

CONCENTRATED LC-MS/MS KIT FOR PETH ANALYSIS IN WHOLE BLOOD.

Comparable analytical performance with ready-to-use kit.

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INTRODUCTION.

Phosphatidylethanol (PEth) is a highly specific biomarker of alcohol consumption formed only in the presence of ethanol [1]. Unlike indirect biomarkers such as carbohydrate-deficient transferrin (CDT), gamma-glutamyl transferase (GGT) and mean corpuscular volume (MCV), PEth formation is directly dependent on ethanol exposure, resulting in high specificity and improved reliability for assessing alcohol intake [1,2]. Due to its detection window of up to four weeks and strong correlation with alcohol consumption, PEth is an established biomarker for alcohol monitoring, forensic applications and alcohol-related research. Accurate quantification of PEth in biological samples is essential for evaluating recent alcohol consumption patterns. This poster presents an LC-MS/MS method for quantifying PEth in human whole blood samples. The method was evaluated for reproducibility and reliability. Additionally, the developed assay was compared with a concentrated version to assess agreement and correlation between both approaches.



Figure 1: Kit composition of the ready-to-use (Ref. 30269139) and concentrated (Ref. 30288761) versions. The ready-to-use kit is shown on the left and the concentrated kit on the right.

METHODS.

The quantification of PEth in human whole blood by LC-MS/MS was evaluated according to the IFU (v.1.4) using the PEth in Whole Blood LC-MS/MS Assay (30269139/1240 M PEth) and its concentrated version (30288761). Both kits are intended for research use only (RUO), are manufactured by Diagnotix and distributed by Tecan. Sample preparation involved the addition of a deproteinization solution which included the internal standards to the whole blood sample, followed by mixing and centrifugation. The resulting supernatant was then transferred for LC-MS/MS analysis, as illustrated in Figure 2. Calibration and quality control (QC) materials were prepared in a human whole blood matrix free of PLPEth and POPeth. The calibration curve consisted of six calibration levels, while three quality control levels were included for analytical evaluation.

