SUPPORTING INFORMATION

Direct Analysis of Biological Tissue by Paper Spray Mass Spectrometry

He Wang[1], Nicholas E. Manicke[2], Qian Yang[1], Lingxing Zheng[3], Riyi Shi[3], R. Graham Cooks[2,4]

and Zheng Ouyang[1,4]*

1. Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA
2. Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA
3. Department of Basic Medical Science, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907, USA
4. Center for Analytical Instrumentation Development, Purdue University, West Lafayette, IN 47907, USA

*** Corresponding Author**
Professor Zheng Ouyang
Weldon School of Biomedical Engineering
Purdue University
West Lafayette, IN, 47907
Tel: (765) 494-2214
Fax: (765) 496-1912
Email: ouyang@purdue.edu

Procedure for Preparing Rat Tissue

The experimental protocols used have been reviewed and approved by the Purdue University Animal Care and Use Committee. Rats were housed and handled in accordance with Purdue University Animal Care and Use Committee guidelines. Briefly, hydralazine was diluted in standard saline solution to reach a concentration of 2.5mg/mL. For every 500g of body weight, 1 mL of this diluted drug solution was injected through peritoneum. The euthanasia of the rat was performed with 1mL of beuthanasia injected into the heart at 130 mins post-drug injection. The following organs/tissues were then dissected out immediately following euthanasia: liver, kidney, spinal cord and brain. All samples were preserved at -20 Celsius before the experiment.

The tissue homogenate was prepared as follows: 0.3 g of the tissue was put into the tissue grinder, and 1 mL of sodium bicarbonate solution (10 mM, pH 7) was added into the grinder. The tissue was homogenized using the pestle and then transferred into centrifuge tubes. After centrifugation, the unhomogenized tissue was removed, and then the supernatant was transferred to fresh tubes and stored at -20ºC.

Experimental Conditions for Paper Spray Mass Analysis

The paper spray experiment has been described previously[31,32]. Briefly, paper was cut into a triangle with height 10 mm and base width 5 mm. A copper clip was used to hold the paper section and to apply the high voltage. The distance between the tip of the paper triangle and the inlet of the mass spectrometer was 5 mm. The paper used was Grade 1 chromatography paper purchased from Whatman (Maidstone, England) without further chemical treatment. The tissue sample was placed in the middle of the paper triangle and solvent was applied to the base. For positive ion mode mass spectrometry, the spray voltage was 4.5 kV and MeOH:H_2O (v:v, 1:1, 10 μL) was used as solvent. In the negative ion mode, the spray voltage was 4 kV and pure MeOH was used as solvent. Quantitation of therapeutic drug hydralazine was performed using a TSQ Quantum triple quadrupole mass spectrometer (Thermo Scientific Inc., San Jose, CA). The detection and identification of lipids in the negative ion mode was performed using an Exactive Orbitrap in the ultra-high resolution mode (100,000). Other mass analyses were performed using a LTQ ion trap mass spectrometer (Thermo Scientific Inc., San Jose, CA).

