

**Table S4.** Analytical validation of the developed method for determination of phosphatidylethanol in VAMS

Analytes	Linearity (R <sup>2</sup> )	LOQ	Intra-Day Precision CV(%)	Inter-Day Precision (CV%)	Accuracy (% bias)	Matrix Effects (%)	Recovery (%)	Hematocrit effects	Stability	Ref
PEth (UGent laboratory)	-	1.7 ng/ml (LOD) 10 ng/ml (LLOQ) 2000 ng/ml (ULOQ)	7.8% (10 ng/ml) 6.2% (30 ng/ml) 7.9% (500 ng/ml) 5.1% (1500 ng/ml) 6.9% (735 ng/ml) 7.9% (244 ng/ml) 8.2% (465 ng/ml) 8.6% (146 ng/ml) 9.6% (15.7 ng/ml)	10.9% (10 ng/ml) 4.8% (30 ng/ml) 7.8% (500 ng/ml) 10.1% (1500 ng/ml) 4.8% (735 ng/ml) 7.5% (244 ng/ml) 7.1% (465 ng/ml) 10.8% (146 ng/ml) 6.6% (15.7 ng/ml)	5.2% (10 ng/ml) -4.5% (30 ng/ml) -5.5% (500 ng/ml) -6.5% (1500 ng/ml)	<b>Non-IS</b> 79.6 % (21% RSD) (low QC) 77.8% (16% RSD) (high QC)  <b>IS</b> 100% (7.9% RSD) (low QC) 95.9% (5.2% RSD) (high QC)	<b>Non-IS</b> 64.2% (12% RSD) (low QC low HCT) 47.6% (14% RSD) (low QC normal HCT) 43.9 (14% RSD) (low QC high HCT)  62.5% (14% RSD) (high QC low HCT) 64.1% (14% RSD) (high QC normal HCT) 61.3% (12% RSD) high QC high HCT  <b>IS</b> 61.0% (10% RSD) (low QC low HCT) 46.6% (9.5% RSD) (low QC normal HCT) 42.5% (13% RSD) (low QC high HCT)  54.5% (15% RSD) (high QC low HCT) 54.0% (8.1% RSD) (high QC normal HCT) 58.6% (10% RSD) (high QC high HCT)	See recovery column.	One week stability at 4°C, RT, and 45° C. One month stability at RT.	[34]
Peth (NICC laboratory)	-	10 ng/ml (LLOQ) 2000 ng/ml (ULOQ)	15.4% (10 ng/ml) 6.2% (30 ng/ml) 9.8% (500 ng/ml) 7.1% (1500 ng/ml)	13.3% (10 ng/ml) 7% (30 ng/ml) 4.6% (500 ng/ml) 5% (1500 ng/ml)	4.4 (10 ng/ml) -10.3% (30 ng/ml) -8.9% (500 ng/ml) 8.5% (1500 ng/ml)	<b>Non-IS</b> 119.5% (24% RSD) (low QC) 107.8% (14% RSD) (high QC)  <b>IS</b> 110.7% (5% RSD) (low QC) 108% (6% RSD) (high QC)	<b>LLE efficiency</b> <b>Non-IS</b> 71.1% (12% RSD) (low QC low HCT) 70.6% (11% RSD) (low QC normal HCT) 79.4% (14% RSD) (low QC high HCT) 70.8% (14% RSD) (high QC low HCT) 78.1 (12% RSD) (high QC normal HCT) 81.1 (6% RSD) (high QC high HCT)	See recovery column.	One week stability at 4°C, RT, and 45° C. One month stability at RT.	[34]

Abbreviations: phosphatidylethanol (PEth), Ghent University Laboratory of toxicology (UGent); National Institute of Criminalistics and Criminology (NICC), low limit of quantitation (LLOQ), upper limit of quantitation (ULOQ); hematocrit effects (HCT), internal standards (IS), liquid liquid extraction (LLE), relative standard deviation (RSD).