product ion scan for 2HG from Case #16, IDH-mutant glioma. Fragmentation of 2HG matches previously reported pattern detected by DESI-MS (20, 22). The fragmentation pattern was matched also against a certified analytical standard. Ion intensities are normalized to the base peak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