Supporting Information

Molecular Imaging of Adrenal Gland by Desorption Electrospray Ionization Mass spectrometry

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False Color Intensity Scale

A rainbow color scale was used in this paper. The red pixel represents the highest signal intensity (100%) of the particular ion, and the black pixel represents the lowest signal (0%). The color scales of two ions are not comparable, because different contrast was used for different ions to optimize visualizability of the relative distribution of each ion. If using the same contrast (normalizing intensities of ions to that of the primary ion in the mass spectrum), the distribution of abundances of ions with low signal intensities could not be easily visualized. However, the relative signal intensities of different ions can be found in the mass spectra (Figure S4).

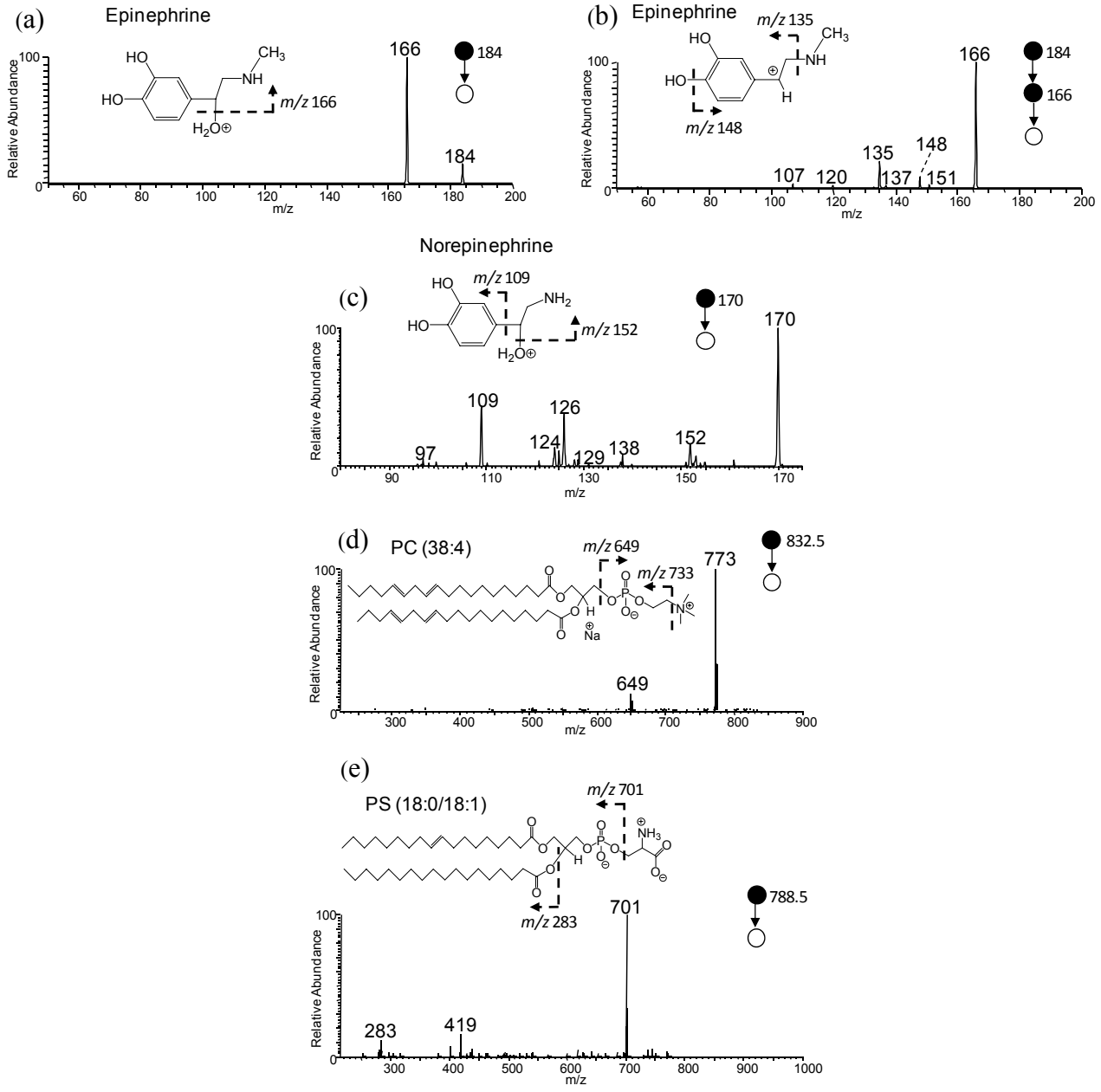

Figure S1. (a) Product ion MS^2 and (b) MS^3 spectra of *m/z* 184 in porcine adrenal gland tissue. Product ion MS^2 spectra of (c) *m/z* 170 (d) *m/z* 832.5 (e) *m/z* 788.5 in porcine adrenal