

Table S6. Analytical validation of the developed method for determination of amino acids and organic acids in VAMS

Analytes	Linearity (R ²)	Precision (CV%)	Recovery (%)	Stability	Additional data	Ref
Amino acids: Ala Arg Asn Asp Betaine Choline Cr Glu Gln His Ile Leu Lys Met Orn Phe Pro Sar Ser Taurine Thr Trp Tyr Val Organic acids 2-HBA 2-OGA 3-HPA CA FUMA GSH	r ² > 0.99	1.6% CV - 8.3% CV (10, 25, 50, and 100 % concentration)	Amino acids: Ala: 93% Arg: 47% Asn: 89% Asp: 87% Betaine: 97% Choline: 92% Cr: 92% Glu: 93% Gln: 85% His: 89% Ile: 95% Leu: 85% Lys: 68% Met: 92% Orn: 86% Phe: 87% Pro: 97% Sar: 90% Ser: 93% Taurine: 96% Thr: 91% Trp: 78% Tyr: 90% Val: 101% (10, 25, 50, and 100 % concentration) Organic acids 2-HBA: 97% 2-OGA: 5% 3-HPA: 74% CA: 60% FUMA: 84% GSH: 89% Glyoxylic acid: 23% LAC: 93%	Stability of metabolites on dried whole blood samples on VAMS tips after storage at ambient temperatures Amino acids and organic acids 4 days: 88% - 130% (+/- < 13 SD) 7 days: 69% - 133% (+/- 12 SD) 15 days: 68% - 138% (+/- 10 SD) No signal difference in VAMS dried and stored at room temperature for 0.5 to 26 hours. Difference in signal in venous blood stored at rt.	Selectivity Isobaric compounds and metabolites with equal nominal mass were separated. No interferences at selected transitions. Note LOQ, accuracy, matrix effects, and HCT effects were not presented.	[38]

Glyoxylic acid LAC Malic acid Pyr SA UA				Malic acid: 42% Pyr: 80% SA: 69% UA: 60% (10, 25, 50, and 100 % concentration)			
MMA SA KMV KIC ILE Leu Phe Tyr Valine	10.0-10,000 ng/ml	Intra-Day LLOQ: < 12.5% LQC: <8.9% MQC: <5.3% HQC: <5.5%	Inter-Day LLOQ: < 5.3% LQC: <4.5% MQC: <4.3% HQC: <4.2%	At 30 ng/ml MMA: 91.8 % SA: 106.67 % KMV: 97.16 % KIC: 77.51 % ILE: 101.34 % Leu: 103.14 % Phe: 105.75 % Tyr: 108.09 % Valine: 103.51 % At 7500 ng/ml MMA: 63.4% SA: 103.07% KMV: 72.77% KIC: 62.87% ILE: 90.59 % Leu: 93.02% Phe: 88.45% Tyr: 90.73% Valine: 94.51%	Seven days in ambient conditions and at -80°C. Three freeze/thaw cycles.	Accuracy Acceptable (< than 15%)	[39]

Abbreviations: Phenylalanine (Phe), room temperature (rt), Tyrosine (Tyr), seconds (s), hematocrit effects (HCT), alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), betaine (BL), choline, creatine (Cr), glutamic acid (Glu), glutamine (Gln), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), Phenylalanine (Phe), proline (Pro), sarcosine (Sar), serine (Ser), taurine, threonine (Thr), tryptophan (Trp), Tyrosine (Tyr), valine (Val), 2-hydroxybutanoic acid (2-HBA), 2-oxo-glutaric acid* (2-OGA), 3-hydroxypropionic acid (3-HPA), citric acid (CA), fumaric acid (FUMA), glutathione (GSH), glyoxylic acid, lactic acid (LAC), malic acid, pyruvic acid (Pyr), succinic acid (SA), uric acid (UA), methylmalonic acid (MMA), 3-methyl-2-oxovaleric acid sodium salt (KMV), sodium 4-methyl 2-oxo valerate (KIC).