Supporting Information

Rapid, Direct Analysis of Cholesterol by Charge Labeling in Reactive Desorption Electrospray Ionization

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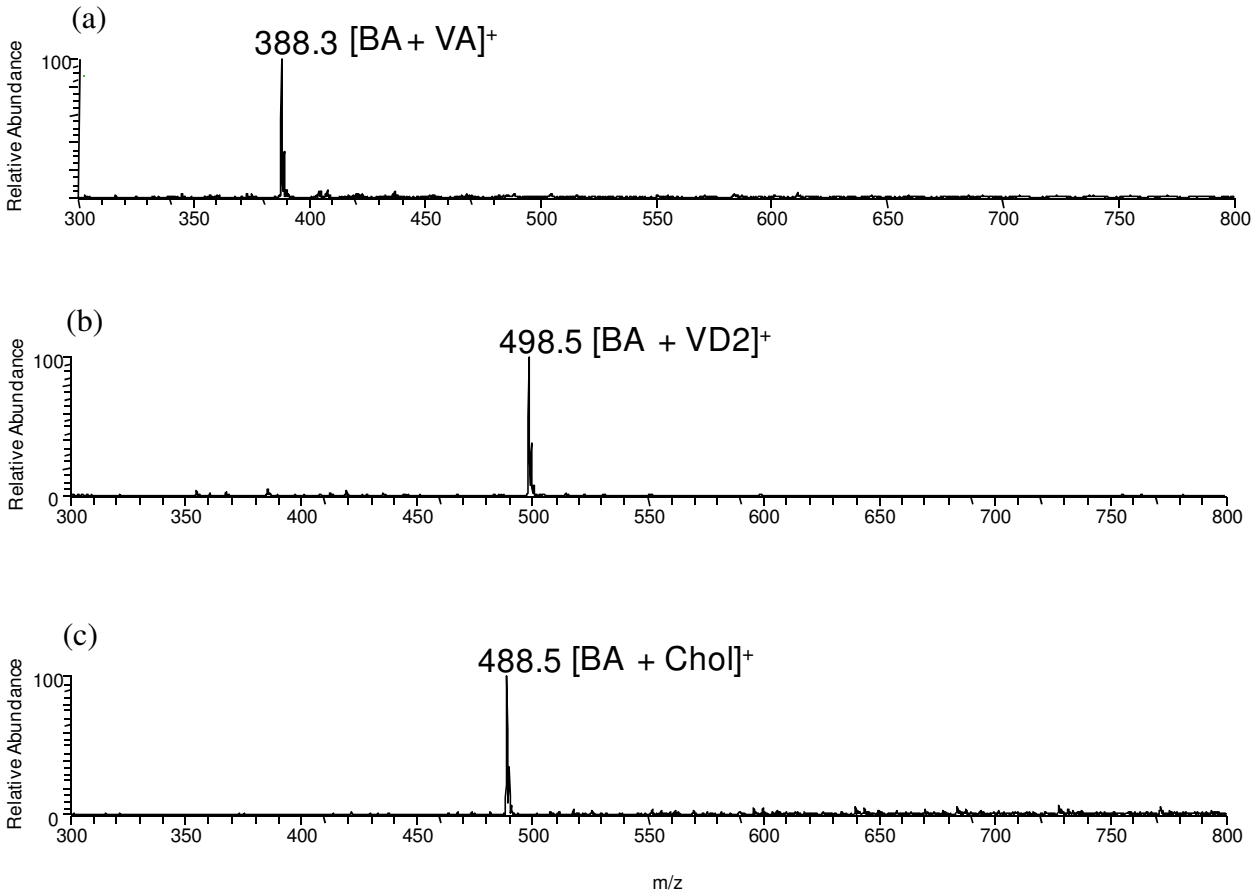


Figure S1. Reactive DESI of 1 μL 50 ppm (a) retinol (vitamin A, MW=286.4). (b) ergocalciferol (vitamin D2, MW=396.6). (c) Cholesterol (MW=386.6) on porous polytetrafluoroethylene (PTFE) surfaces. Spray solution of ACN/$CHCl_3$ (1:1) containing 50 ppm betaine aldehyde chloride was used.