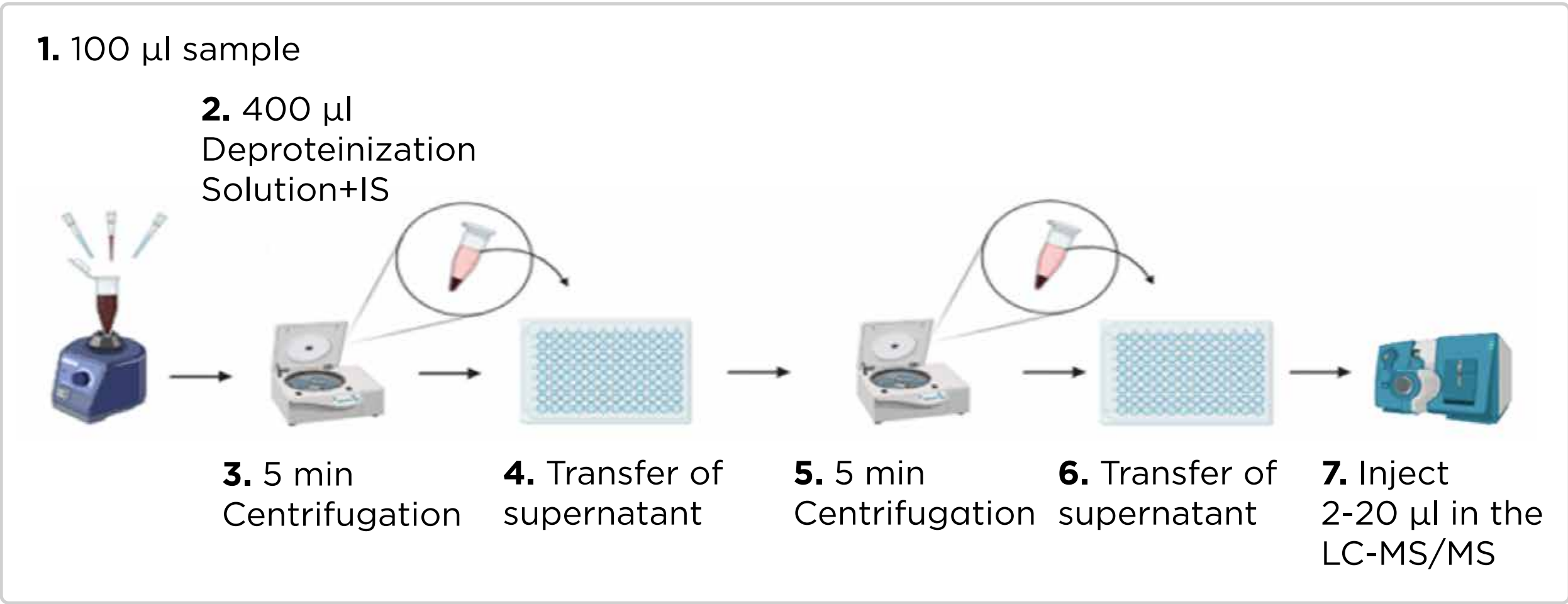


Figure 2: Sample preparation workflow for the quantification of PEth in human whole blood by LC-MS/MS.

REFERENCES.

- Varga A, Hansson P, Lundqvist C, Alling C. Phosphatidylethanol in blood as a marker of ethanol consumption in healthy volunteers: comparison with other markers. Alcohol Clin Exp Res. 1998;22(8):1832-1837.
- Hill-Kapturczak N, et al. The utility of phosphatidylethanol in the assessment of alcohol use and abuse. Alcohol Clin Exp Res. 2018.

RESULTS.

Figure 3 shows an example of the chromatographic analysis of PEth, which is representative of both kits, as the LC-MS/MS setup was identical. Good separation and detection of PLPEth and POPeth, together with their respective isotopically labeled internal standards, were achieved. PLPEth eluted at approximately 1.5 min, followed by POPeth at approximately 1.8 min.