gland tissue. Note that hydrogen rearrangements needed to produce the indicated fragment ions are not shown. Note too that the position of the double bonds in the structure of PC (38:4) and PS (18:0/18:1) were not confirmed. The type of phospholipids was determined by the characteristic head group loss (e.g. a neutral loss of 87 is the indication of PS). If MS^2 of a lipid (e.g. PS 18:0/18:1) shows sufficient fragmentation from fatty acid chains, the assignment with individual chain lengths and double bonds can be made, otherwise the assignment with the chain lengths (and number of double bonds) summed was reported (e.g. PC 38:4).

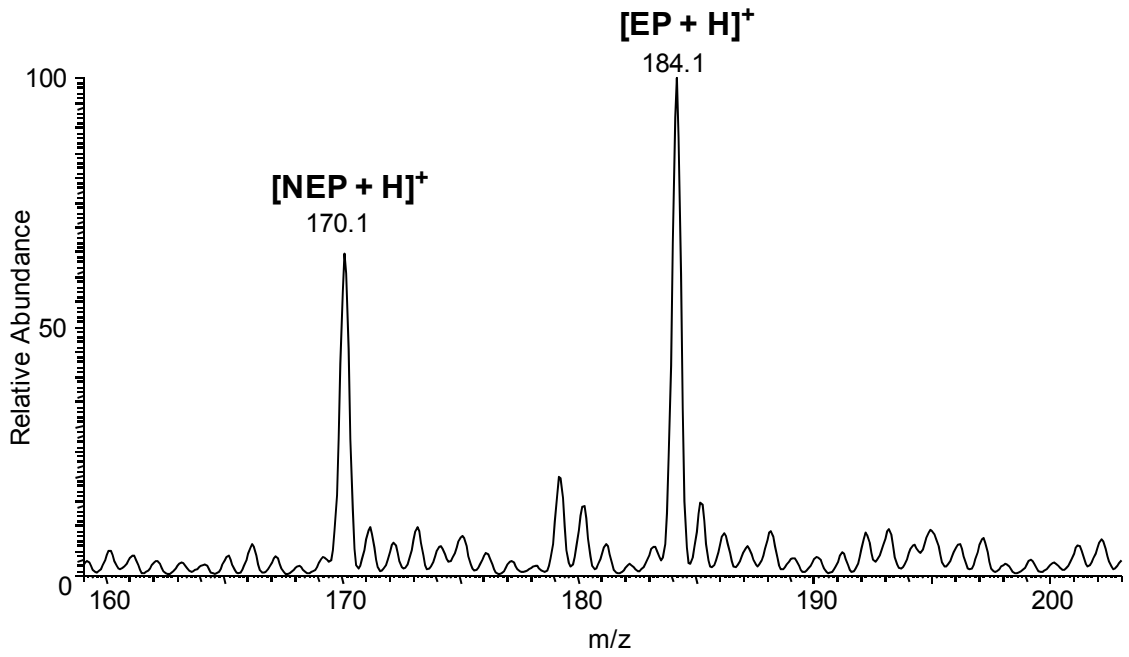

Figure S2. Normal DESI mass spectrum of the mixture of equal amounts of norepinephrine and epinephrine show that the peak intensities are similar in intensity. An aliquot (1μL) of the mixture solution of norepinephrine and epinephrine at a concentration of 5×10^{-11} M, prepared in MeOH/ H_2O (1:1), was spotted onto a microscope glass slide and dried before analysis. The spray solvent of MeOH/H_2O (1:1) was used.