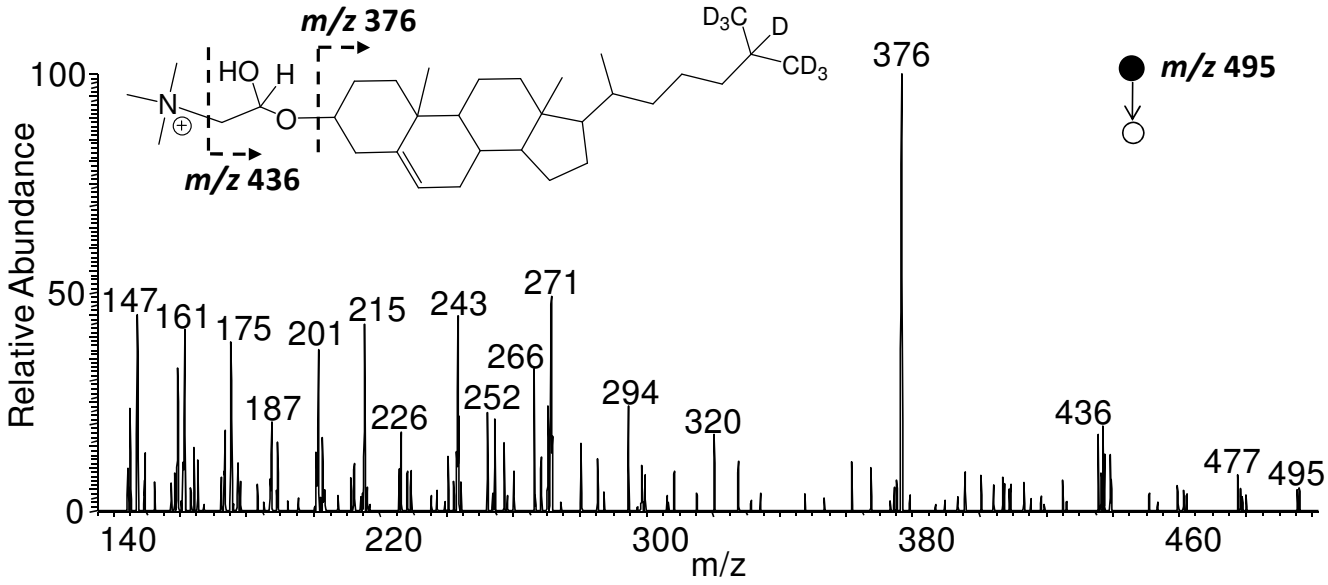


Figure S2. Product ion MS/MS spectrum of the internal standard [BA + Chol-d7]$^+$ at *m/z* 495.

