Supporting Information For

Using Ambient Ozone for Assignment of Double Bond Position In Unsaturated Lipids

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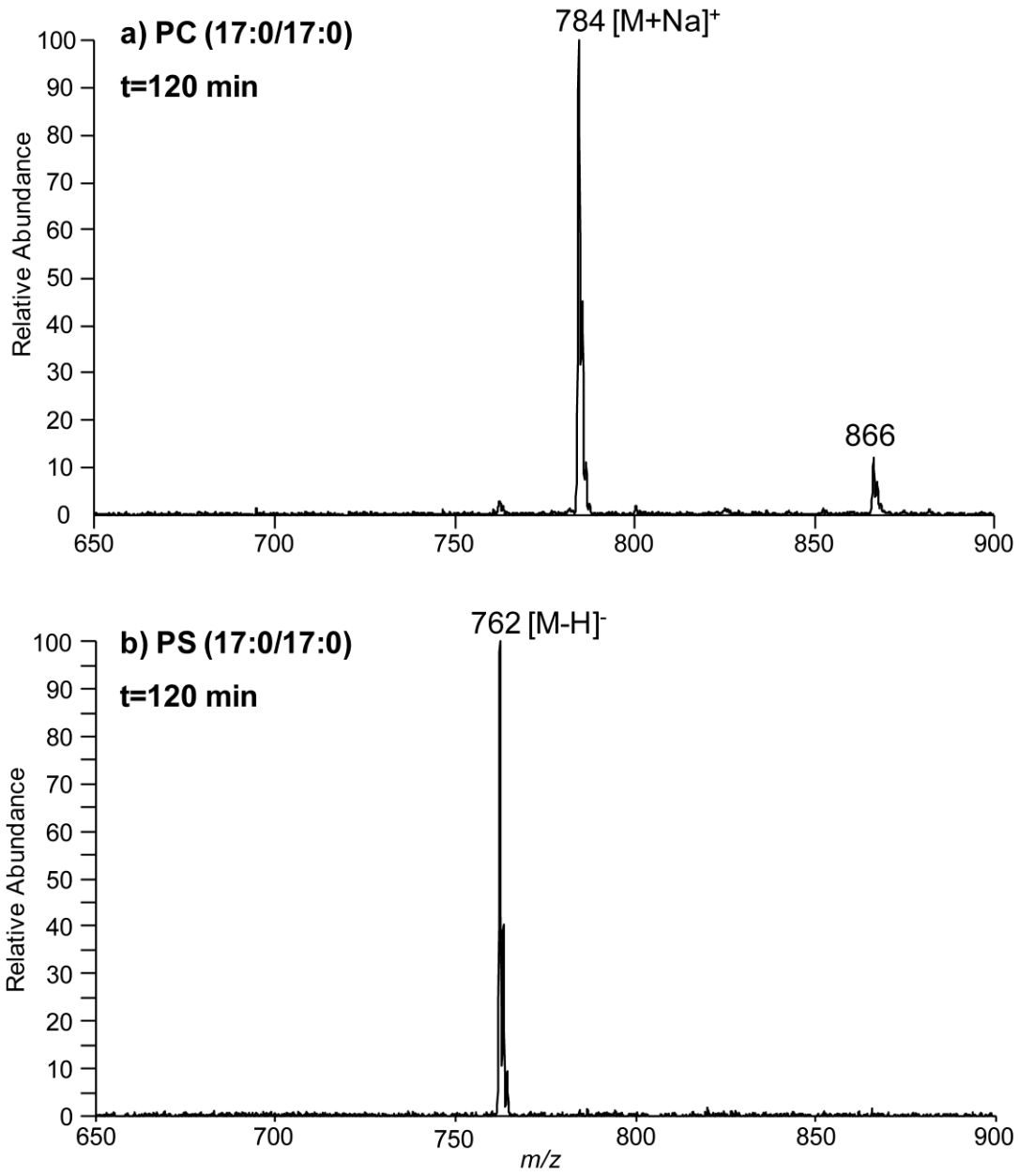

Figure S1. (a) Positive ion DESI-MS spectrum acquired from PC (17:0/17:0) and (b) negative ion DESI-MS spectrum acquired from PS (17:0/17:0) following deposition onto PTFE coated slide and exposure to laboratory air for 2 hours prior to analysis. As expected no ozonolysis products are observed for these saturated lipids.

