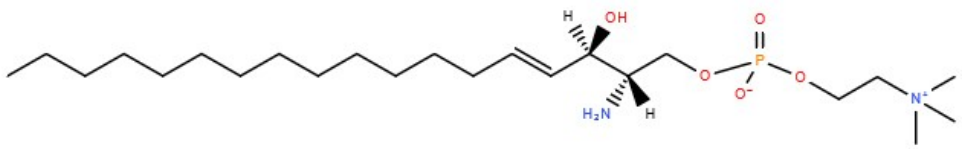
Electronic Supplementary Information for *Anal. Meth.*

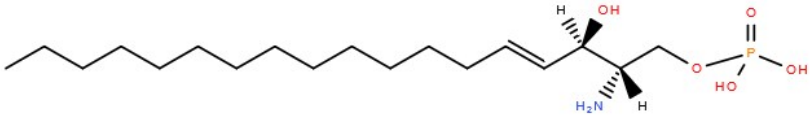
Proton-Propelled Methyl-*tert*-butyl Ether Extraction for High Efficient Mass Spectrometric Analysis of Phospholipid

1) Phospholipid structure:

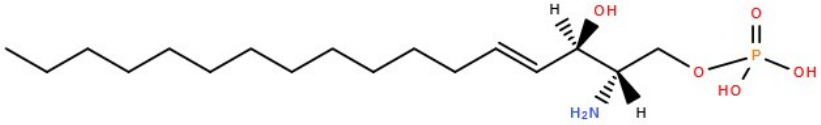
i. Sphingosine-1-phosphocholine (S1PC) $C_{23}H_{49}N_2O_5P$ $[M-H]^-$ 463.3



ii. Sphingosine-1-phosphate (S1P) $C_{23}H_{49}N_2O_5P$ $[M-H]^-$ 378.2



iii. Sphingosine-1-phosphate (C17) (S1P-C17) $C_{22}H_{47}N_2O_5P$ $[M-H]^-$ 364.2



2) Dependence of S1P recovery on acidity

The acidic-MTBE extraction solution with different percentage of formic acid was prepared by series dilution. The acid concentration ranged from 0.0001% to 1% (v/v). The human plasma from same lot # was used as matrices for all MTBE-extractions, following the same protocol described in experiment section. Fig. S1 shows a series of resulting chromatograms of S1P under the different acidity. With a gradual increase of percentage of formic acid, it significantly improves the recovery from human plasma, attaining the maximal efficiency at approximately

0.2% FA. An analogue phospholipid (nominal concentration 100ng/mL) with C17 carbohydrate chain that is not exist in normal human plasma was spiked in matrices as internal standard. The MS chromatograms were outlined in Fig. S2, which exhibited the same propensity on density of protons.