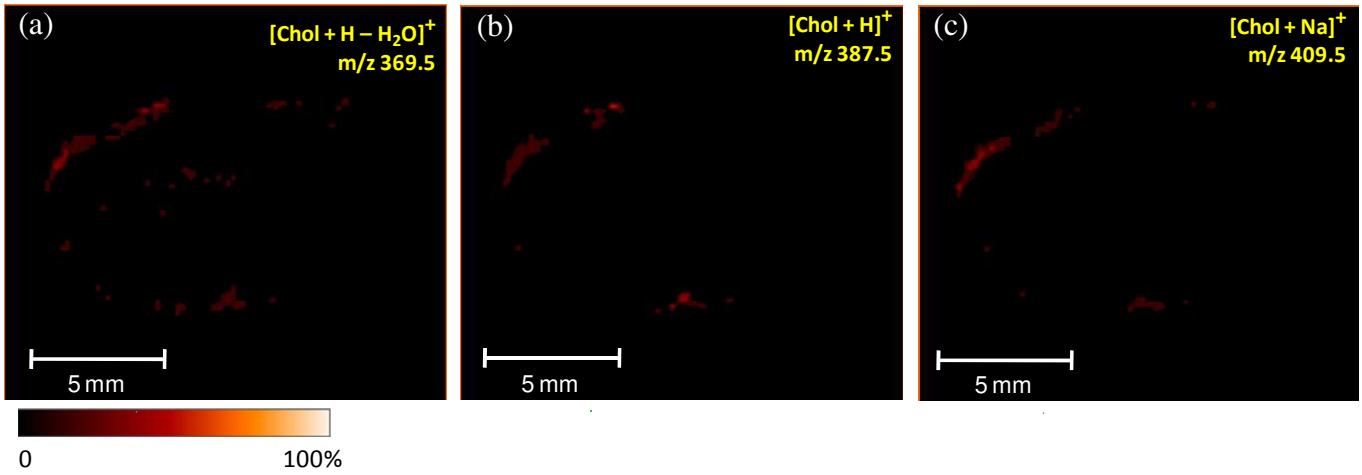


Figure S3. Image of (a) $[Chol + H - H_2O]^+$ (b) $[Chol + H]^+$ (c) $[Chol + Na]^+$ in coronal section of rat brain with normal DESI using a spray solvent of MeOH/H_2O (1:1). Possible cholesterol related peaks: protonated peak [Chol + H]+ (m/z 387), water loss peak [Chol + H – H2O]+ (m/z 369) and sodium adducts [Chol + Na]+ (m/z 409) were not observed. Note the low sensitivity indicated by barely visible images; contrast these data with the reactive DESI data of Figure 4a.

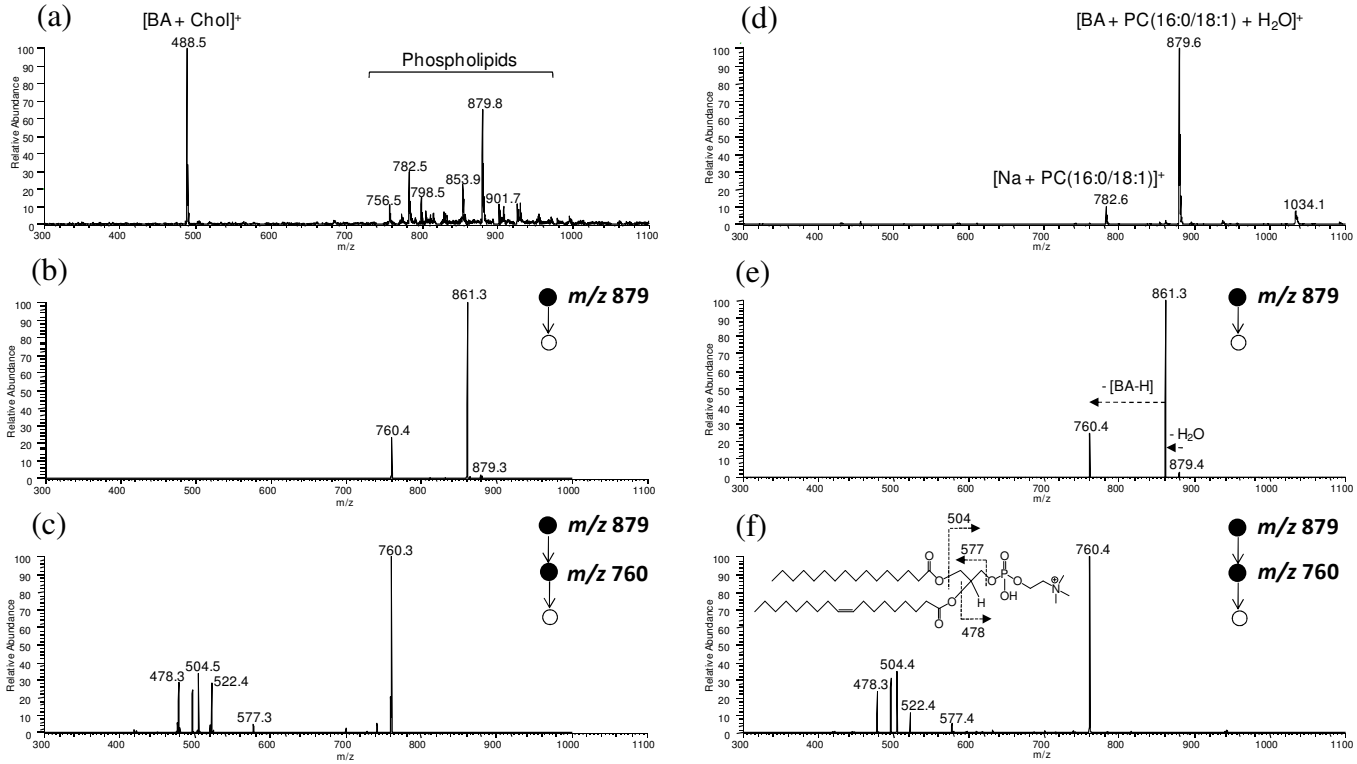


Figure S4. (a) Typical reactive DESI mass spectrum of rat brain tissue.(b) MS^2 and (c) MS^3 of *m/z* 879 observed in the reactive DESI mass spectrum of rat brain tissue. (d) Reactive DESI mass spectrum of the standard lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC 16:0/18:1). (b) MS^2 and (c) MS^3 of *m/z* 879 observed in the reactive DESI mass spectrum of PC 16:0/18:1. A solvent of ACN/H_2O/DMF (8:3:1) doped with 65 ppm BA was used in each case.

