Supporting information

Miniature Mass Spectrometer-Based Point-Of-Care Assay for Measuring Phosphatidylethanol in Blood

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Analysis of dried blood spot (DBS) samples

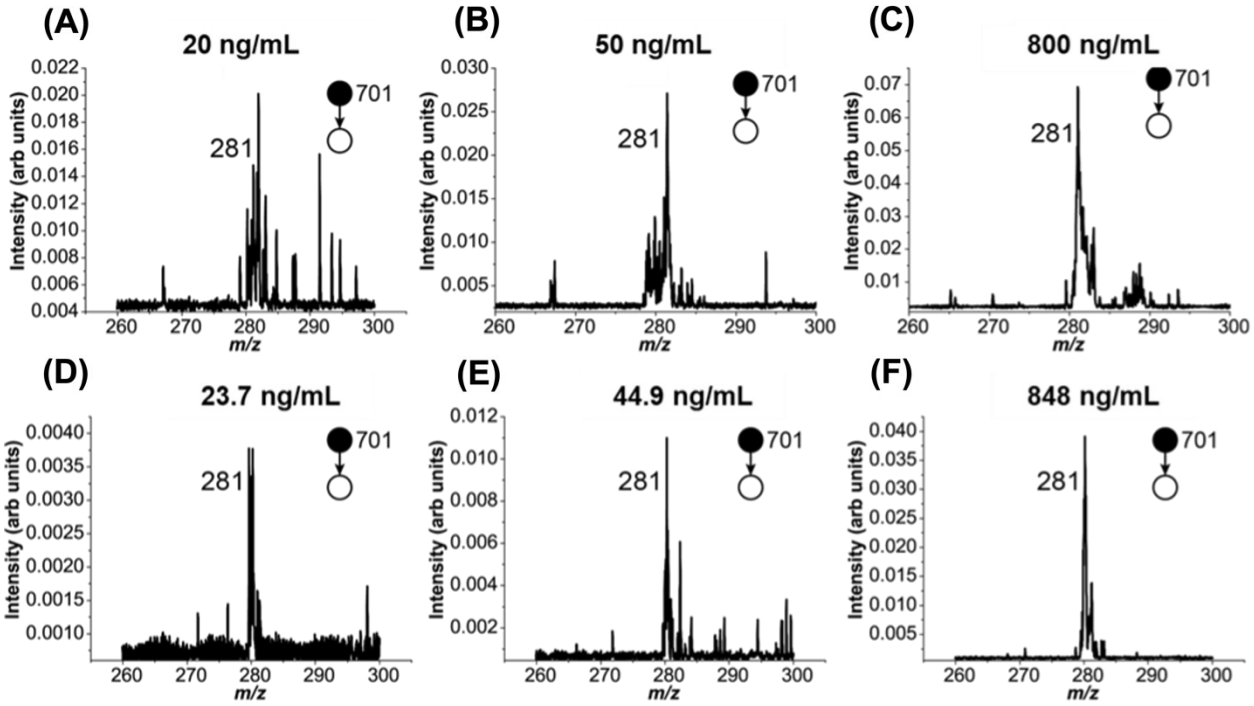

Figure S1. MS/MS spectra of 16:0-18:1 PEth in DBS. (A), (B) and (C) represent data collected with simulated dry blood spot samples containing 20 ng/mL, 50 ng/mL and 800 ng/mL of 16:0-18:1 PEth, respectively; (D), (E) and (F) are spectra collected with extracts of patient DBS samples with 23.7, 44.9 and 848 ng/mL of 16:0-18:1 PEth, respectively.

Challenges encountered in choosing a suitable internal standard

Choosing a suitable internal standard is crucial to the success of a quantitation experiment. The best quantitative performance is obtained by employing isotopic analogues of the analyte, which correct for ion suppression and signal instability due to their structural similarity to the analyte. The portion of the isotopically labelled molecule that contains the internal standard determines the *m/z* of the product ion that is used for quantitation. In an ideal case, the isotopic D or ^{13}C atoms are present in portions of the molecule that are retained in the fragment ions. However, oftentimes, the isotopic label is present in the portion of the precursor ion that is not retained in the MS/MS product ion. This does not pose a problem when quantitation is performed in the MRM mode, where the ion abundance data is recorded as the instrument cycles through each individual transition of the precursor-product ion pair for the analyte and the internal standard. Monitoring *different* channels with MRMs yields high quality results for quantitation experiments with a triple quadrupole, because the dwell time for each transition is usually in the 5-100 ms

range, a time scale which is small enough to correct for any major changes in the electrospray efficiency by dividing the intensity of the analyte with that of the internal standard. On the other hand, when quantitation is performed using the Mini-12, ion abundances of the analyte and the isotopic internal standard should be measured from the *same* scan for obtaining reasonable quantitative performance. While it is certainly possible to monitor ion abundances of the analyte and internal standard in different scans, it is important to note that the discontinuous nature of the ion introduction method used with the Mini-12 system would involve fragmenting precursor ions of the analyte and internal standards arising from different ion packets, which is likely to decrease precision.

The commercially available isotopic internal standard of 16:0-18:1 PEth, has the deuterium labels present on the head-group of the phospholipid-like molecule (Figure S2 (B)), a portion of the precursor ion that was not retained in the fragment ion. To address this issue of interference between product ions of the analyte and the internal standard, three structural analogs of 16:0-18:1 PEth, namely 12:0 phosphatidylethanolamine (12:0 PE), 17:0 phosphatidylethanolamine (17:0 PE) and 16:0 phosphatidylpropanol were evaluated for their potential use as internal standard (structures shown in Figure S2 (C), (D), and (E), respectively).