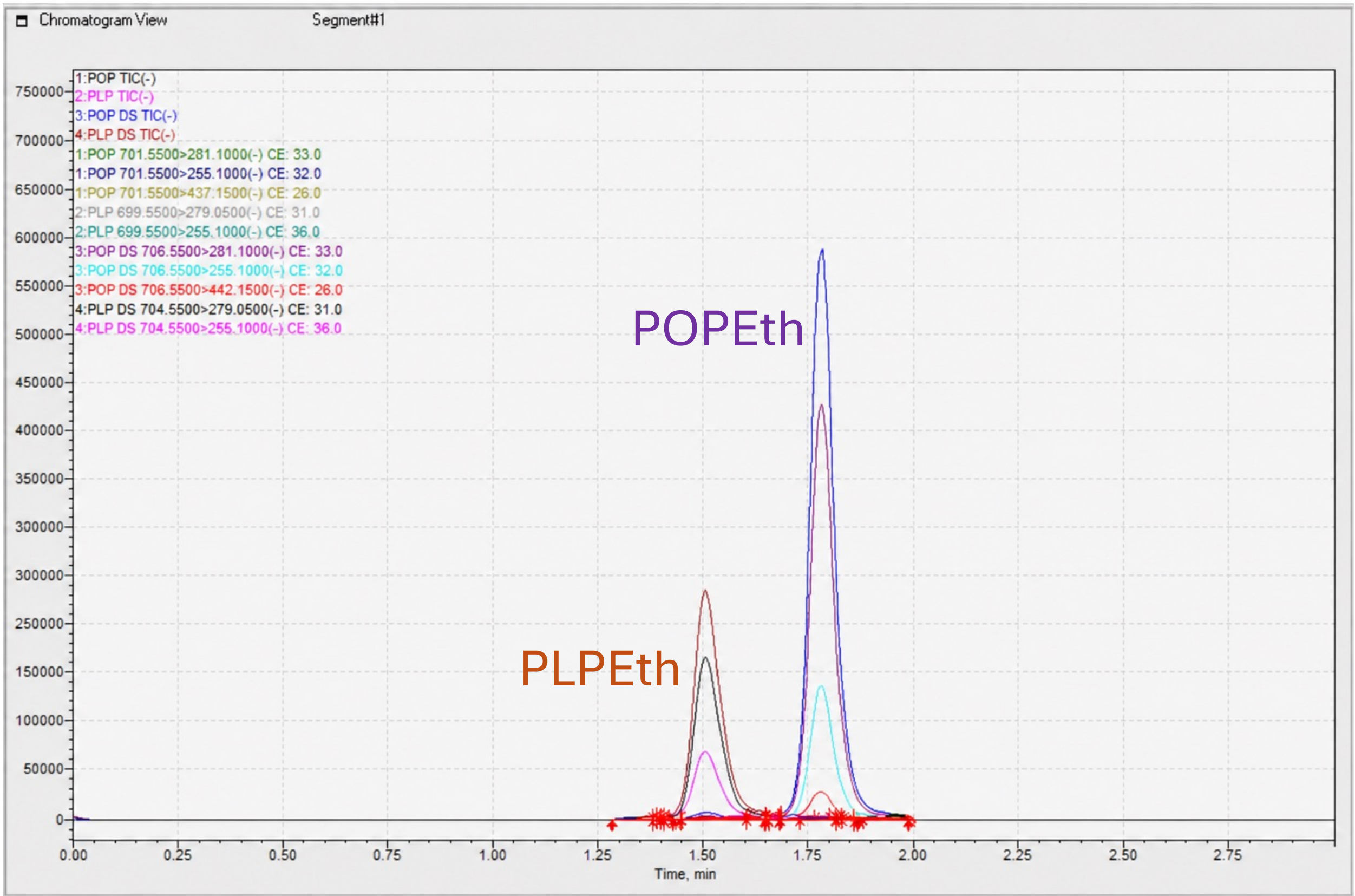


Figure 3: Representative LC-MS/MS chromatogram of PLPEth and POPeth with their corresponding internal standards.

The newly developed concentrated kit was compared with the ready-to-use kit. Both kits are available for Research Use Only. The concentrated format uses the same reagent composition as the ready-to-use kit while reducing storage space, transport volume and reagent consumption. A method comparison study was performed to assess the agreement and correlation of the developed assay using the concentrated format and the ready-to-use kit.

Passing-Bablok regression.

Passing-Bablok regression plots evaluated the relationship between PLPEth, and POPeth concentrations measured with concentrated and ready-to-use kits. Data points are shown as blue dots, the regression line as red and the identity line as green dashed. Both analytes showed strong correlation and good agreement between methods, with no apparent substantial systematic bias (Figure 4A and B; Table 1).

Bland-Altman analysis.

Bland-Altman plots assessed agreement between concentrated and ready-to-use kits across the measured concentration range. Blue dots represent individual samples; the red dashed line shows mean bias and black dotted lines indicate the limits of agreement. PLPEth showed a slight negative bias, while POPeth showed a slight positive bias. Most samples remained within the limits of agreement, supporting good consistency between both methods (Figure 4C and D; Table 1).