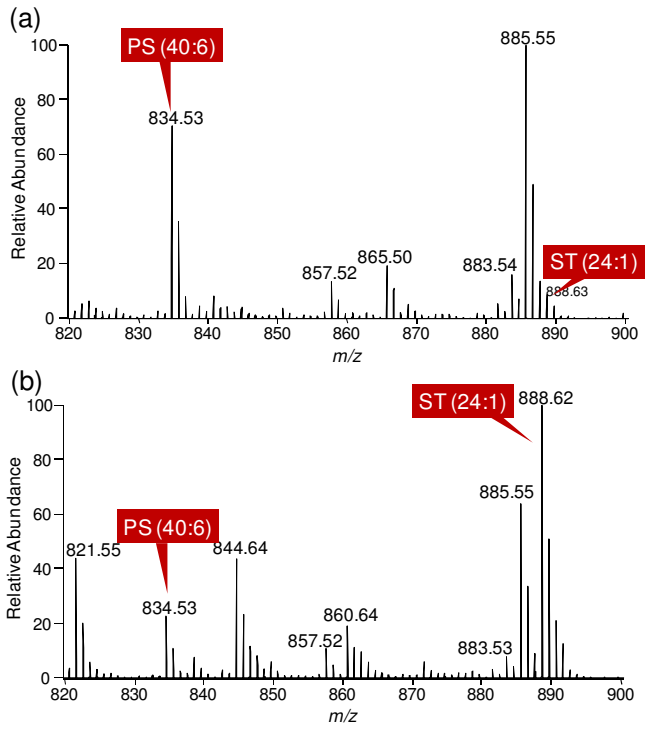

Figure S-1. Mass spectra of rat brain tissue in the (a) grey-matter and (b) white-matter regions, negative ion mode using Exactive Orbitrap. The major characteristic lipid PS (40:6) in the grey-matter region and the major lipid ST (24:1) in the white-matter region have substantially different relative abundances in the two samples.

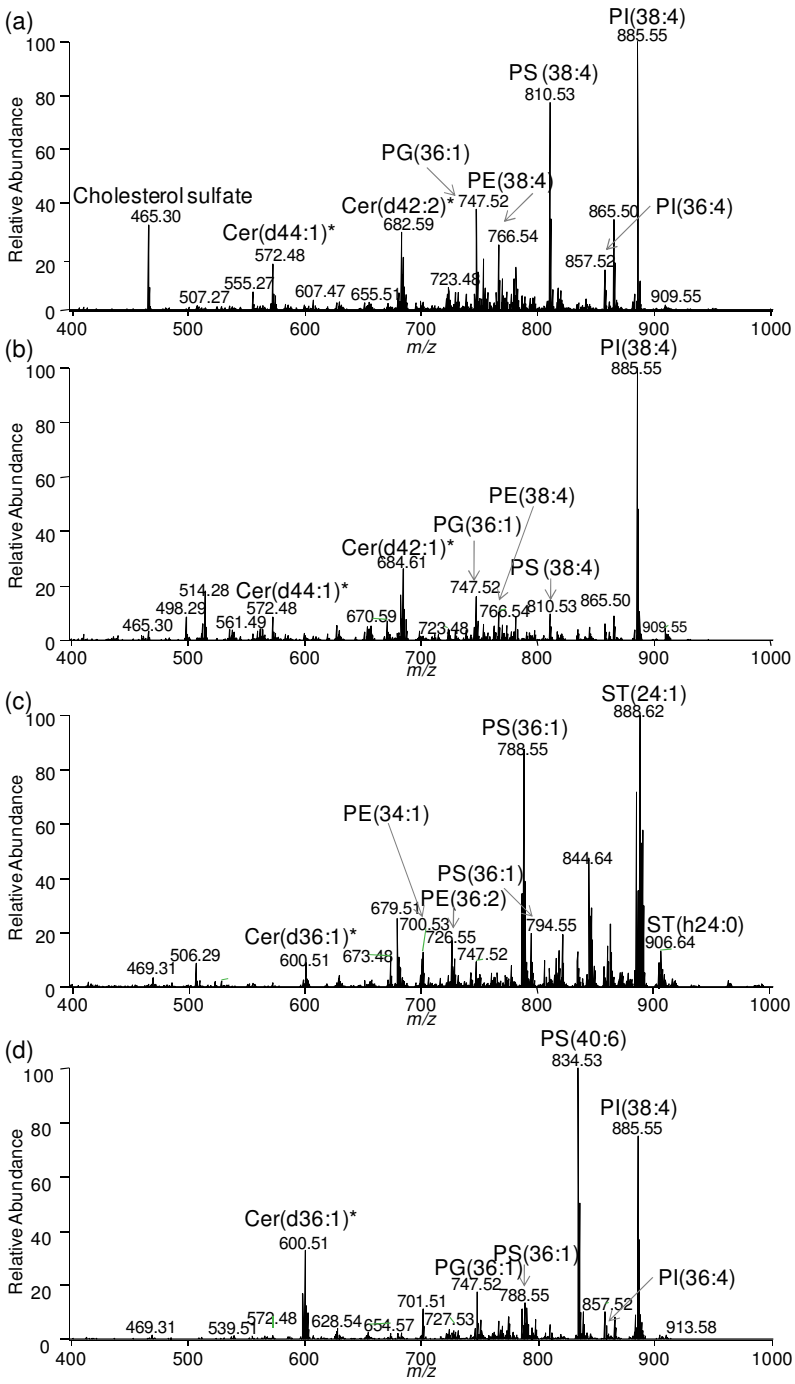

Figure S-2. Paper spray mass spectra of rat bulk tissue from (a) kidney, (b) liver, (c) spinal cord, and (d) brain in grey-matter region. Negative ion mode using Exactive Orbitrap.