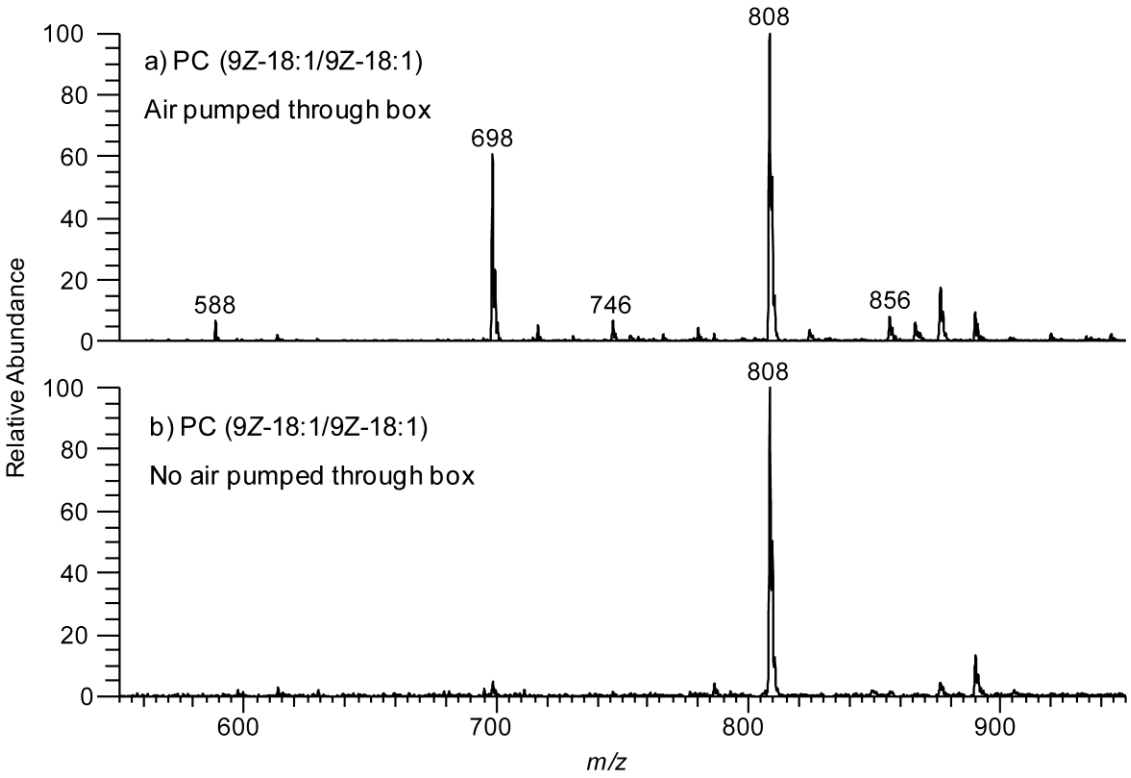

Figure S2. DESI-MS spectra acquired from PC (9Z-18:1/9Z-18:1) deposited onto PTFE slides and placed in an environmental chamber sealed to external air with (a) ambient air continuously pumped through the box for 1 hour and (b) no air pumped through box for 1 hour prior to analysis.

Analysis of the CID spectrum of $[M+Na+2O_3-C_9H_{18}O_2]^+$ formed from [PC (9*Z*-18:1/9*Z*-18:1)+Na]$^+$

The CID spectrum of the $[M+Na+2O_3-C_9H_{18}O_2]^+$ at *m/z* 746 (Figure S3) reveals an reveals two ions at *m/z* 588 and 614 corresponding to the aldehyde and Criegee ions formed following further oxidative cleavage of the intact 9*Z*-18:1 fatty acid chain, thus providing strong evidence for the assignment of this ion as an ozonide. This *m/z* 588 ion, assigned as the di-aldehyde species, is identical to the *m/z* 588 ion observed in the DESI-MS spectrum in Figure 1(a).The ions at *m/z* 687, 529 and 545 correspond to the loss of $N(CH_3)_3$ from the aldehyde and Criegee ions, respectively, while the *m/z* 700 ion corresponds to the loss of formic acid (*cf. m/z* 856). The *m/z* 559 ion is assigned to the decomposition of the secondary ozonide via an identical mechanism to the formation of the *m/z* 669 ion following CID of the $[M+Na+O_3]^+$ ion. Further evidence for the assignment of *m/z* 588 and 746 as products originating from ozonolysis of both fatty acid chains is provided by their presence in the CID spectrum of $[M+Na+2O_3]^+$ observed at *m/z* 904 in Figure 1(a) (data not shown).

