A sensitive and rapid method for amino acid quantitation in malaria biological samples using AccQ•Tag UPLC-ESI-MS/MS with multiple reaction monitoring

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***Plasmodium Falciparum* Culture Protocol.** *P. falciparum* clone 3D7 was grown at 2-3% haematocrit in RPMI 1640 medium supplemented with 10% (v/v) human serum. Uninfected red blood cells (RBCs) for parasite culture were obtained fresh weekly from a limited stable pool of screened O+ donors. Parasite stage synchrony was maintained using sorbitol lysis. Ring and trophozoite stage parasites were purified by saponin lysis and then washed twice with ice cold PBS. The hemozoin content for each ring and trophozoite stage parasite pellet was determined colorimetrically.[44] Independent replicates (flasks) were prepared. After centrifugation at 8000g, the pellets containing the whole mature parasites were stored at -80 °C before being analyzed for amino acid content. Parasite growth and preparation was done at Johns Hopkins University.

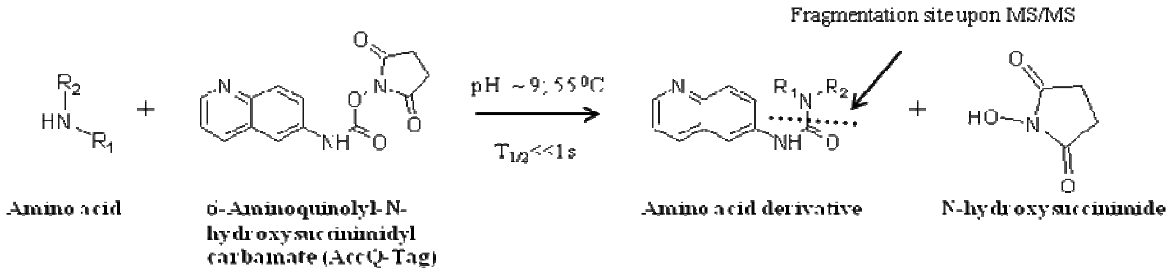

Figure S-1. Schematic reaction of amino acid derivatization with AccQ•Tag reagent.