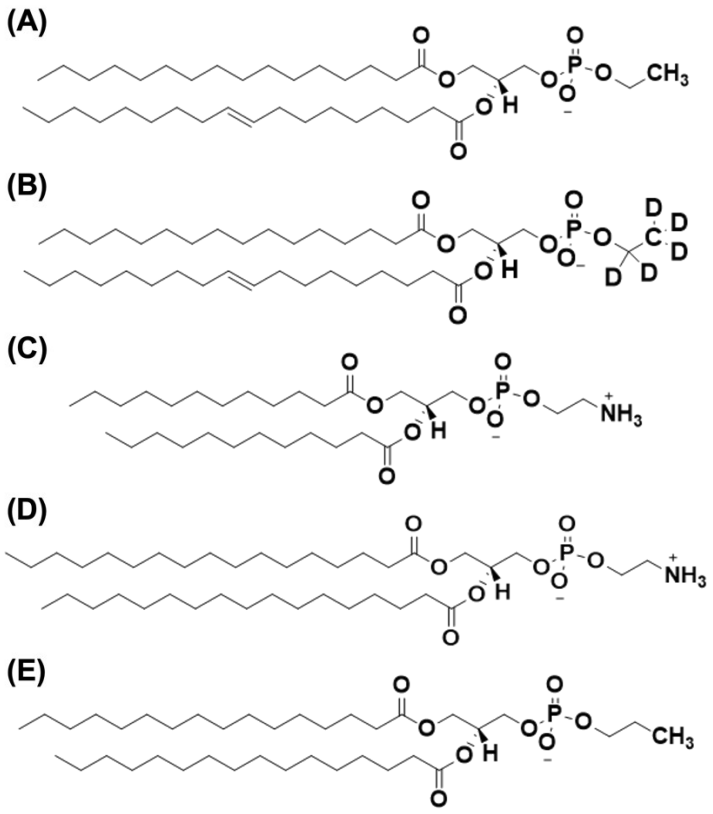

Figure S2. Chemical structures of (A) 16:0-18:1 PEth (B) 16:0-18:1 PEth-d5, (C) 12:0 PE, (D) 17:0 PE, and (E) 16:0 phosphatidylpropanol

Quantitation of 16:0-18:1 PEth with 12:0 PE as internal standard

First, we attempted to quantify 16:0-18:1 PEth (with transition *m/z* 701→281) in whole blood with 12:0 PE (with transition *m/z* 578→396) as internal standard. Large interferences were observed at the quantifier ion of the analyte (product ion at *m/z* 281) when the analyte and internal standard were fragmented in the same scan due to endogenous lipids present in blood. Monitoring these species in different scans gave reasonable quantitative performance (*m/z* 701→281 monitored for analyte and *m/z* 578→396 for internal standard) with R^2=0.9835 and RSD values <30%, as shown in Figure S3. These promising preliminary data encouraged us improve quantitative performance by using a different internal standard so that we could monitor the analyte and internal standard from the same ion packet.

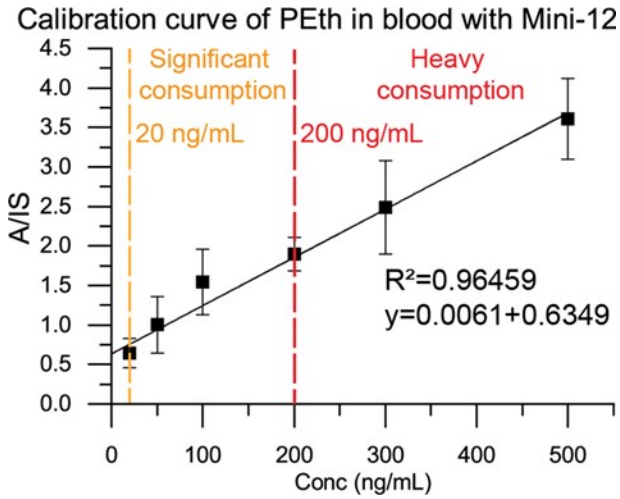

Figure S3. Calibration curve obtained for PEth spiked in blood. Transitions m/z 701→281 and *m/z* 578→396 were used to monitor PEth and 12:0 PE, respectively. Orange and red lines represent the threshold concentrations that distinguish between significant and heavy alcohol consumption. n=4 for each concentration.

We then explored the performance of a non-endogeneous lipid, 17:0 PE (*m/z* 719, product ion at *m/z* 269). While its fragments did not interfere with the analyte response, we faced significant challenges in making a solution of this in solvent systems that were compatible with the extraction of PEth. Additionally, the inter-day responses for the same concentration were not reproducible. Next, we tested the performance of 16:0 phosphatidylpropanol as internal standard. No issues were encountered with solubility, however, the fragment ion of 16:0 phophatidylpropanol at *m/z* 255 overlapped with one of the product ions of 16:0-18:1 PEth. Since the the product ion of 16:0-18:1 PEth at *m/z* 255 was not used as the quantifier, we proceeded by ejecting this product ion before fragmenting the precursor ion of the internal standard. This eliminated interferences from the analyte and enabled us to monitor the analyte (*m/z* 701→281) and internal standard (*m/z* 689→255) in the same scan and is used as the internal standard in the manuscript.