Table S-1. Lipid molecular species detected from the grey region of mouse brain tissue in the negative ion mode (m/z 500-1000). The masses of the lipids were compared with data in an online database http://www.lipidmaps.org/data/structure/index.html.

Measured *m/z*	Proposed formula	Molecular spiecies[a]	Relative intensity(%)	Mass error(ppm)
598.49784	C36H69NO3	Cer(d36:2) [b]	17.1	3.908467143
600.51335	C36H71NO3	Cer(d36:1) [b]	32.5	3.662213428
747.51883	C40H77O10P	PG(36:1)[c]	17.1	1.632336127
750.54499	C43H78NO7P	PE(O-38:5) [c]	7.1	1.632149992
766.53977	C43H78NO8P	PE(38:4) [c]	6.8	1.422498341
774.54532	C45H78NO7P	PE(P-40:6) [c]	8.2	2.007633487
786.52965	C42H78NO10P	PS(36:2) [c]	10.9	1.450932785
788.54553	C42H80NO10P	PS(36:1) [c]	13.3	1.738773897
790.53982	C45H78NO8P	PE(40:6) [c]	11.4	1.442560661
797.53469	C44H79O10P	PG(38:4) [c]	7.2	1.793154056
810.52968	C44H78NO10P	PS(38:4) [c]	5.6	1.444983103
834.52924	C46H78NO10P	PS(40:6) [c]	100	0.876183368
838.56084	C46H82O10NP	GPSer(40:4) [c]	10.2	1.229488927
857.51878	C45H79O13P	PI(36:4) [c]	10	0.905287115
885.54967	C47H83O13P	PI(38:4) [c]	74.1	0.413528641

[a] (X:Y) represents the different number of carbon atoms and different number of double bonds in the fatty acid chains. Only the peaks with the relative intensity higher than 5% were listed.

[b] Species were detected as chloride adducts.

[C] Species were detected as deprotonated forms.