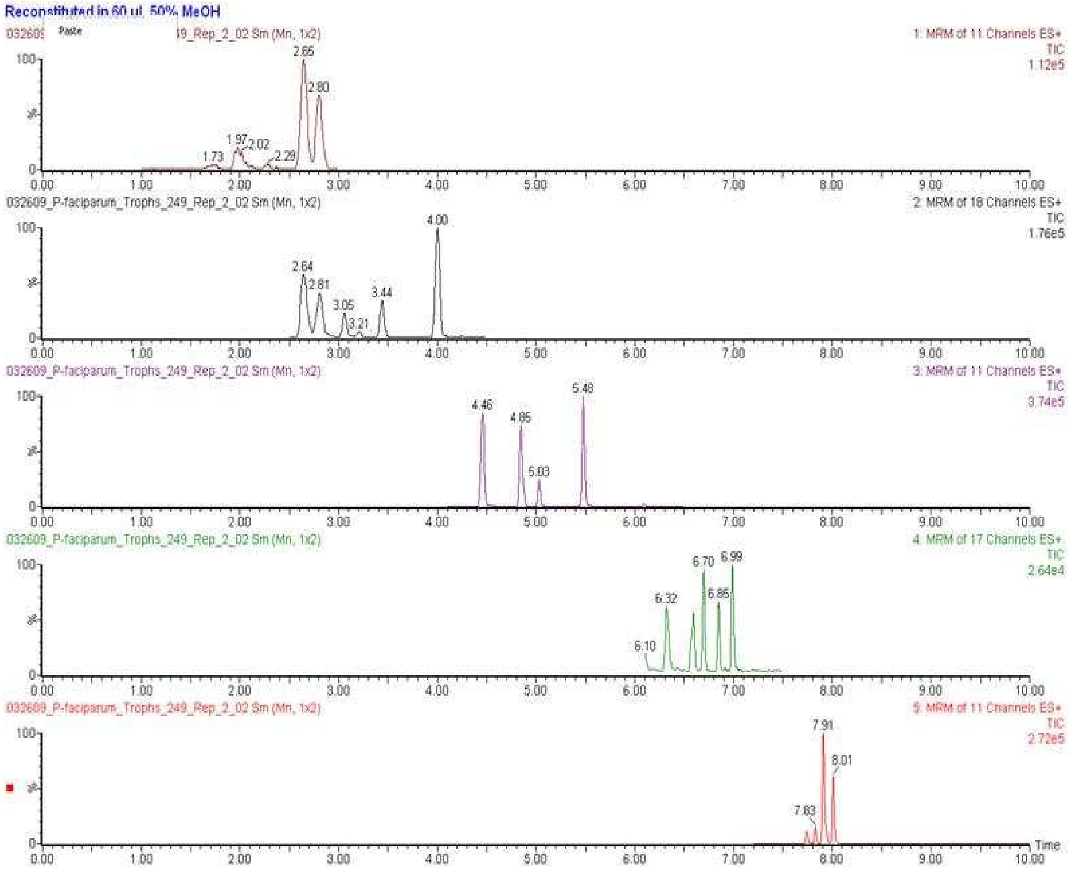

Figure S-2. Typical chromatograms for AccQ•Tag tag derivatized amino acids detected in *P. falciparum* trophozoites, obtained with UPLC-MS/MS using multiple reaction monitoring. Experimental conditions were the same as in Table S-3.

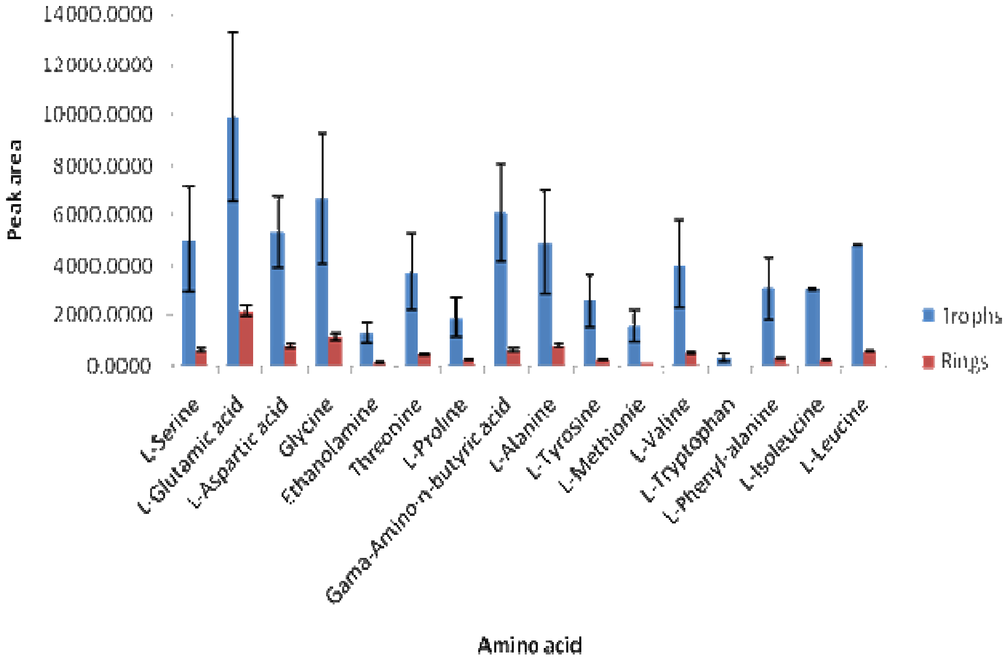

Figure S-3. Biological reproducibility for AccQ•Tag derivatized amino acids detected in *P. falciparum* rings or trophozoites. Experimental conditions were the same as in Table S-3.