As shown in Figure S4d, the standard lipid PC (16:0/18:1) reacts with BA and water to form a product at *m/z* 879 in reactive DESI. MS^2 of *m/z* 879 indicates loss of water and BA in Figure S4e. MS^3 of *m/z* 879 in Figure S4f shows that *m/z* 760 is corresponding to protonated PC (16:0/18:1). Figure S4a is a reactive DESI mass spectrum of rat brain tissue, from which *m/z* 879 is isolated and fragmented with tandem mass spectrometry. MS^2 and MS^3 of this peak (Figure S4b and S4c) match exactly with that of the standard PC (16:0/18:1) in Figure S4e and S4f. The tandem mass data prove that *m/z* 879 observed in the reactive DESI of rat brain tissue is due to the reaction of PC with BA and water. Assignments of other peaks between the mass range *m/z* 850 to 1000 in Figure S4a were proved the same way. The possible structure the product ion [BA + PC (16:0/18:1) + H_2O]$^+$ is the covalent phosphate shown in Scheme S1. The reaction possibly occurs between the phosphate group and BA, and the phosphorus electrophile induces the nucleophilic addition of water through intermolecular electronic induction.

Direct Analysis of Vitamin D3 Tablets and Cod Liver Oil

Reactive DESI using the BA reagent was also tested on vitamin D3 tablets and cod liver oil. Again, no sample preparation was needed before analysis. Cod liver oil is known as a good source of vitamin A and vitamin D. The cod liver oil purchased contains about 320 µg/ml vitamin A and 2 µg/ml vitamin D, but detailed information about the types of vitamins and cholesterol was not available. Figure S5a shows the BA derivatized vitamin D3 at m/z 486.5 and the BA derivatized cholesterol at m/z 488.6 in cod liver oil using reactive DESI. However, retinol (a form of vitamin A) expected at *m/z* 388.3 was not observed. This could be because retinol is not the major vitamin A source due to its instability. So, other forms like retinyl acetate or palmitate are usually produced and administered.[1] Figure S5b shows reactive DESI on a vitamin D3 tablet (containing 10 µg vitamin D3 in a 400 mg tablet) after removal of the coating. Vitamin D in the form of cholecalciferol (vitamin D3) was successfully observed at *m/z* 486.5 (S/N >8) and confirmed by tandem mass spectrometry (Figure S6).