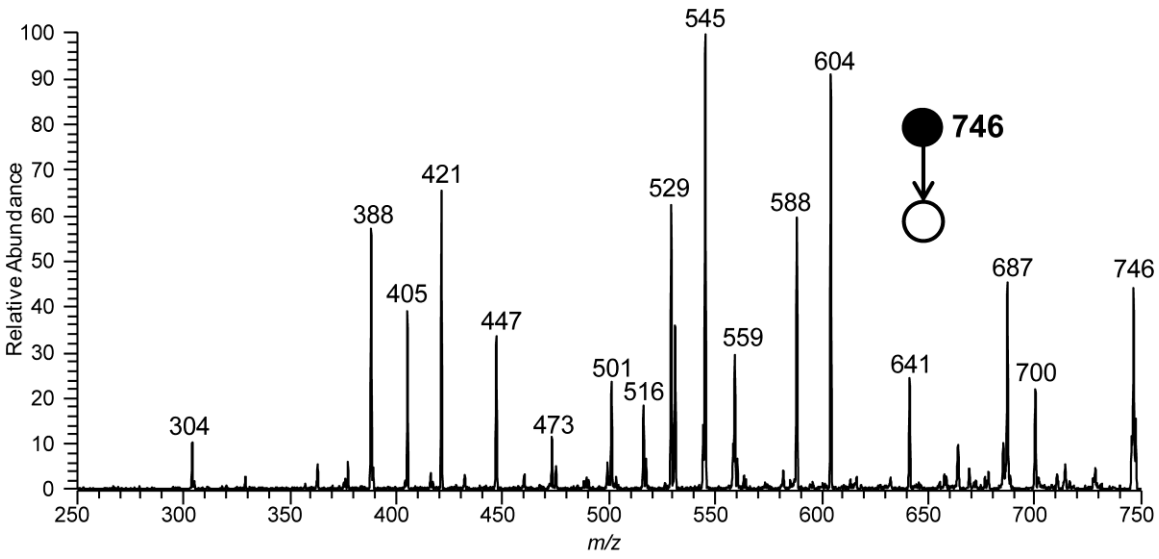

Figure S3. CID spectrum of the $[M+Na+2O_3\text{-}C_9H_{18}O_2]^+$ ion formed following ambient ozonolysis of PC (9*Z*-18:1/9*Z*-18:1) deposited onto a PTFE coated slide.

Assignment of additional PC lipids observed by TLC/DESI-MS of human lens extract

The ion at *m/z* 810 in Figure 5(b) is assigned as the $[M+Na]^+$ ion of PC (18:0/18:1) which represents *ca*. 8% of total lens PC.[S1] Upon ozonolysis this lipid would be expected to exhibit ions at *m/z* 700, 732, 728 and 760 corresponding to the aldehdye and hemiacetal ions formed from *n*-9 and *n*-7 double bonds. The *m/z* 760 is present in the DESI spectrum at very low abundance, while the ions at *m/z* 700 and 732 are assigned as ozonolysis products of the more abundant PC (16:0/18:1) and *m/z* 728 can also be assigned as the $[M+Na]^+$ ion of the known lens lipids PC (14:0/16:0).[S1] However, it is likely that a small fraction of these ion populations are due to ozonolysis of PC (18:0/18:1). the DESI spectrum also reveals an ion at *m/z* 768 which could correspond to the sodiated ion of the ether lipid, PC (16:0e/18:1).[S1] The low mass ions at *m/z* 758, 690, 686 and 718 can then be tentatively assigned as the aldehdye and hemiacetal ions following ozonolysis of the isomeric lipids PC (16:0e/9*Z*-18:1) and PC (16:0e/11*Z*-18:1), respectively.