Table S-1. Retention times and parent ions observed for AccQ•Tag derivatized physiological amino acid standards and isotopically labeled amino acids, analyzed by UPLC-MS.

Amino acid	**FW**	**AccQ•Tag fragment (m/z)**	**Predicted parent ion (m/z)**	**Observed parent ion (m/z)**	**RT (min)**
4-Hydroxy-L-proline	131.11	171	302.11	302.14	1.86
Histidine (ring 2-^{13}C)	156.21	171	327.21	327.09	1.76
L-Histidine	155.21	171	326.21	326.13	1.82
1-Methyl-histidine	169.21	171	340.21	340.22	2.23
Taurine	125.11	171	296.11	296.08	2.32
3-Methyl-histidine	169.21	171	340.21	340.22	2.48
Serine,2,3,3-d_3	108.11	171	279.11	279.08	2.69
L-Serine	105.11	171	276.11	276.12	2.75
Arginine (guanido-$^{15}N_2$)	176.36	171	347.36	347.13	2.95
L-Arginine	174.21	171	345.21	345.16	3.07
Glycine-d_5	77.25	171	248.25	248.03	3.11
Glycine	75.08	171	246.08	246.11	3.13
L-Carnosine	226.21	171	397.21	397.15	3.2
Ethanolamine	61.09	171	232.09	232.16	3.29
L-Asparagine-^{15}-N_2	134.27	171	305.27	305.11	3.47
L-Aspartic acid	133.11	171	304.11	304.14	3.47
L-Sarcosine	89.17	171	260.17	260.17	3.91
Citrulline-ureido-^{13}C	176.35	171	347.35	347.14	4.01
L-Glutamic acid -2,4,4-d_3	150.11	171	321.11	321.06	4.03
L-Glutamic acid	147.11	171	318.11	318.15	4.03
L-Citrulline	175.21	171	346.21	346.15	4.06
β-Alanine	89.17	171	260.17	260.1	4.29
L-Threonine (^{13}C, ^{15}N)	124.11	171	295.11	295.06	4.52
L-Threonine	119.11	171	290.11	290.18	4.52
L-Alanine	89.1	171	260.1	260.1	4.9
D-L-Alanine-2,3,3,3-d_4	93.11	171	264.11	264.13	4.91
γ-Amino-n-butyric acid	103.11	171	274.11	274.15	5.07
D-L-β-Aminoisobutyric acid	103.11	171	274.11	274.14	5.52
Proline-2,5,5-d_3	118.32	171	289.32	289.12	5.56
L-Proline	115.16	171	286.16	286.16	5.58
δ-hydroxylysine	161.31	171	33.31	333.18	5.72
L-α-Amino-n-butyric acid	103.11	171	274.11	274.16	6.11
Ornitine-3,3,4,4,5,5-d_6	308.22	171	479.22	479.22	6.23
L-Ornitine	132.21	171	303.21	-	6.24
Lysine-3,3,4,4,4,5,5,6,6-d_8-01	324.58	171	495.58	495.26	6.62
L-Lysine	146.21	171	317.21	315.15	6.63
4-Hydroxyphenyl-2,6-d_2-alanine-2-d_1-01	184.22	171	355.22	355.22	6.68
Tyrosine	181.21	171	352.21	352.17	6.74
Methionine-methyl-d_3	152.13	171	323.4	323.13	6.85
L-Methionine	149.21	171	320.21	320.16	6.91
Valine-d_8	125.11	171	296.11	296.21	7.02
L-Valine	117.21	171	288.21	288.16	7.02
L-Isoleucine	131.21	171	302.21	302.19	7.83
Leucine-d_{10}	141.24	171	312.24	312.24	7.84
L-Leucine	131.21	171	302.21	302.16	7.89
Phenyl-d_5-alanine	170.21	171	341.21	341.11	7.97
Phenylalanine	165.21	171	336.21	336.24	8.01
Tryptophan-2’,4’,5’,6’,7’-d_5(indole-d_5)-01	209.21	171	380.21	380.21	8.07
Tryptophan	204.21	171	375.21	375.17	8.11

Note: Separation was performed on a 2.1 mm i.d. x 100 mm AccQ•Tag colum; MS data was acquired using full scan MS mode; the concentration of each derivatized amino acid standard was 0.25 μmol/mL, and 1 μL of sample was injected. Separation conditions were the same as reported in the experimental section-UPLC analysis.

