

Table S5. Analytical validation of the developed method for determination of phenylalanine, tyrosine, and homogentisic acid in VAMS.

Analytes	Linearity (R ²)	LOQ	Intra-Day Precision (CV%)	Inter-Day Precision (CV%)	Accuracy (% bias)	Matrix Effects (%)	Recovery (%)	Hematocrit effects	Stability	Additional data	Ref
Phe and Tyr	–	–	Phe: 5.6% CV (endogenous, 508, and 811) µmol/L Tyr: 6.2% CV (endogenous, 349, and 557) µmol/L		Phe: 0.1% bias (endogenous) -15.8% bias (508 µmol/L) -20.4% bias (811 µmol/L) Tyr: -12% to -25% bias (349 – 557) µmol/L	–	–	Phe: -12.8% bias (508 µmol/L; 44.4% and 63.3% HCT) Tyr: -17.3% bias (349 µmol/L; 44.4% and 63.3% HCT)	15 days at rt and 4°C. p>0.01.	Effect of application of different volumes and contact time after tip is filled Phe: No effect on concentrations, p > 0.01 (20, 50, and 200 µl; 508 µmol/L; 0, 2, 5, and 10 s) Tyr: No effect on concentrations, p > 0.01 (349 µmol/L; 0, 2, 5, and 10 s)	[35]
Phe and Phe-surrogate	30 - 3000 µmol/L, r ² = 0.9894	30 µmol/L	Phe: 3.1% - 7.7% CV (75, 450, and 2250) µmol/L Phe-surrogate: 3.5% - 9.3% CV (75, 450, and	Phe: 4.9% - 6.2% CV (75, 450, and 2250) µmol/L Phe-surrogate: 6.0% - 8.8% CV (75, 450, and	Intra accuracy Phe: -3.9% - 13.0% bias (75, 450, and 2250) µmol/L Phe-surrogate: -5.3% - 10.9% bias (75, 450, and 2250) µmol/L	Phe: no impact on accuracy and precision (75, 450, and 2250) µmol/L Phe-surrogate: -13.5% to 4.9% bias.	Phe-surrogate: 88% to 108.4% (75, 450, and 2250) µmol/L	Phe: -10.7 to 3.1% bias; 2.5% to 9.3% CV (75 and 2250 µmol/L; 0.3, 0.5, and 0.7 HCT)	3.25 hrs. in WB at 37°C and room temperature. 232 days (in the VAMS clamshell) at rt. 15 days at 45°C. 200 hrs. in extract at rt. 208.75 hrs. for reinjection precision at rt.	Lipemic effect Phe-surrogate: 0.4% to 3.6% bias; 1.9% to 7.9% CV. (75 and 2250 µmol/L) Selectivity Phe and Phe-surrogate:	[36]

			2250) μmol/L	2250) μmol/L	Inter accuracy Phe: 0.9% - 11.4% bias (75, 450, and 2250) μmol/L Phe-surrogate: -1.3% - 9.9% bias (75, 450, and 2250) μmol/L	1.2% to 12.6% CV (75 and 2250) μmol/L				No meaningful effect in quantification. Method comparison Phe VAMS vs plasma samples Pearson Correlation (Significant correlation. p < 2.2*10 e^6, R = 0.9756). Passing Bablock (Intercept ~ 0; 95% CI contained 0, slope ~ 1, value range < LLOQ (30 μmol/L), % bias < 5%). Bland Altman (Proportional mean difference = -0.04246; 95% CI (-0.05904 to - 0.02589)).	
Phe; Tyr; HGA in urine VAMS	—	—	Sampling precision 3.7% CV (absorb till tip is filled and wait 2 additional seconds) 4.1% CV (sample for 6 s) 4.6% CV (sample for 10 s) 1.2% CV (good pipetting) 4.6% CV (bad pipetting)	Sampling accuracy measured vols (actual vol = 10 μL); <0.47 SD 10.3 μL (absorb till tip is filled and wait 2 additional seconds) 10.4 μL (sample for 6 s) 10.6 μL (sample for 10 s)	—	Phe (< 9.3 SD): 83.1% (5 min agitation) 95.4% (15 min agitation) 99% (5 min no agitation) 101.1% (15 min no agitation) Tyr (< 7.0 SD) 86.9% (5 min agitation) 104.3% (15 min agitation)	—	Phe: Stable for up to 28 days (20°C day light/ no light; 4°C no light, 37°C no light). Small increase in overall stability at 37°C for up to 28 days (AC vs non-AC). Tyr:	Mitra drying time: Constant Mitra tip weight reached after drying for 45 mins. Method comparison (urine VAMS vs liquid urine) Phe:	[37]	

				<p>10.1 µL (good pipetting) 10.2 µL (bad pipetting)</p>		<p>106.4% (5 min no agitation) 103.9% (15 min no agitation)</p> <p>HGA (<11.9 SD) 82.2% (5 min agitation) 93.5% (15 min agitation) 98.2% (5 min no agitation) 98.8% (15 min no agitation)</p>		<p>Deterioration from 14 to 21 days (20°C light/no light).</p> <p>Stable for 28 days (4°C no light).</p> <p>Rapid deterioration within 24 hours (37 °C no light).</p> <p>No statistical impact on (AC vs non-AC)</p> <p>HGA: Poor stability (20°C light/no light).</p> <p>Stability in non-acidified dried urine VAMS significantly > than non-acidified liquid urine (4 °C no light).</p> <p>Poor stability (37°C no light).</p> <p>No statistical impact on stability (AC vs non-AC).</p> <p>Additional notes:</p>	<p>1.014 (slope) 0.279 (intercept) 0.985 (R²)</p> <p>Tyr: 0.965 (slope) 1.593 (intercept) 0.97 (R²)</p> <p>HGA 0.94 (slope) 62.127 (intercept) 0.983 (R²)</p>	
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								<p>No effect of AC on Mitra device tip Significant improvement in stability at lower temperatures except for PHE in acidified conditions.</p> <p>Increased stability observed at 4°C for approximately 28 days for all analytes (recommended transport and storage temperature).</p>		
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Abbreviations: Phenylalanine (Phe), 2,3-13C215N-Phenylalanine (Phe-surrogate), whole blood (WB), room temperature (rt), Tyrosine (Tyr), seconds (s), hematocrit effects (HCT), homogentisic acid (HGA), volume (vol), and standard deviation (SD), AC(AC).