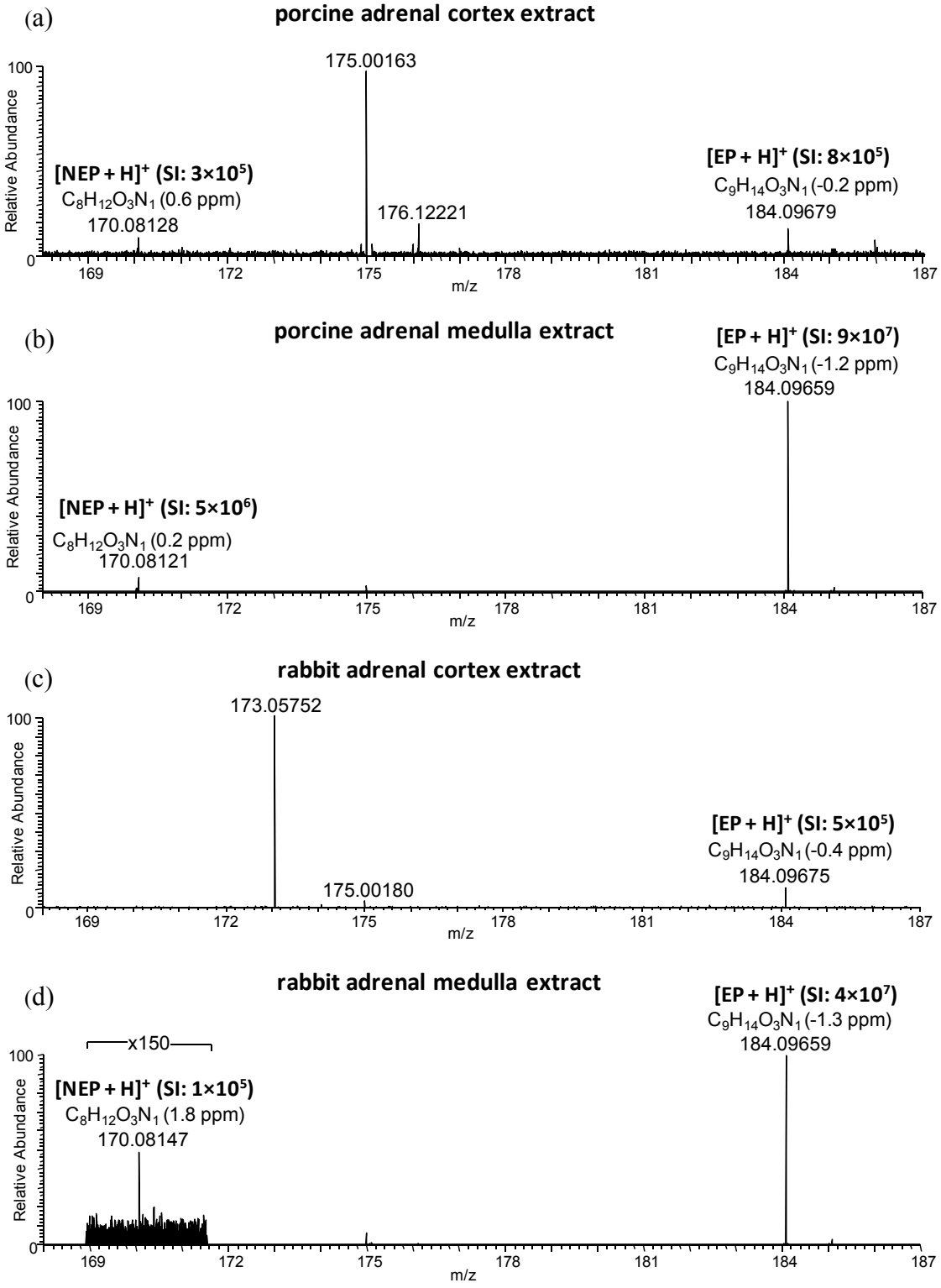

Figure S3. LTQ Orbitrap exact mass measurements of catecholamines using ESI in (a) porcine adrenal cortex extract (b) porcine adrenal medulla extract (c) rabbit adrenal cortex extract (d) rabbit adrenal medulla extract. The lock mass function was used with arginine (*m/z* 175.11895) as the internal standard. Spectra shown are an average of about 20 consecutive scans. The formulas were assigned by the software in Xcalibur. The errors of assigning these formulas are given in parenthesis with ppm as the unit. SI: signal intensity.