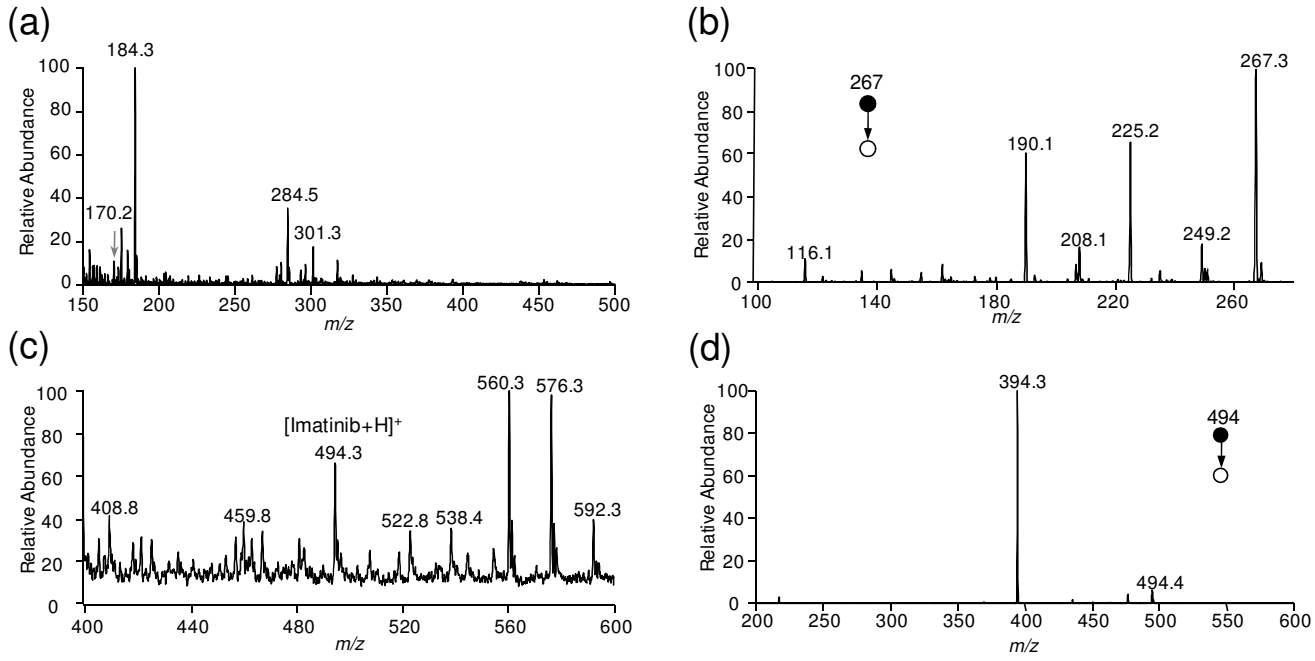

Figure S-3. Analysis of therapeutic drugs in tissue section (a and b) and tissue homogenate (c and d) by PS-MS. 10 μL of an atenolol solution (1 μg/mL) was deposited onto a mouse adrenal gland section (4mm × 7mm) and allowed to dry, resulting in a surface concentration of approximately 0.36 ng/mm^2; then 1 mm^2 of the tissue section was punched out and transferred onto the paper triangle. (a) The MS spectrum of atenolol deposited on adrenal tissue gland. (b) 0.36 ng atenolol could be identified by the tandem MS spectrum of m/z 267. Imatinib was spiked into mouse liver tissue homogenate. Then 0.5 μL of the tissue homogenate was applied on the paper surface to form a dried spot. The MS (c) and MS/MS (b) spectra were recorded for imatinib in tissue homogenate samples at 5 μg/mL and 500 ng/mL, respectively. The spectra were recorded using LTQ ion trap mass spectrometer.

