

Table 1. Estimated intracellular metabolite concentrations^a in saponin-isolated malaria parasites: comparison of four extraction methods

Metabolite	Perchloric acid (n = 13)	Methanol/water (n = 12)	Methanol/chloroform/water (n = 10)	Methanol (n = 13)
Amino acids				
Alanine (Ala)	0.48 ± 0.12	2.1 ± 0.8	0.40 ± 0.10	0.47 ± 0.15
GABA	2.3 ± 0.5	2.1 ± 0.5	1.9 ± 0.4	2.1 ± 0.5
Arginine (Arg)	4.2 ± 1.1	2.9 ± 1.3	3.0 ± 0.7	1.6 ± 0.4
Asparagine (Asn)	0.7 ± 0.4	2.6 ± 1.3	0.51 ± 0.19	0.65 ± 0.16
Aspartate (Asp)	2.0 ± 0.5	1.4 ± 0.8	1.7 ± 0.5	1.73 ± 0.29
Glutamate (Glu)	11 ± 4	9.1 ± 2.5	9.9 ± 3.1	8.8 ± 2.1
Glutamine (Gln)	1.34 ± 0.30	1.7 ± 0.4	1.12 ± 0.29	1.4 ± 0.5
Glycine (Gly)	0.43 ± 0.08	1.6 ± 0.6	0.34 ± 0.07	0.4 ± 0.1
Histidine (His)	0.16 ± 0.04	0.60 ± 0.23	0.14 ± 0.04	0.15 ± 0.07
Isoleucine (Ile)	0.16 ± 0.05	1.1 ± 0.6	0.13 ± 0.06	0.17 ± 0.05
Leucine (Leu)	0.37 ± 0.12	2.8 ± 1.1	0.33 ± 0.12	0.44 ± 0.12
Lysine (Lys)	1.3 ± 0.3	3.4 ± 1.2	1.16 ± 0.22	0.93 ± 0.20
Methionine (Met)	nd ^b	0.43 ± 0.29	nd	nd
Phenylalanine (Phe)	0.16 ± 0.05	0.9 ± 0.4	0.13 ± 0.02	0.14 ± 0.03
Serine (Ser)	nd	1.5 ± 0.8	nd	nd
Threonine (Thr)	0.43 ± 0.10	1.8 ± 0.9	0.42 ± 0.15	0.49 ± 0.29
Tyrosine (Tyr)	0.19 ± 0.04	0.8 ± 0.3	0.14 ± 0.05	0.17 ± 0.04
Valine (Val)	0.29 ± 0.08	1.4 ± 0.5	0.24 ± 0.07	0.28 ± 0.08
Total amino acids	28 ± 6	44 ± 12	25 ± 5	23 ± 4
Glutathione				
Reduced (GSH)	0.6 ± 1.2 ^c	5.4 ± 1.2	3.4 ± 0.7	3.2 ± 1.3
Oxidised (GSSG)	1.7 ± 0.7	nd	0.3 ± 0.3 ^c	0.6 ± 0.4
Soluble membrane precursors				
Glycerophosphocholine (GPC)	nq ^b	nq	nq	nq
Glycerophosphoethanolamine (GPE)	nq	nq	nq	nq
myo-Inositol (Ins)	0.24 ± 0.08	0.22 ± 0.07	0.15 ± 0.07	0.17 ± 0.04
Phosphocholine (PC)	1.49 ± 0.32	1.36 ± 0.28	1.4 ± 0.5	1.29 ± 0.23
Phosphoethanolamine (PE)	4.5 ± 1.5	3.9 ± 1.2	3.9 ± 1.1	3.1 ± 0.7
Nucleotides and related compounds				
AMP	0.36 ± 0.15	0.55 ± 0.33	0.27 ± 0.18	0.16 ± 0.10
ADP	1.1 ± 0.4	0.65 ± 0.28	0.46 ± 0.17	0.11 ± 0.10
ATP	2.0 ± 0.9	1.0 ± 0.4	1.0 ± 0.7	0.12 ± 0.06
GMP ^d	nq	nq	nq	nq
GDP	nq	nq	nq	nq
GTP ^d	nq	nq	nq	nq
Hypoxanthine ^d	nq	nq	nq	nq
IMP ^d	nq	nq	nq	nq
UMP, CMP, (U/C MP) ^e	0.8 ± 0.4	1.3 ± 0.8	0.51 ± 0.24	0.40 ± 0.22
UDP ^d , CDP, UTP ^d , CTP, (U/C D/T P) ^e	2.8 ± 0.9	2.6 ± 1.1	1.7 ± 0.9	1.2 ± 0.5
NAD ⁺	2.2 ± 0.5	2.9 ± 0.5	2.0 ± 0.6	2.0 ± 0.4
NADP ⁺ d	nq	nq	nq	nq
Carboxylates				
Acetate	0.36 ± 0.12	0.6 ± 0.5	0.8 ± 0.9	0.5 ± 0.4
Citrate	nq	nq	nq	nq
Formate ^d	0.22 ± 0.07	0.14 ± 0.06	0.16 ± 0.06	0.13 ± 0.04
Fumarate	0.16 ± 0.04	0.21 ± 0.07	0.13 ± 0.03	0.14 ± 0.03
α-Ketoglutarate (α-KG)	0.24 ± 0.07	0.5 ± 0.4	0.27 ± 0.10	0.26 ± 0.07
Lactate (Lac)	1.1 ± 0.8	1.0 ± 0.6	0.8 ± 0.7	1.1 ± 0.7
Malate (Mal)	1.8 ± 0.5	1.6 ± 0.4	1.5 ± 0.5	1.6 ± 0.4
Pyruvate	nq	nq	nq	nq
Succinate (Succ)	0.41 ± 0.07	0.33 ± 0.08	0.29 ± 0.07	0.30 ± 0.06

(Continues)

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Metabolite	Perchloric acid (n = 13)	Methanol/water (n = 12)	Methanol/chloroform/water (n = 10)	Methanol (n = 13)
Other compounds				
Ethanol ^f	0.5 ± 0.3	0.38 ± 0.32	0.21 ± 0.11	0.23 ± 0.10
HEPES	12 ± 2	10 ± 4	7 ± 2	7 ± 3
Lipids	nq	nq	nq	nq
Putrescine (Put)	2.9 ± 0.8	0.4 ± 0.2	0.6 ± 0.4	0.15 ± 0.07
Spermidine (Spd)	5.7 ± 1.6	1.4 ± 0.5	1.0 ± 0.5	0.74 ± 0.24
Spermine (Spm)	0.52 ± 0.11	nq	nq	nq
Total metabolite concentration	70 ± 13	74 ± 19	49 ± 12	44 ± 9

^aThe estimated millimolar concentrations can be converted into millimoles/10¹⁵ cells by multiplying the concentration by a factor of 28 (L/10¹⁵ parasites). Concentrations (mM) were averaged from n replicates and are shown as mean ± SD.

^bnd, not detected; nq, not quantified.

^cThe compound was not detected in all of the samples from which the average was derived.

^dThe identification of these compounds was largely reliant on a limited number of spectral correlations or signals and should be regarded as tentative.

^eConcentration determined by comparing the integral of the NMR signal with that of the reference compound, TSP, as described in Experimental.

^fEthanol is a contaminant from the ethanol/dry ice bath used during the processing of the parasites prior to extraction.