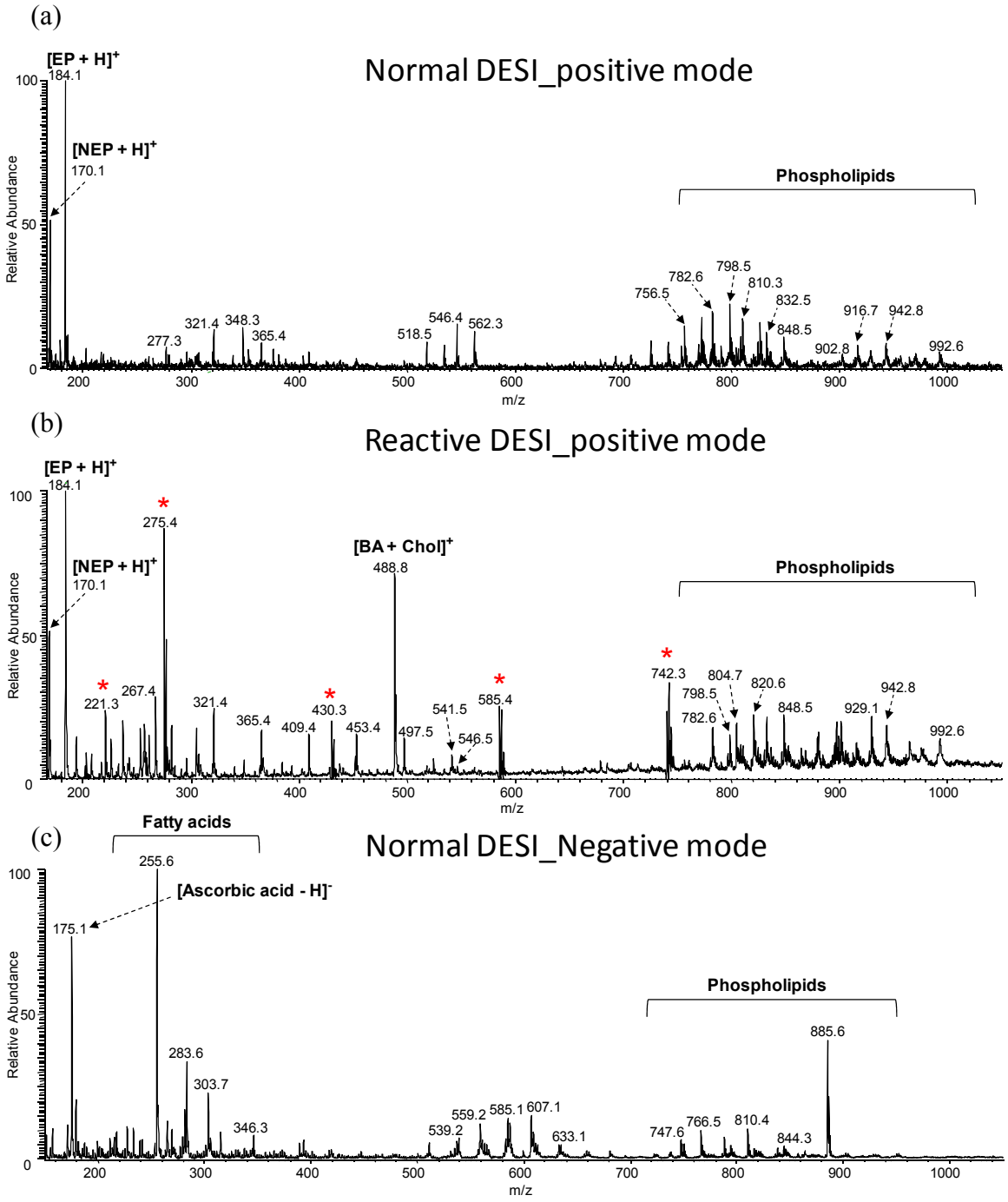

Figure S4. (a) Normal DESI mass spectrum of porcine adrenal medulla tissue in the positive ion mode using MeOH/H_2O (1:1) as solvent. (b) Reactive DESI mass spectrum of porcine adrenal medulla tissue in the positive ion mode using a solvent of ACN/H_2O/DMF (8:3:1) doped with 65 ppm BA. The new peak at *m/z* 488.8 corresponds to the reaction product of endogenous cholesterol with BA, while the catecholamines and lipids can still be detected in the reactive DESI spectrum. The peaks marked with red stars are background peaks due to the solvent used. (c) Normal DESI mass spectrum of porcine adrenal medulla tissue in the negative ion mode using MeOH/H_2O (1:1) as solvent.

