Supporting Online Material for

Lysophosphatidylcholine is Generated by Spontaneous Deacylation of Oxidized Phospholipids

*Jaewoo Choi, Wujuan Zhang, Xiaodong Gu, Xi Chen, Li Hong, James M. Laird and Robert G. Salomon**

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio, 44106, USA

All solvents were distilled under a nitrogen atmosphere prior to use, and all materials were obtained from Aldrich unless specified. Flash chromatography was performed using ACS grade solvents from Fisher Scientific (Hanover Park, IL). R_f values are quoted for TLC plates of thickness 0.25 mm from Whatman (Florham Park, NJ). Plates were visualized using iodine, dinitrophenylhydrazine, or phosphomolybdic acid reagent. Flash column chromatography was performed on 230-400 mesh silica gels supplied by Whatman and Sorbent Technologies Inc. (Atlanta, GA).

Table S1. Elution gradient for separating phospholipids by HPLC

Time (min)	CH_3OH (%)	H_2O (%)	Curve
0	85	15	*
6	85	15	6
18	88	12	6
20	100	0	6
36	100	0	6
37	85	15	6
43	85	15	1

Table S2. Optimized parameters for the mass spectrometric detection of phospholipids and lactone derivatives

	AA-PAF, LA-PAF	HOHA-PC, HOOA-PC, HODA-PC, KOHA-PC oxPCs-furan	DNPH derivs
Ion mode	positive	positive	negative
Capillary (kV)	5.00	5.00	4.00
Cone (V)	30	50	35
Hex 1 (V)	30	50.0	30.0
Aperture (V)	0.0	0.0	0.0
Hex 2 (V)	1.0	1.0	1.0
LM 1 resolution	15.0	15.0	15.0
HM 1 resolution	15.0	15.0	15.0
Ion energy 1	1.0	1.0	1.0
LM 2 resolution	15.0	15.0	15.0
HM 2 resolution	15.0	15.0	15.0
Ion energy 2	2.0	2.0	2.0

Table S3. MRM transition ion pair and optimal collision energy for the analytes

Analytes	MRM (m/z) transition ion pair	Collision Energy (eV)
KOHA-PC	634 > 184	35
AA-PAF	745 > 184	24
LA-PAF	678 > 184	24
LysoPAF	510 > 184	24
HOHA-PC	636 > 184	40
HOOA-PC	651 > 184	35
HODA-PC	706 > 184	35
oxPC-furan (2)	618 > 184	40
oxPC-furan (3)	632 > 184	20
oxPC-furan (7)	688 > 184	20
LysoPC	496 > 184	30
HOHA lactone-DNPH	319 > 152	18
HOOA lactone-DNPH	333 > 152	14
Cinnam-DNPH	311 > 181	24

Figure S1

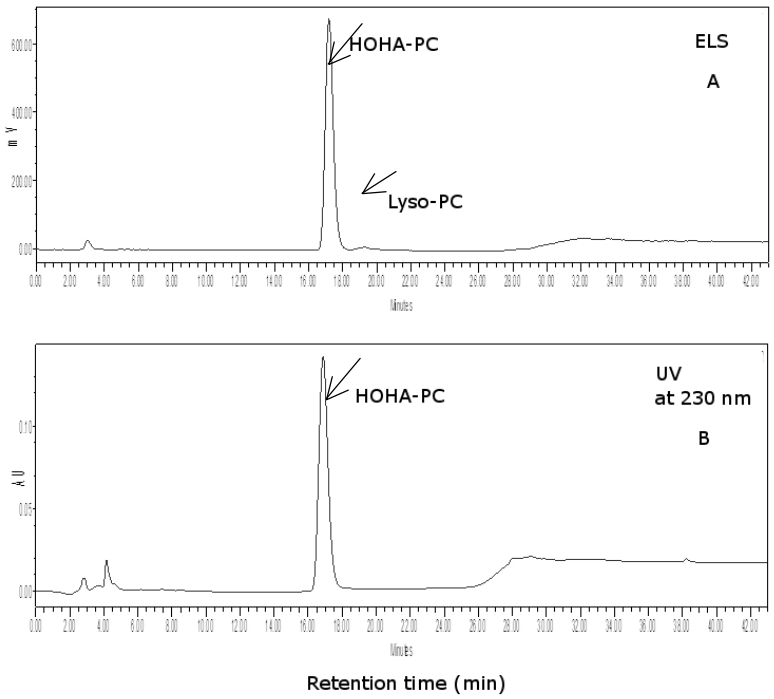

Figure S1. HPLC chromatogram of authentic HOHA-PC containing traces of lysoPC with (A) ELS or (B) UV detection.