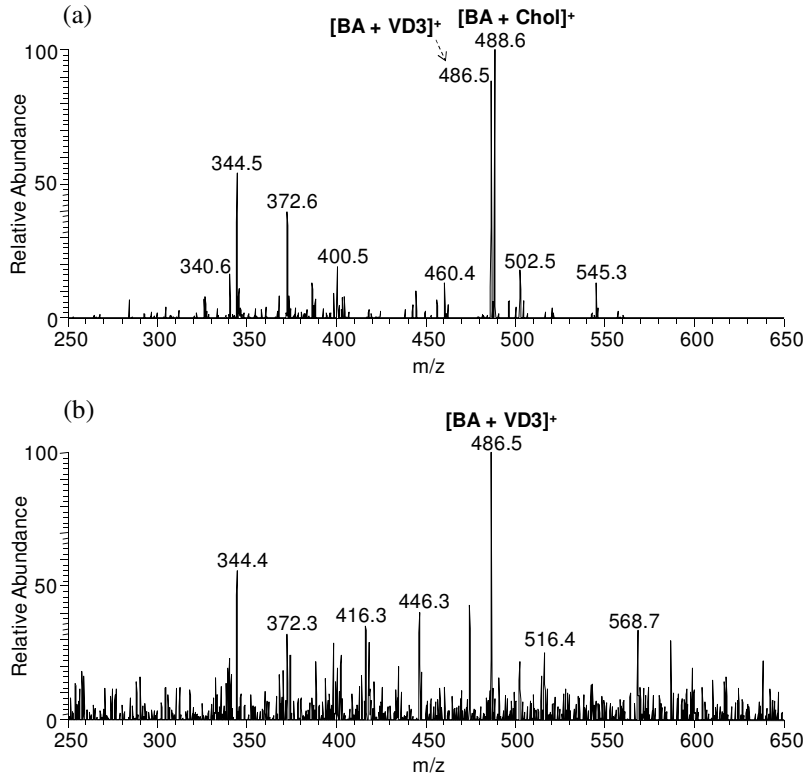


Figure S5. Reactive DESI of (a) cod liver oil (b) vitamin D3 tablet recorded using ACN/$CHCl_3$ (1:2) doped with 50 ppm BA.

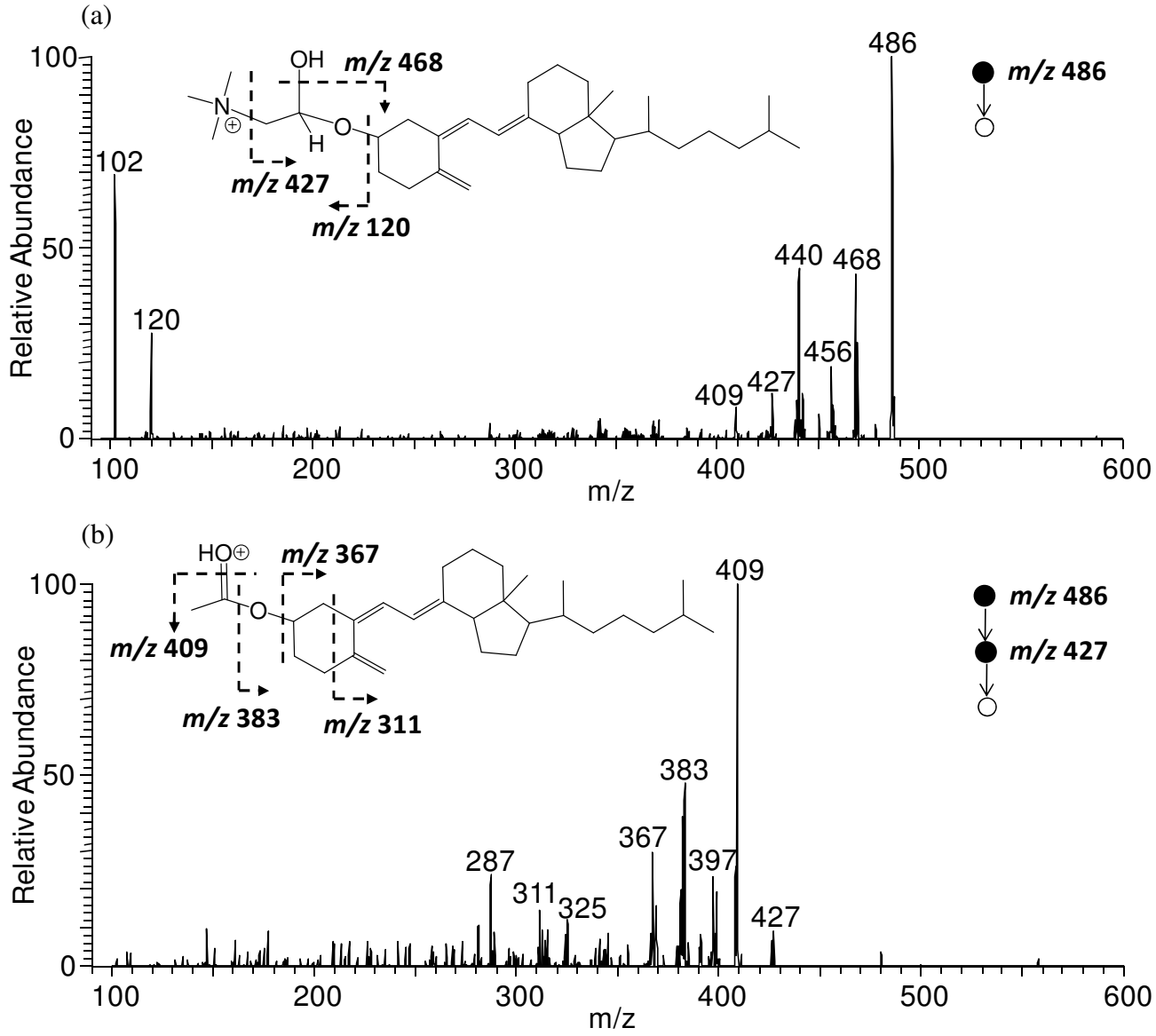
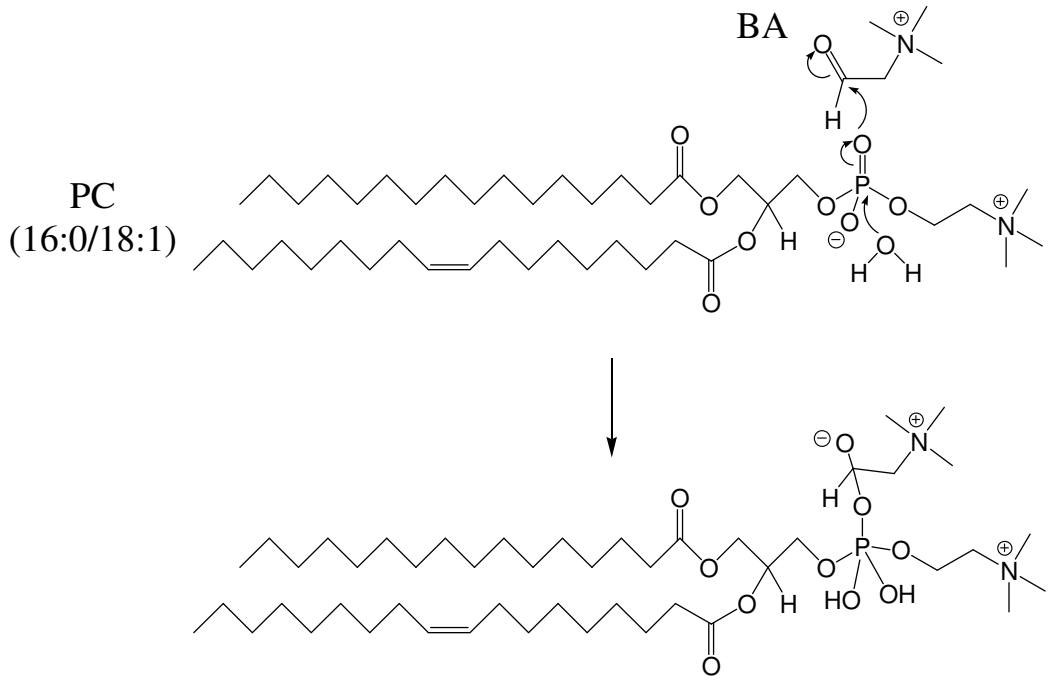