Table S-2. Optimal cone voltage and collision energy for selected AccQ•Tag derivatized amino acid standards analyzed by ESI-MS/MS, using a triple quadrupole mass spectrometer, and, either, low flow (20 μL/min) or combined flow (200 μL/min total flow).

Amino acid (symbol)	Side chain polarity/ charge at pH 7. .0	Parent/ Daughter transition	Run #	Experimental values		Values implemented	
				Cone (V)	CE (eV)	Cone (V)	CE (eV)
Alanine (A)	Non-polar/neutral	260/171	1	30	24	32	25
			2	33	27		
Arginine (L)	Polar/acidic	345.23/171	1	34	18	35	19
			2	35	21		
Glutamic acid (E)	Polar/basic	318.11/171	1	32	24	32	24
			2	32	24		
Glycine (G)	Non-polar/neutral	246.16/171	1	34	22	33	20
			2	33	19		
4-Hydroxy-L-Proline	Polar	302.20/171	1	34	21	33	22
			2	33	23		
Isoleucine (I)	Non-polar/neutral	302.20/171	1	40	33	39	35
			2	38	38		
Proline (P)	Non-polar/neutral	286.16/171	1	30	23	29	21
			2	29	20		
Serine (S)	Polar/neutral	276.12/171	1	37	19	37	19
			2	37	19		
Threonine (T)	Polar/neutral	290.18/171	1	31	22	31	22
			2	33	20		
Valine (V)	Non-polar/neutral	288.23/171	1	36	25	35	24
			2	34	26		

Table S-3. Peak area reproducibility for AccQ•Tag labeled physiological amino acid standards analyzed by UPLC-MS/MS using 001 s and 002 s dwell time. Experimental conditions: 2.1 x 100 mm AccQ•Tag column; 0.25 µmol/mL AccQ•Tag derivatized physiological amino acids, 1 µL sample injection volume; positive ionization mode.

