The Reproducibility of Phospholipid Analyses by MALDI-MSMS: Supporting Information

Catherine J. Pridmore,[a] Jackie A. Mosely*[a] and John M. Sanderson*[a]

Mass Isolation Windows for MSMS

Table S1 Summary of precursor ion isolation windows for MSMS analyses.[a]

	$[POPC + H]^+$	$[POPE + H]^+$	$[POPC + Li]^+$	$[POPE + Li]^+$
Manual LIFT	± 11	± 11	+ 11, – 5	+ 5, – 5
Automated LIFT	± 15	± 14	+ 9, – 5	+ 6, – 7
Automated CID	± 8	n/a	± 8	n/a

[a] isolation windows are given in Da.

MALDI-MS Spectra of POPC and POPE

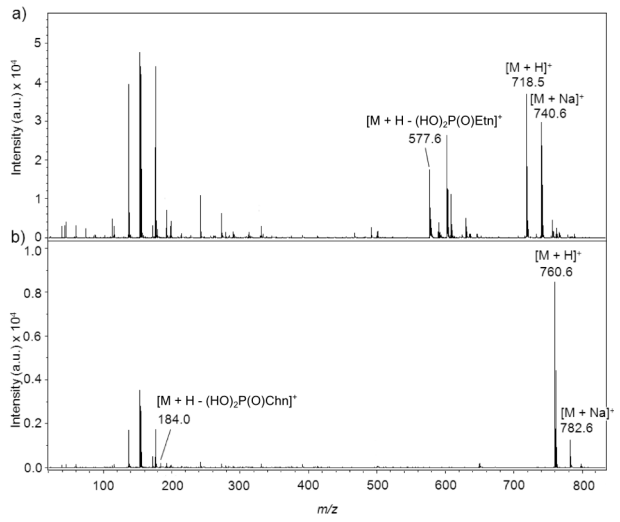

Fig. S1 MALDI-MS spectra of a) POPE b) POPC showing molecular ions and fragments observed. Each sample was prepared (in the absence of lithium) by mixing a solution of the lipid in $CHCl_3$ (1 mg/ml) 1:9 (*v/v*) with a solution of DHB (30 mg/mL) in EtOH/H_2O (50 % *v/v*).

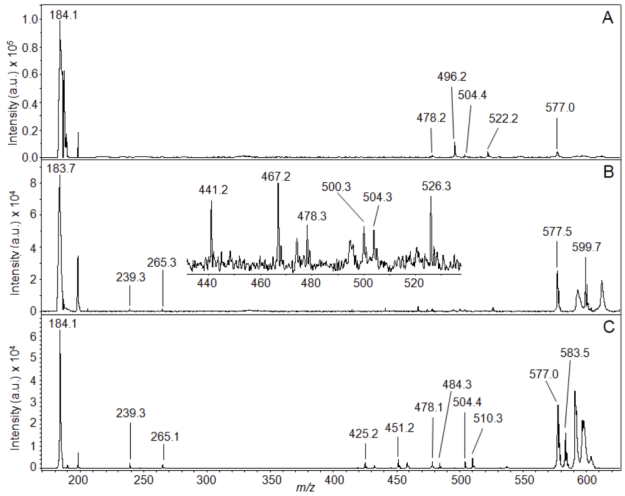

Fig. S2 MALDI-MSMS spectra of A) $[POPC + H]^+$; B) $[POPC + Na]^+$; and C) $[POPC + Li]^+$. The samples were prepared by mixing a solution of the lipid in $CHCl_3$ (1 mg/mL) 1:9 (*v/v*) with solutions of DHB (30 mg/mL) in EtOH/H_2O (50:50 (*v/v*)), or NaCl (100 mM) or LiCl (100 mM) in EtOH/H_2O (50:50 (*v/v*)) respectively.

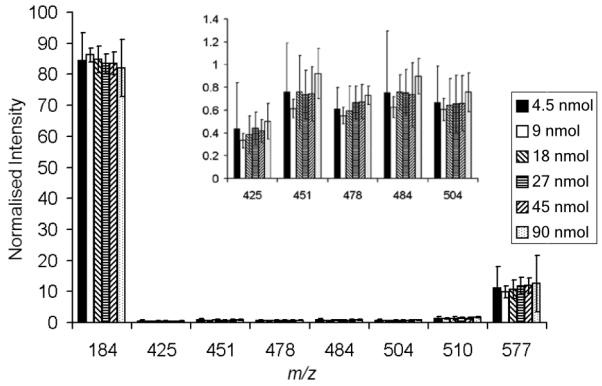

Fig. S3 Normalized intensities of $[POPC + Li]^+$ fragment peaks with varying amounts of lithium chloride per target spot (4.5, 9, 18, 27, 45, 90 nmol) and fixed amounts of DHB (0.2 μmol) and lipid (0.2 nmol). Error bars represent 2× the standard deviation from 8 repeat scans in each case.

MALDI-MS Data for OPPC

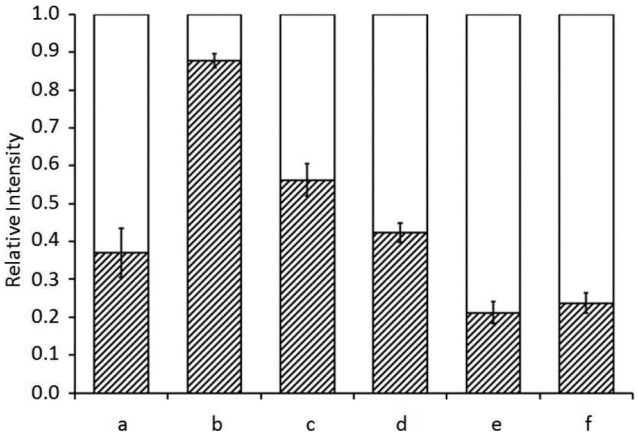

Fig. S4 Relative peak intensities for loss of acyl chain fragments from the *sn*-1 and *sn*-2 positions of OPPC in MALDI-MSMS spectra of $[OPPC + H]^+$ and $[OPPC + Li]^+$. The white sections correspond to a loss from *sn*-1 position, shaded to the *sn*-2 position. Refer to Table S2 for identification of *a-f*. The error bars correspond to 2× the standard deviation of the data.