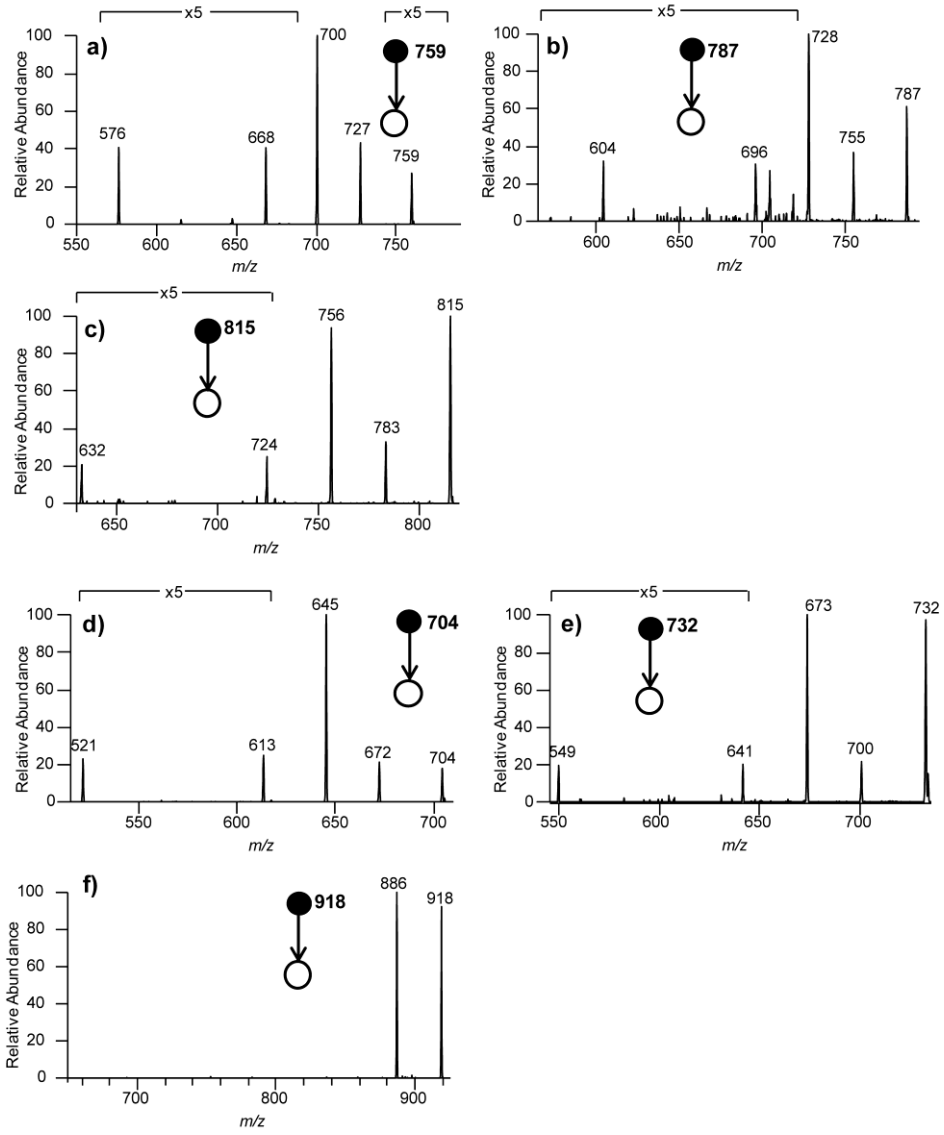

Figure S4. CID spectra of the hemiacetals generated following TLC separation and ambient ozonolysis of a human lens lipid extract formed from (a) [SM (d18:0/15Z-24:1)+Na]$^+$, (b) [SM (d18:0/17Z-24:1)+Na]$^+$, (c) [SM (d18:0/19Z-24:1)+Na]$^+$, (d) [PC (16:0/9Z-18:1)+Na]$^+$, (e) [PC (16:0/11Z-18:1)+Na]$^+$ and (f) [LacCer (d18:0/15Z-24:1)+Na]$^+$. In all cases a loss of methanol (-32 Da) is observed confirming the assignment of these ions as hemiacetals and thus confirming they are ozonolysis products.

References

(S1) Deeley, J. M., Mitchell, T. W., Wei, X., Korth, J., Nealon, J. R., Blanksby, S. J., Truscott, R. J. W., *Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids* **2008**, *1781*, 288-298.