Figure S6. (a) MS^2 (b) MS^3 of of *m/z* 486 observed in the reactive DESI of vitamin D3 tablet.

Scheme S1. Plausible estimation of the product structure of PC (16:0/18:1) reacting with BA and water.



$[BA + PC\ (16:0/18:1) + H_2O]^+$ *m/z* 879.7

Table S1. Assignments of lipids detected from reactive DESI of rat brain tissue in positive ion mode.

Mass-to-charge ratio (*m/z*)	Corresponding ions	Lipid[a]
756.5	$[M + Na]^+$	16:0/16:0 PC
772.5	$[M + K]^+$	16:0/16:0 PC
782.5	$[M + Na]^+$	16:0/18:1 PC
798.5	$[M + K]^+$	16:0/18:1 PC
804.6	$[M + Na]^+$	36:4 PC
820.6	$[M + K]^+$	36:4 PC
810.7	$[M + Na]^+$	36:1 PC
826.7	$[M + K]^+$	36:1 PC
853.9	$[M+BA+H_2O]^+$	16:0/16:0 PC
879.8	$[M+BA+H_2O]^+$	16:0/18:1 PC
901.7	$[M+BA+H_2O]^+$	36:4 PC
907.8	$[M+BA+H_2O]^+$	36:1 PC

[a] (X:Y) represents the different number of carbon atoms and the different number of double bonds in the fatty acid chains. (X1:Y1/X2:Y2) indicates 2 fatty acid chains, and the number of carbon atoms and double bonds in each chain.

REFERENCES

(1) Murphy, P. A. *Food Technol.* **1996**, *50*, 69-74.