		001 s Dwell time (method # 1)			**0.02 s Dwell time (method # 2)**		
Amino acid	**RT**	**Area**	**STD**	**CV**	**Area**	**STD**	**CV**
4-Hydroxy-L-Proline	1.64	5444.3256	271.1593	4.98	5566.8097	646.6690	11.62
L-Histidine	1.78	500.9280	29.4190	5.87	488.3001	62.8618	12.87
1 Methyl-histidine	2.19	572.4806	39.9383	6.98	548.2807	64.9612	11.85
Taurine	2.33	1409.6150	91.6646	6.50	1398.6956	130.2501	9.31
3-Methyl histidine	2.43	616.5313	50.6220	8.21	620.9461	53.2337	8.57
L-Serine	2.74	7519.0145	480.3950	6.39	6271.4555	440.3902	7.02
L-Carnosine	2.97	1654.0044	174.6027	10.56	1587.3623	137.3719	8.65
L-Arginine	2.99	769.2371	128.9220	16.76	789.6934	177.4694	22.47
Glycine	3.11	10279.6715	777.3611	7.56	10452.1492	1167.6755	11.17
Ethanolamine	3.26	21888.1744	1317.5921	6.02	21197.9733	1411.7032	6.66
L-Aspartic acid	3.49	3551.6079	275.4630	7.76	3439.9399	273.6357	7.95
L-Sarcosine	3.92	6131.3008	372.8854	6.08	6100.9862	521.2789	8.54
L-Glutamic acid	4.06	5853.6262	360.9170	6.17	5837.1004	483.4357	8.28
L-Citrulline	4.07	2845.7597	243.6547	8.56	2668.5124	302.5678	11.34
β-Alanine	4.28	12007.7519	987.0927	8.22	11343.6462	1262.2200	11.13
Threonine	4.50	9210.8077	717.7032	7.79	8910.4477	720.4672	8.09
L-Alanine	4.91	11642.4342	624.2984	5.36	11221.9654	831.0872	7.41
γ-Amino-n-butyric acid	5.07	18697.5293	1153.9400	6.17	18948.8163	1452.7136	7.67
Amino adipic acid	5.28	8627.1619	527.4358	6.11	9085.8054	623.4739	6.86
δ-hydroxylysine	5.28	337.5054	26.8940	7.97	370.3647	33.4606	9.03
D-L-β-Aminoisobutyric acid	5.52	18053.7697	954.1240	5.28	18133.5832	1254.2389	6.92
L-Proline	5.54	6326.2104	418.1214	6.61	6147.9922	339.2061	5.52
L-α-Amino-n-butyric acid	6.13	9715.2046	1182.5514	12.17	7804.3374	2328.0226	29.83
L-Lysine	6.62	442.1846	48.1525	10.89	417.6766	59.7404	14.30
L-Tyrosine	6.71	7888.3505	790.2071	10.02	7035.4064	890.3845	12.66
L-Methionie	6.87	7535.2930	580.4547	7.70	6727.8577	819.6171	12.18
L-Valine	7.01	8312.5817	484.0699	5.82	8299.6486	764.0349	9.21
L-Isoleucine	7.76	7208.9950	503.8162	6.99	6715.6008	655.1755	9.76
L-Leucine	7.84	7503.3079	297.0700	3.96	6774.5927	418.1853	6.17
L-Phenylalanine	7.95	8739.0914	483.1840	5.53	8157.0873	709.8919	8.70
L-Tryptophan	8.05	7330.4634	557.2566	7.60	6766.0857	728.1215	10.76

Table S-4. Dynamic range, high limits of linearity, low limits of linearity, and detection limits for AccQ•Tag physiological amino acids standards. Experimental conditions were the same as in Table S-3.

Amino acid	**Dynamic range**	**High limits of linearity (pmol/µL)**	**Low limits of linearity (fmol/µL)**	**Detection limit (fmol)**	**R^2**
Hydroxy-L-proline	100	6.25	24.41	1.65	0.9943
L-Histidine	100	6.25	97.65	13.28	0.9967
1-Methyl-histidine	100	12.5	97.65	17.95	0.9925
Taurine	100	12.5	195.31	3.95	0.9993
3-Methyl-histidine	1000	25	97.65	12.05	0.998
L-Serine	1000	25	24.41	1.65	0.9949
L-Arginine	100	25	390.6	11.9	0.9979
Glycine	10000	25	3.05	1.65	0.9929
L-Carnosine	1000	25	97.65	5.88	0.988
Ethanolamine	1000	12.5	48.82	1.65	0.9959
L-Aspartic acid	100	6.25	24.41	3.49	0.9715
L-Sarcosine	1000	25	97.65	1.65	0.9976
L-Glutamic acid	100	6.25	12.22	5.4	0.983
L-Citrulline	100	25	390.62	3.98	0.985
β-Alanine	1000	12.5	24.41	2.27	0.9902
L-Threonine	1000	25	48.82	2.63	0.9988
L-Alanine	1000	25	48.82	1.85	0.9997
γ-Amino-n-butyric acid	1000	12.5	12.2	1.65	0.9958
Amino adipic acid	1000	12.5	97.65	1.79	0.9894
D-L-β-Aminoisobutyric acid	1000	12.5	24.41	1.65	0.9955
L-Proline	1000	25	24.41	2.83	0.9993
L-α-Amino-n-butyric acid	1000	25	12.2	1.71	0.9956
Tyrosine	1000	25	48.82	3.85	0.994
L-Methionine	1000	25	12.2	1.65	0.9952
L-Valine	1000	25	24.41	1.65	0.9977
L-Isoleucine	1000	12.5	48.82	1.65	0.998
L-Leucine	1000	25	48.82	1.65	0.9983
Phenylalanine	10000	25	6.1	4.01	0.9997
Tryptophan	1000	25	97.65	7.57	0.9993