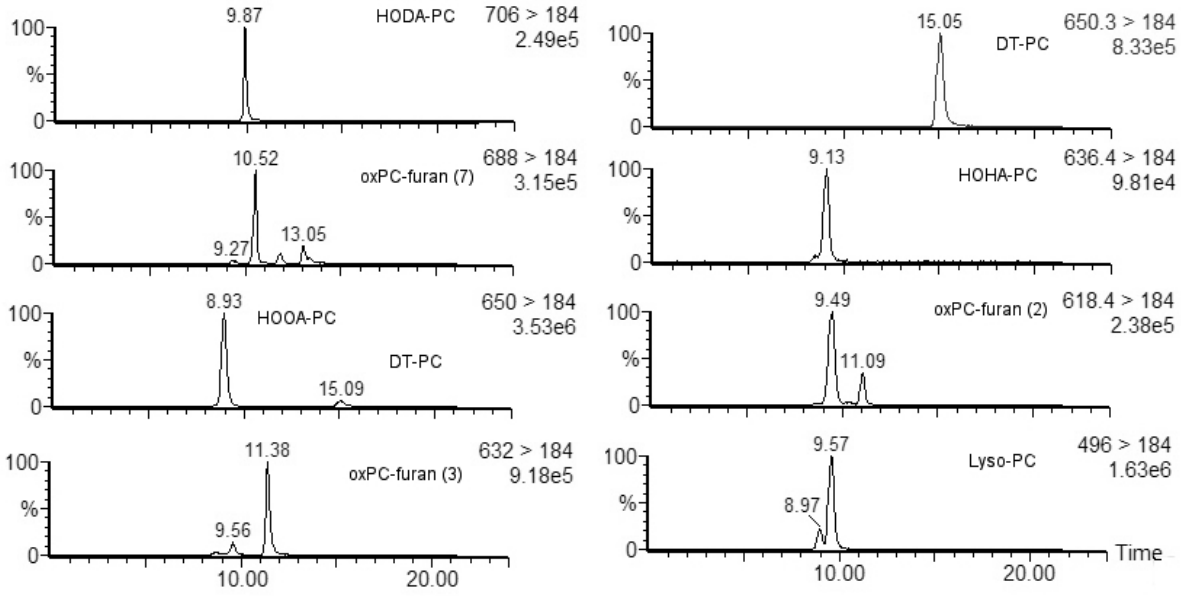

Fig S2. MRM HPLC chromatogram of phospholipids

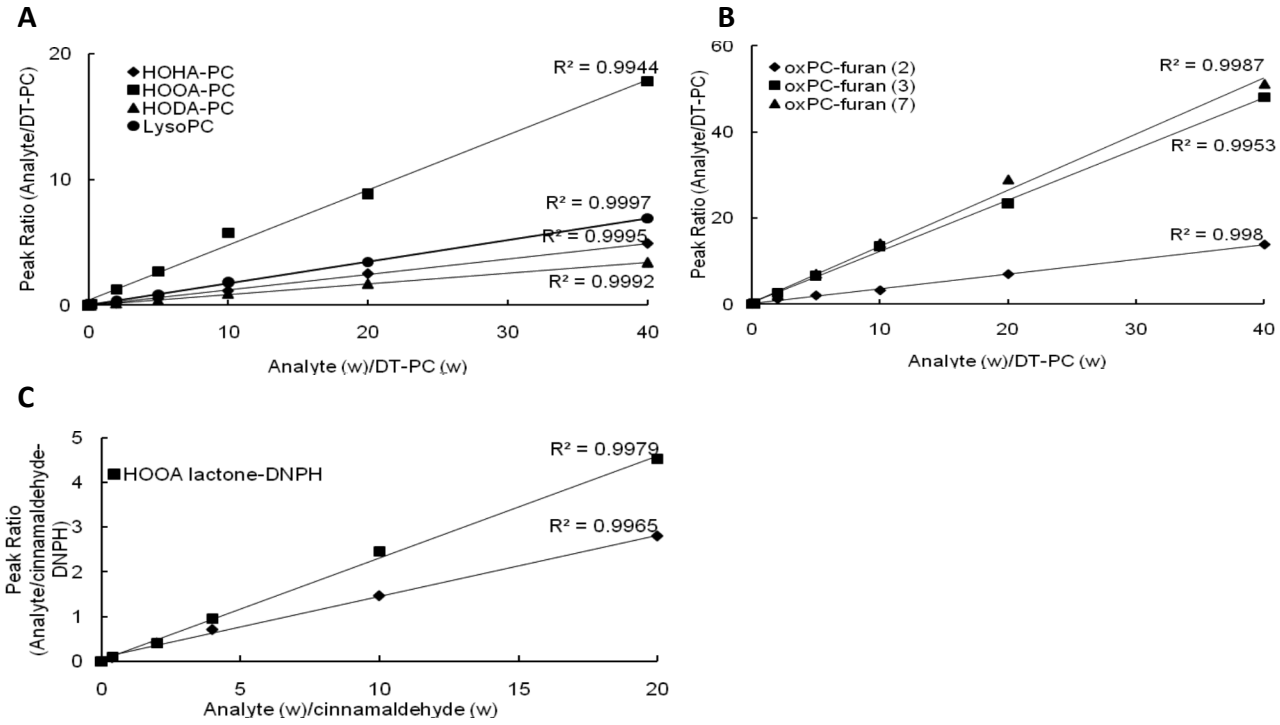

Fig S3. LC/ESI/MS/MS calibration curves of lysoPC, oxPC-furan, HOHA lactone-DNPH and HOOA lactone-DNPH and their precursors. Calibration curves for quantitative analyses of (A) and (B) were constructed by adding a 50 ng of internal standard DT-PC into various amounts of the indicated authentic synthetic phospholipids prior to extraction and LC/MS/MS analysis. In the case of (C), a constant amount of internal standard, cinnamaldehyde, was added before incubation procedure.