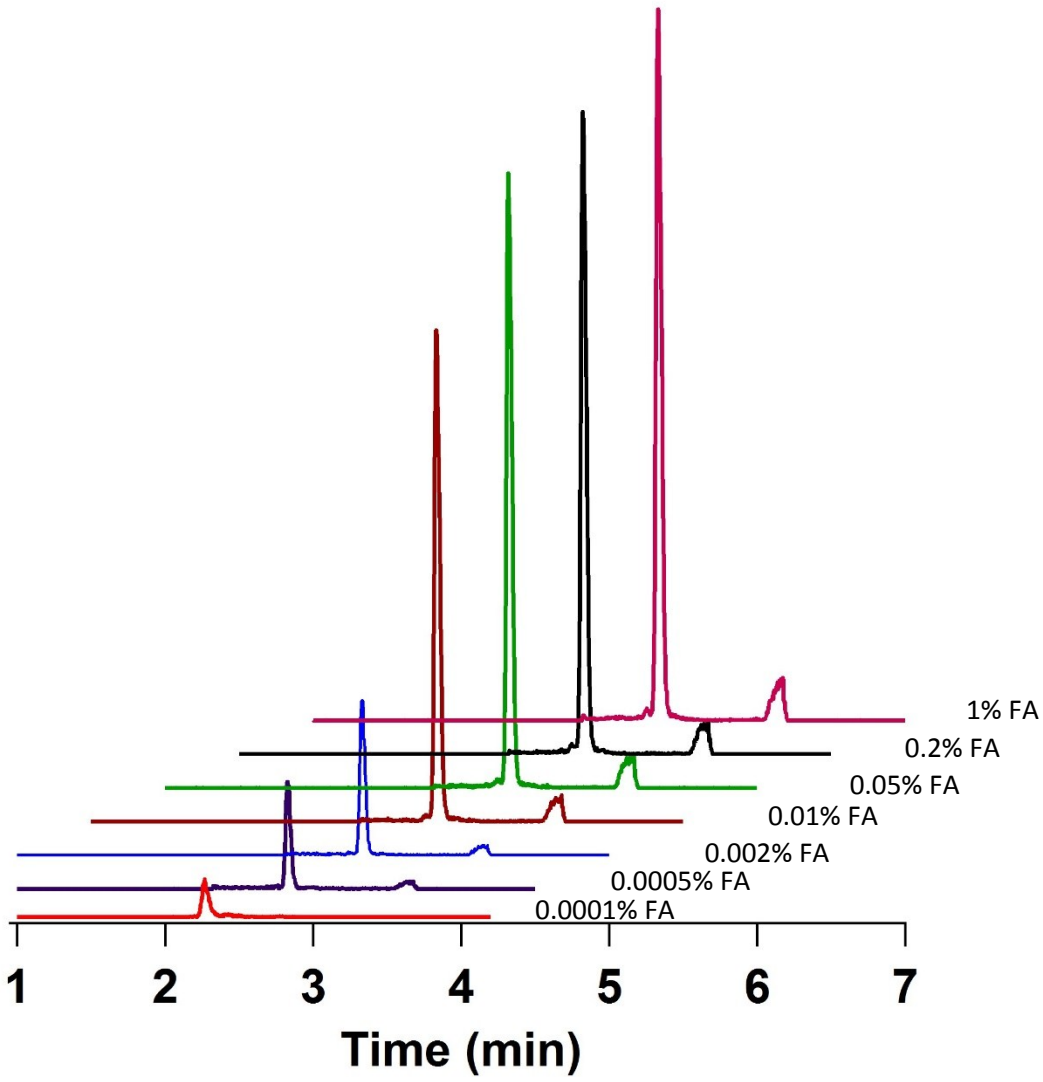


Figure S1. A series of MS chromatogram of extraction of S1P with gradient concentration of formic acid in MTBE, ranged from 0.0001% to 1% (v/v).

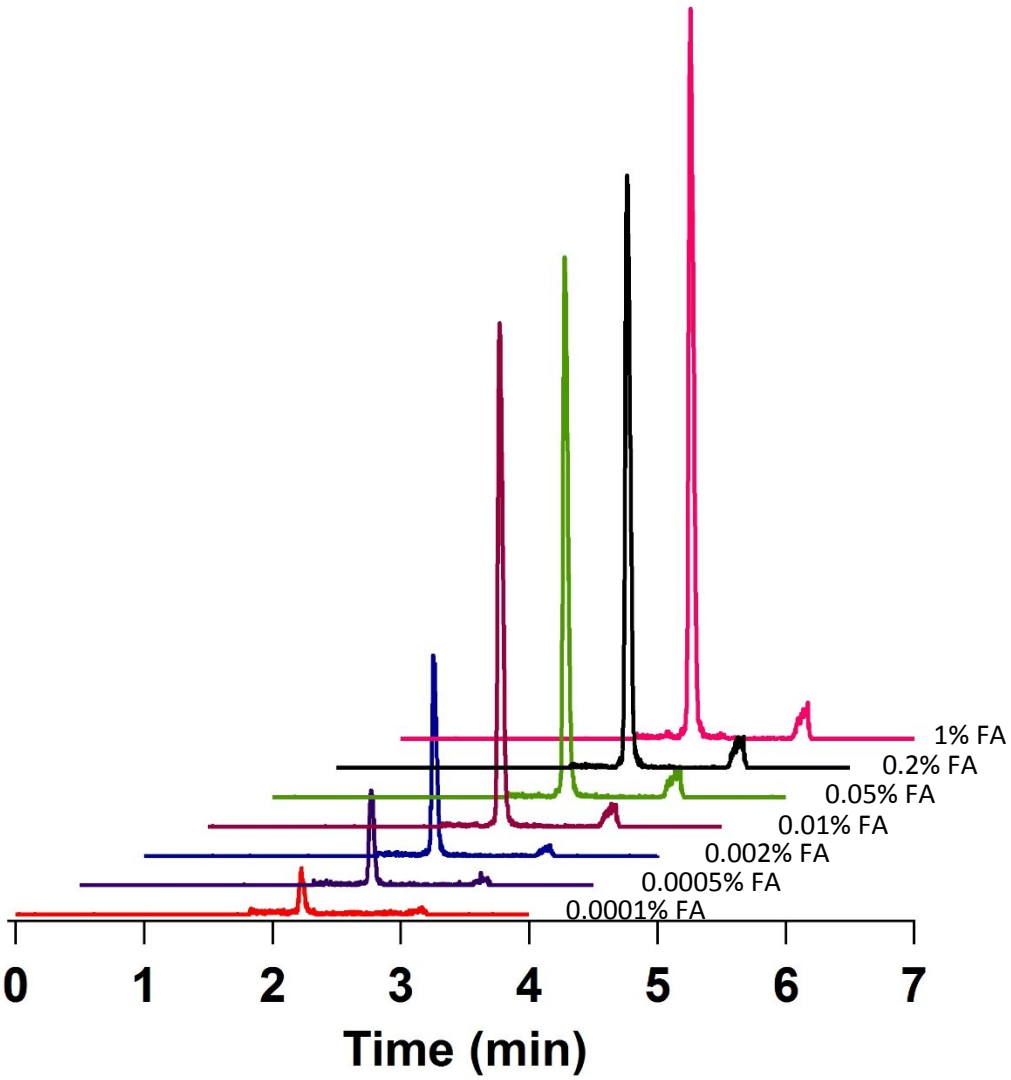


Figure S2. A series of MS chromatogram of extraction of spiked S1P-C17 (nominal, 100ng/mL) with gradient concentration of formic acid in MTBE, ranged from 0.0001% to 1% (v/v).