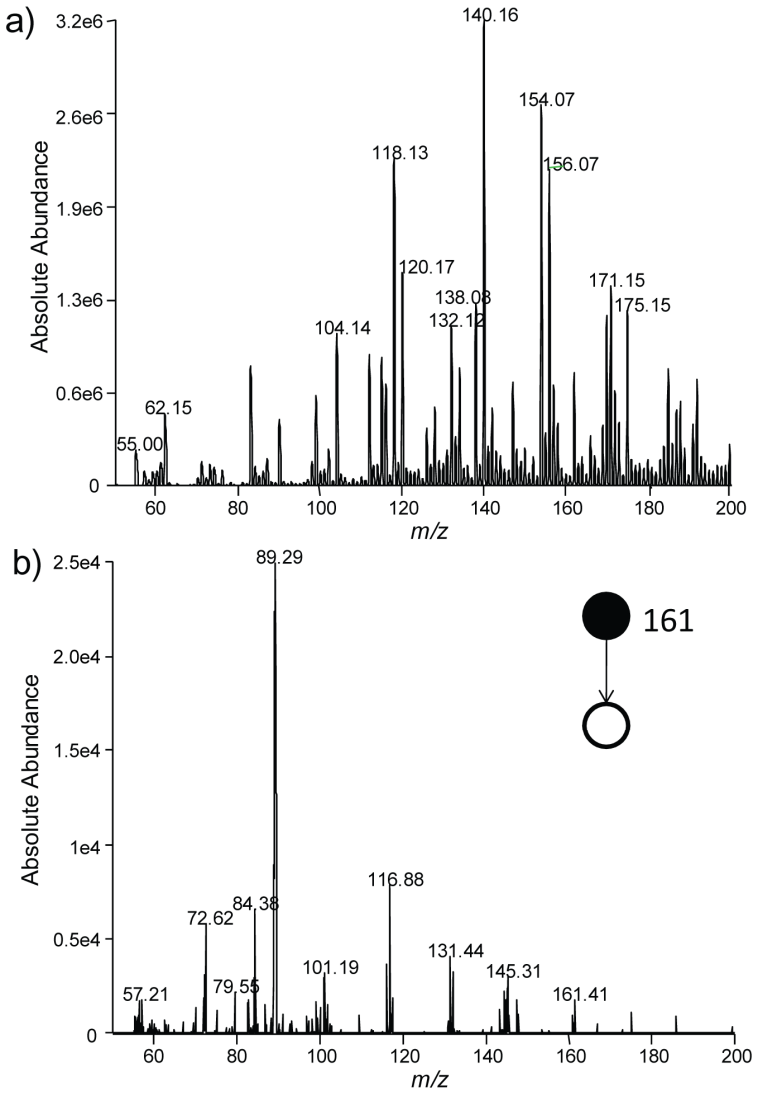

Figure S-4. Paper spray mass spectra of hydralazine in bulk tissue from rat kidney. (a) Mass spectrum and (b) MS/MS spectrum. The spectra were recorded using a triple quadrupole TSQ. The amount of tissue used in this experiment is about 10 times more than that for the tissue homogenate experiment (Figure 4).

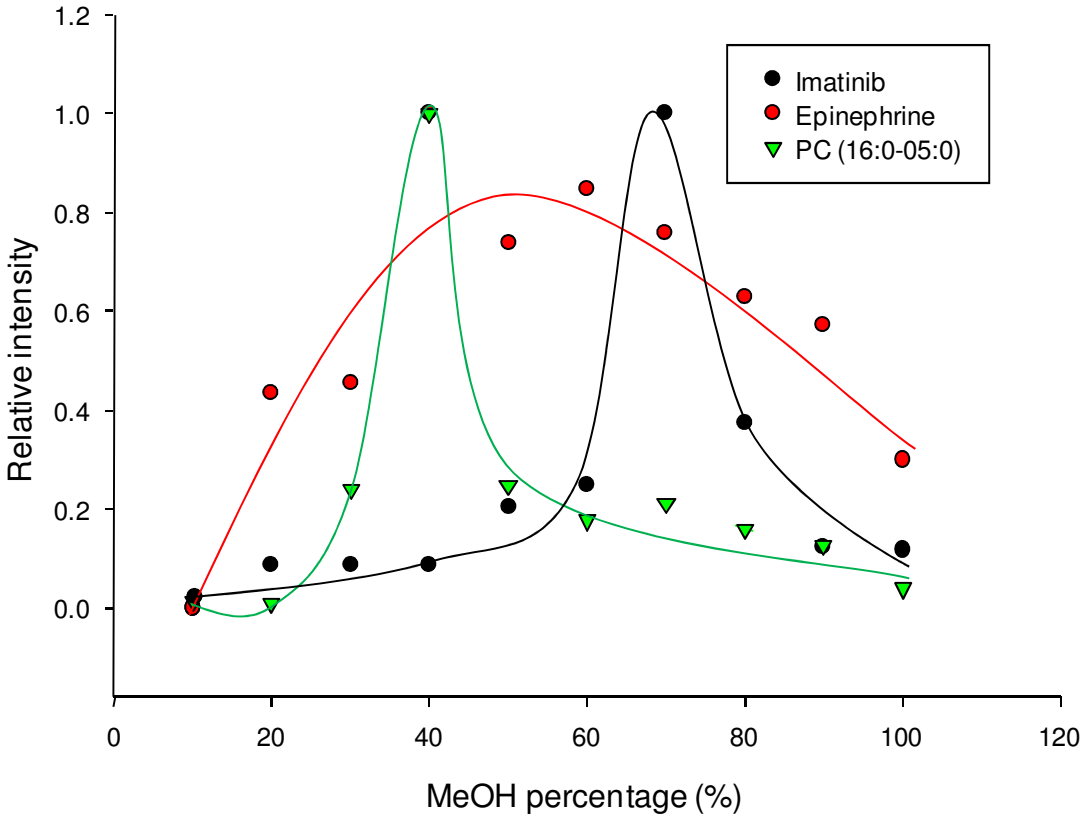

Figure S-5. The effect of spray solvent for different pure chemicals in paper spray ionization. Methanol/water solution (10 µL) containing a single compound, imatinib (500 ng/mL), epinephrine (10 µg/mL) or PC (16:0-05:0) (10 µg/mL), was deposited on a paper triangle to generate the spray. The percentage of the methanol was varied and the peak intensities of the molecular peaks were recorded and normalized.