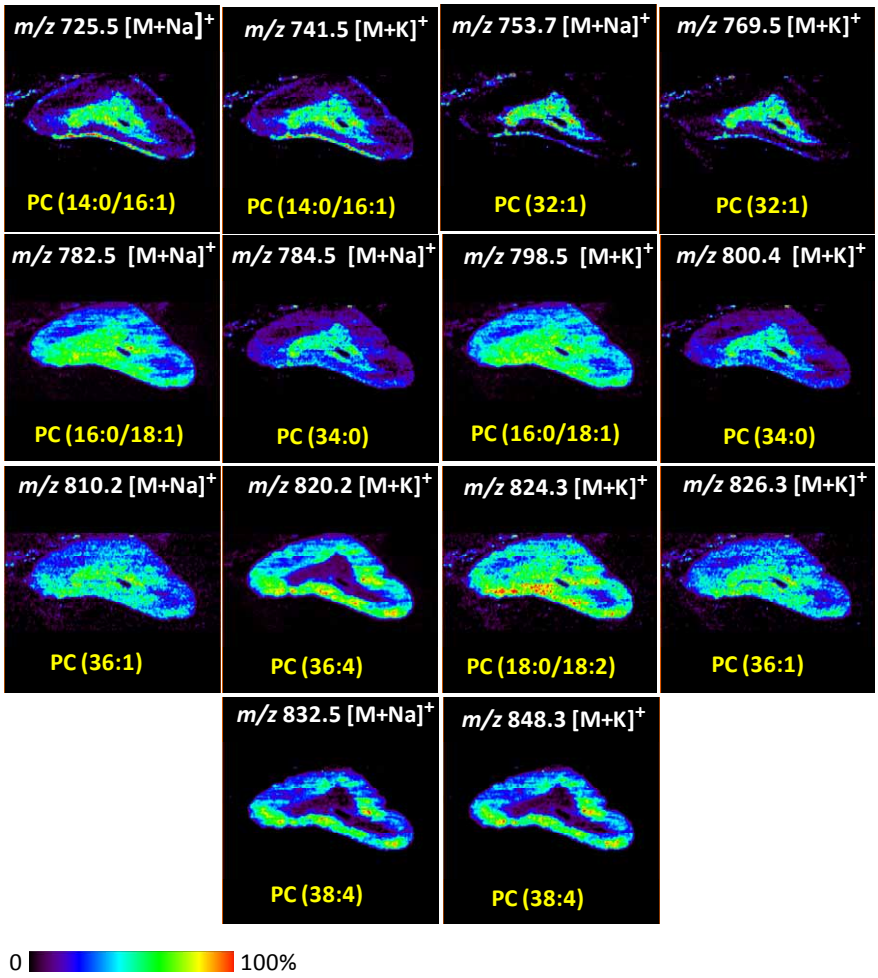

Figure S5. Ion images of PC (14:0/16:1, sodium adduct), PC (14:0/16:1, potassium adduct), PC (32:1, sodium adduct), PC (32:1, potassium adduct), PC (16:0/18:1, sodium adduct), PC (34:0, sodium adduct), PC (16:0/18:1, potassium adduct), PC (34:0, potassium adduct), PC (36:1, sodium adduct), PC (36:4, potassium adduct), PC (18:0/18:2, potassium adduct), PC (36:1, potassium adduct), PC (38:4, sodium adduct), and PC (38:4, potassium adduct) obtained in the positive ion mode.

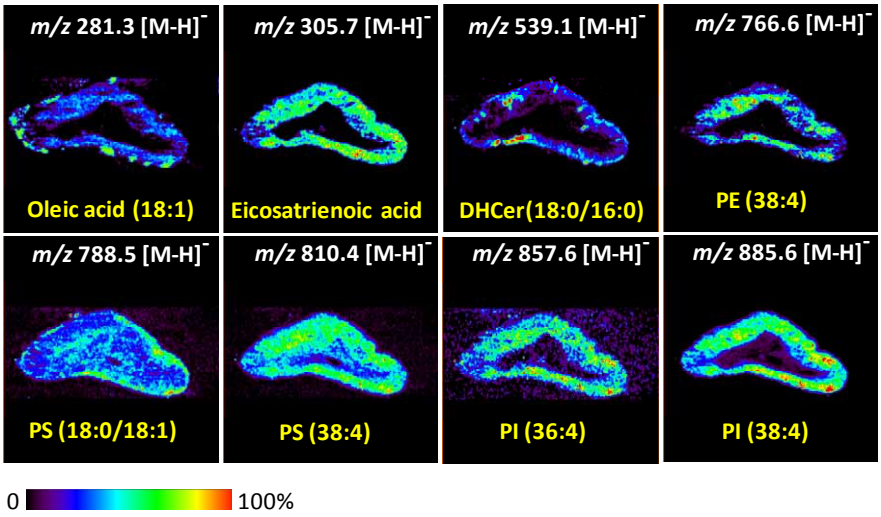

Figure S6. Ion images of oleic acid, eicosatrienoic acid, DHCer (18:0/16:0), PE (38:4), PS (18:0/18:1), PS (38:4), PI (36:4), and PI (38:4) obtained in the negative ion mode.