Table S-5. Technical reproducibility for AccQ•Tag derivatized amino acids detected and measured in *P. falciparum* ring and trophozoite developmental stages. Experimental conditions were the same as in Table S-3.

Amino acid	***P. falciparum* rings**			***P. falciparum* trophozoites**		
	Area	**CV (%)**	**STError**	**Area**	**CV (%)**	**STError**
Serine	477.279	9.18	25.283	19898.23	3.40	390.496
Glutamic acid	1797.766	8.77	90.979	35119.162	1.51	306.028
Aspartic acid	723.568	8.79	36.708	15799.672	3.85	350.793
Glycine	1588.287	5.70	52.301	25272.032	3.78	550.982
Ethanolamine	35.475	-	-	4054.876	3.77	88.296
Threonine	371.736	10.97	23.545	14843.529	3.60	308.860
Proline	252.636	11.63	16.969	7050.015	5.16	210.157
γ-Amino-n-butyric acid	847.659	5.11	24.986	20764.828	3.42	409.662
Alanine	757.857	6.20	27.147	20275.084	2.63	307.478
Tyrosine	249.737	6.94	10.004	10160.909	5.42	317.880
Methionine	112.121	11.77	7.619	6187.525	9.70	346.406
Valine	522.668	18.23	55.020	17250.195	4.87	484.700
Tryptophan	31.945	17.12	3.158	1440.356	6.85	56.984
Phenylalanine	276.702	15.33	24.496	12079.774	3.85	268.163
Isoleucine	225.678	10.21	13.301	13221.078	4.93	376.061
Leucine	596.329	3.29	11.311	20383.451	5.45	641.467

Table S-6. Biological reproducibility across peak areas measured for AccQ•Tag derivatized isotopically labeled amino acid internal standards spiked into red blood cells. Experimental conditions were the same as in Table S-3. The biological reproducibility was calculated across 6 biological replicates, each analyzed three times by UPLC-MS/MS.

Isotopically labeled amino acid	Area	STD	CV (%)
Serine-2,3,3-d_3	633.933	77.011	12.15
Glycine-d_5	350.138	38.025	10.86
L-Asparagine-^{15}-N_2	279.149	37.790	13.54
L-Glutamic acid-2,4,4-d_3	220.429	26.247	11.91
Threonine-d_5	625.824	60.447	9.66
D-L-Alanine-2,3,3,3-d_4	788.569	72.753	9.23
Proline-2,5,5-d_3	583.957	55.182	9.45
Ornitine-3,3,4,4,5,5-d_6	209.369	30.879	14.75
4-Hydroxyphenyl-2,6-d_2-alanine-2-d_1-01	292.036	42.140	14.43
Methionine-methyl-d_3	747.010	56.910	7.62
Valine-d_8	487.745	64.640	13.25
Leucine-d_{10}	983.879	29.082	2.96
Phenyl-d_5-alanine	537.870	75.172	13.98
Tryptophan-2’,4’,5’,6’,7’-d_5(indole-d_5)-01	376.856	49.308	13.08

Equation S-1

The detection limit, which is the lowest concentration that produces a signal within a certain confidence level, was calculated using the following equation.

$$\mathrm{LOD} = 3 \times \mathrm{STD}_{\mathrm{blank}} / m$$

Where: LOD is the limit of detection; $\mathrm{STD}_{\mathrm{blank}}$ is the standard deviation of the blank and m is the slope of the calibration curve.