Table S2 Product ions corresponding to the loss of the acyl chain from the *sn*-1 or *sn*-2 positions of OPPC in MALDI-MSMS spectra of $[OPPC + H]^+$ and $[OPPC + Li]^+$. The relative intensities of peaks corresponding to the loss of the acyl chains from the *sn*-1 and *sn*-2 positions have been normalized with respect to one another for each type of fragmentation.

	Parent Ion	Fragment[a]	RI, x = 1[b,c]	RI, x = 2[b,c]
a	$[M + H]^+$	$[M + H - R_xCO_2H]^+$	0.63 ± 0.06	0.45 ± 0.06
b	$[M + H]^+$	$[M + H - R_x{=}C{=}O]^+$	0.12 ± 0.02	0.88 ± 0.02
c	$[M + H]^+$	$[R_xCO]^+$	0.44 ± 0.04	0.56 ± 0.04
d	$[M + Li]^+$	$[M + Li - R_xCO_2Li]^+$	0.58 ± 0.03	0.42 ± 0.03
e	$[M + Li]^+$	$[M + Li - R_xCO_2H]^+$	0.79 ± 0.03	0.21 ± 0.03
f	$[M + Li]^+$	$[M + Li - (R_xCO_2H + NMe_3)]^+$	0.76 ± 0.03	0.24 ± 0.03

[a] x = 1 and x = 2 correspond the *sn*-1 and *sn*-2 positions of the lipid respectively.

[b] RI = relative intensity.

[c] The error is calculated as 2× the standard deviation from 24 spectra.

CID MSMS Data

Table S3 Product ions corresponding to the loss of the acyl chain from the *sn*-1 or *sn*-2 positions of POPC in the CID MSMS spectra of $[POPC + H]^+$ and $[POPC + Li]^+$. Entries *a-e* correspond to the data in Fig. S5.

	Parent Ion	Fragment
a	$[POPC + H]^+$	$[M + H - R_xCO_2H]$
b	$[POPC + H]^+$	$[M + H - R_x{=}C{=}O]$
c	$[POPC + Li]^+$	$[R_xCO]$
d	$[POPC + Li]^+$	$[M + Li - (R_xCO_2H + NMe_3)]$
e	$[POPC + Li]^+$	$[M + Li - R_xCO_2Li]$

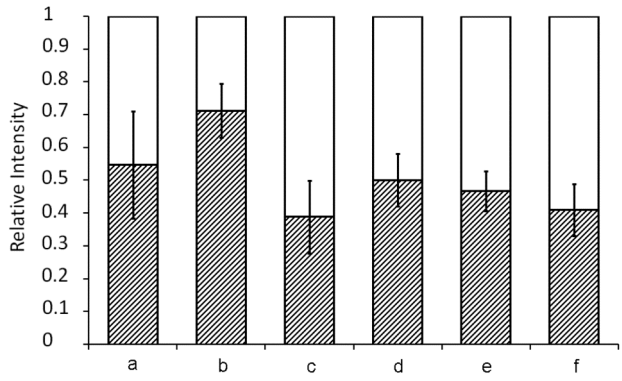

Fig. S5 The relative intensities of peaks corresponding to loss of the acyl chain from the *sn*-1 and *sn*-2 positions of different types of fragments of POPC from CID MSMS spectra of $[POPC + H]^+$ and $[POPC + Li]^+$. The white sections correspond to a loss from *sn*-1 position, shaded to the *sn*-2 position. Refer to Table S3 for identification of *a-e*. The error bars correspond to two times the standard deviation of the data from 25 repeat scans.

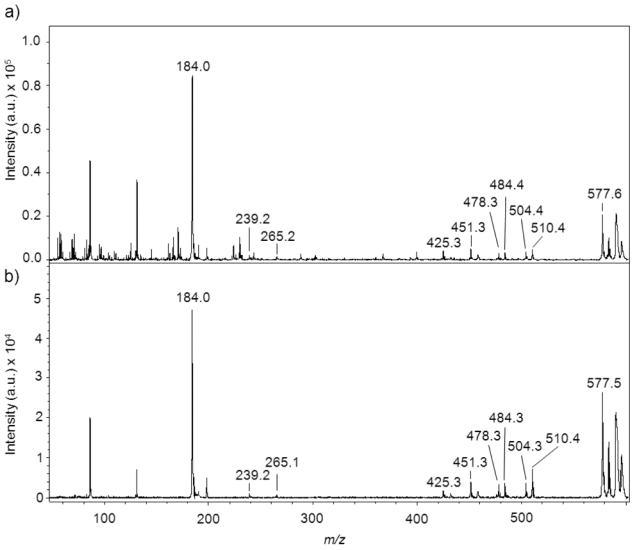

Fig. S6 CID (a) and LIFT (b) spectra of $[POPC + Li]^+$. Ion masses are identified in Table 3 of the main paper.

Notes and references

[a] *Department of Chemistry, Durham University, Durham, DH1 3LE, UK. Fax: +44 (0)191 3844737; Tel: +44 (0)191 3342107; E-mail: j.m.sanderson@durham.ac.uk; jackie.mosely@durham.ac.uk*