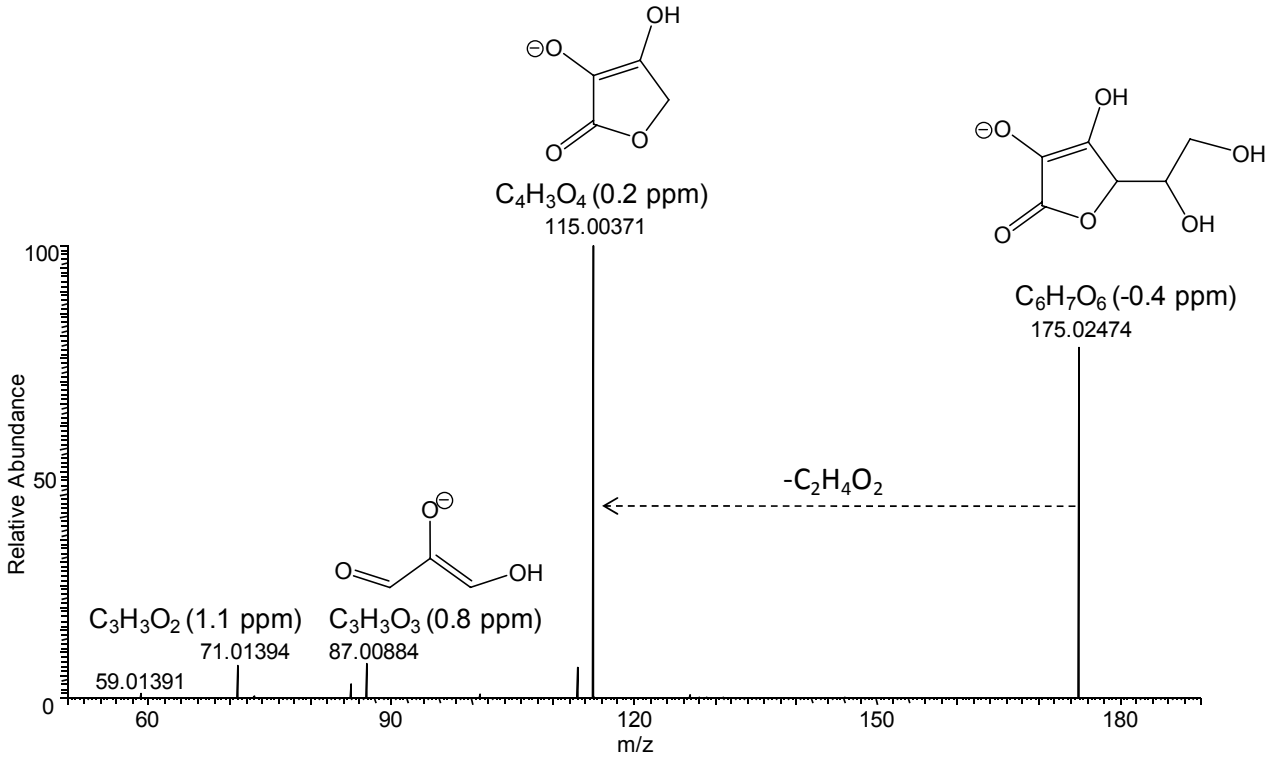

Figure S7. Tandem MS and exact mass measurements of *m/z* 175 in porcine adrenal tissue with DESI coupled to the LTQ Orbitrap to identify *m/z* 175 as ascorbic acid. Authentic ascorbic acid provided the same fragmentation pattern.