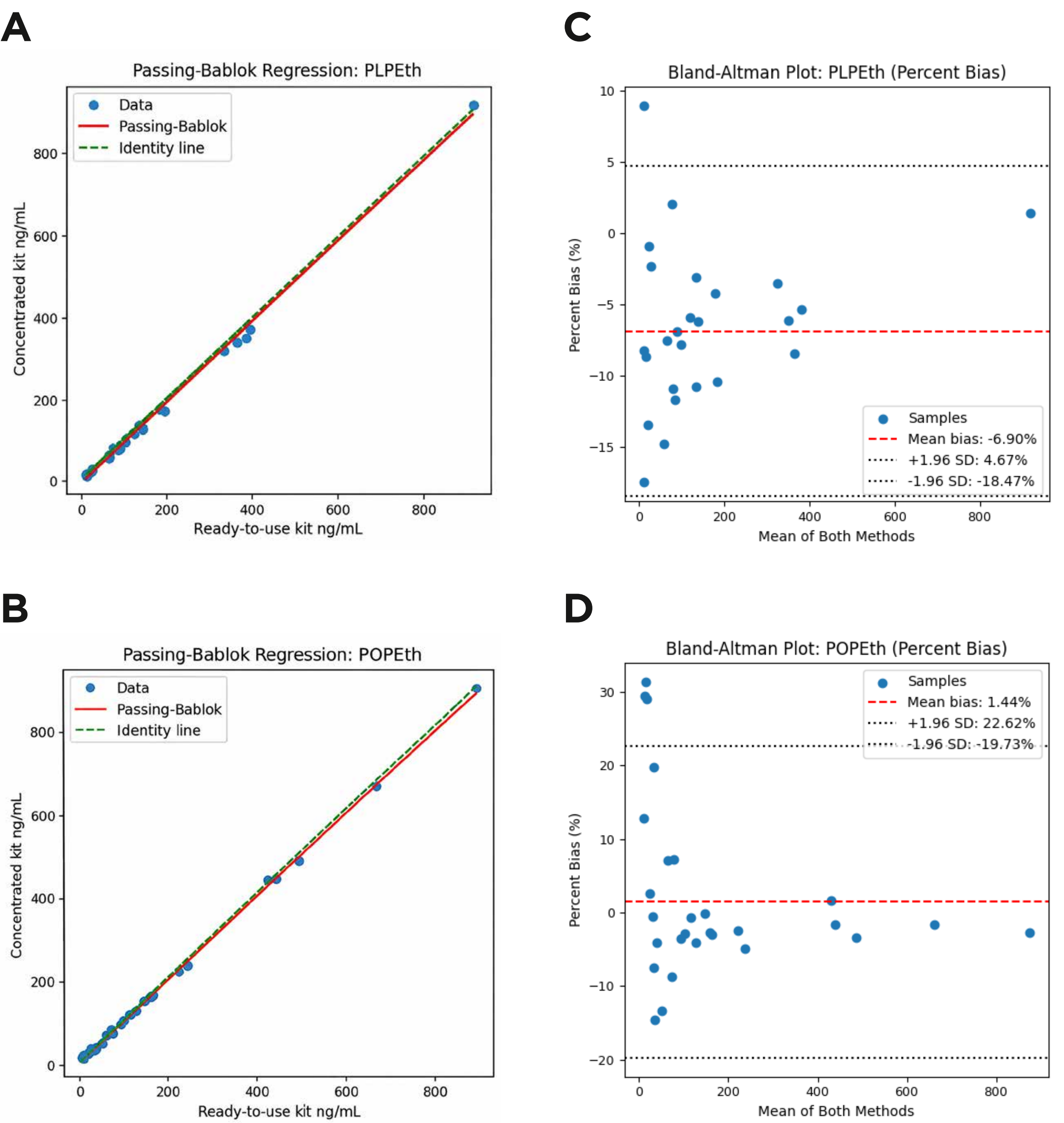


Figure 4: Passing-Bablok regression (A, B) and Bland-Altman plots (C, D) for PLPEth and POPeth.

Table 1. Passing-Bablok regression analysis and bias assessment for the comparison of PLPEth and POPeth quantification methods

Analyte	Passing-Bablok Slope	Passing-Bablok Intercept	Corr Coef (R)	Mean Bias	Percent Bias (%)
PLPEth	0.997	7.1279	0,9989	-7.68	-4.81
POPeth	0.977	0.8476	0,9997	-3.07	-1.79

CONCLUSION.

The PEth in whole blood LC-MS/MS Assay provides a sensitive, precise, and reliable method for PEth analysis in whole blood.

The concentrated reagent kit delivers comparable analytical performance to the ready-to-use kit, while improving laboratory efficiency through reduced storage, transport and reagent use. These benefits, along with strong method agreement and high data quality, make the assay a good choice for scientific laboratory use and large-scale alcohol biomarker studies.