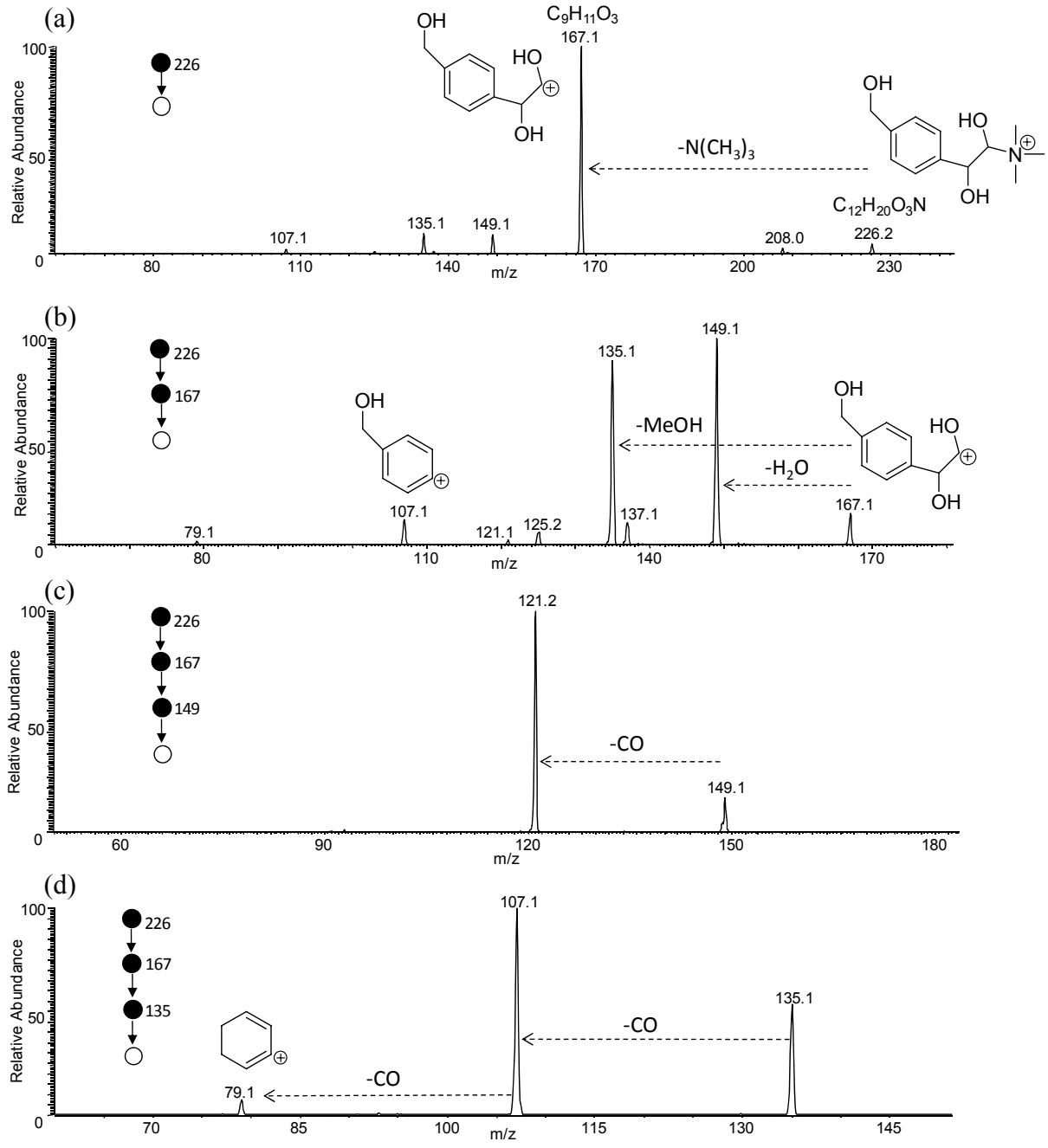

Figure S8. (a) MS^2 of *m/z* 226 ($C_{12}H_{20}O_3N$) in porcine adrenal tissue with DESI coupled to the LTQ. The elemental composition was determined by Orbitrap. The ion of *m/z* 226 is proposed to be the trimethylamino-hydroxymethylphenylethane-diol. (b)- (d) MS^3 and MS^4 of *m/z* 226. The ion structures shown are for convenience, alternatives like the phenonium ion (in a) and the benzylic cation (in b) are likely.

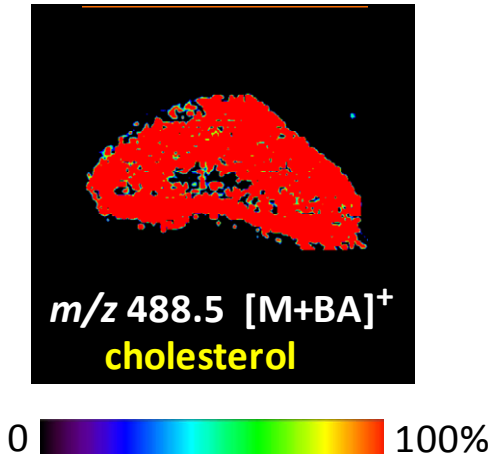

Figure S9. Ion image of cholesterol (the reaction product with betaine aldehyde, BA) obtained using reactive DESI in the positive mode, with a solvent of ACN/H_2O/DMF (8:3:1) doped with 65 ppm BA.