

Table S1: Information on target analytes and respective abbreviations, including retention time on employed column and accurate mass of monoisotopic and its fully ¹³C labeled analogue.

Abbreviation	Compound Name	polarity	monoisotopic m/z	U ¹³ C analogue m/z	Column	Retention time [min]
Val	Valine	+	118.0863	123.1030	HILIC	1.7
Tyr	Tyrosine	+	182.0812	191.1114	HILIC	1.7
Ser	Serine	+	106.0499	109.0599	HILIC	1.8
Pro	Proline	+	116.0706	121.0874	HILIC	2.0
Thr	Threonine	+	120.0655	124.0789	HILIC	2.1
Gln	Glutamine	+	147.0764	152.0932	HILIC	2.2
Glu	Glutamate	+	148.0604	153.0772	HILIC	2.4
Arg	Arginine	+	175.1190	181.1391	HILIC	4.1
Lys	Lysine	+	147.1128	153.1329	HILIC	4.3
His	Histidine	+	156.0768	162.0969	HILIC	4.4
Asn	Asparagine	-	131.0462	135.0596	RPLC	2.2
Asp	Aspartate	-	132.0302	136.0437	RPLC	2.2
Ala	Alanine	-	88.0404	91.0505	RPLC	2.3
PEP	Phosphoenolpyruvate	-	166.9751	169.9852	RPLC	3.3
Pyr	Pyruvate	-	87.0088	90.0188	RPLC	3.5
Mal	Malate	-	133.0142	137.0277	RPLC	3.6
Val	Valine	-	116.0717	121.0885	RPLC	3.6
5'CMP	Cytidine 5'-monophosphate	-	322.0446	331.0748	RPLC	3.8
I-Cit	I-Citrate	-	191.0197	197.0399	RPLC	3.8
Lac	Lactate	-	89.0244	92.0345	RPLC	4.5
Met	Methionine	-	148.0438	153.0605	RPLC	4.6
AKG	α-Ketoglutarate	-	145.0142	150.031	RPLC	5.1
5'UMP	Uridine 5'-monophosphate	-	323.0286	332.0588	RPLC	5.8
5'AMP	Adenosine 5'-monophosphate	-	346.0558	356.0894	RPLC	6.1
Cit	Citrate	-	191.0197	197.0399	RPLC	6.2
NADP	Nicotinamide adenine dinucleotide phosphate	-	742.0682	763.1386	RPLC	6.5
Ile	Isoleucine	-	130.0874	136.1075	RPLC	6.5
5'GMP	Guanosine 5'-monophosphate	-	362.0507	372.0843	RPLC	6.6
NAD	Nicotinamide adenine dinucleotide	-	662.1018	683.1712	RPLC	6.7
Leu	Leucine	-	130.0874	136.1075	RPLC	6.7
Fum	Fumarate	-	115.0037	119.0171	RPLC	6.8
Suc	Succinate	-	117.0193	121.0328	RPLC	6.9
3'AMP	Adenosine 3'-monophosphate	-	346.0558	356.0894	RPLC	7.2
Aco	Aconitate	-	173.0092	179.0293	RPLC	7.5
Phe	Phenylalanine	-	164.0717	173.1019	RPLC	8.9
Trp	Tryptophane	-	203.0826	214.1195	RPLC	10.5
Ri-Fl	Riboflavine	-	375.1310	392.1880	RPLC	13.6

Table S2: Results on recovery of the off-line dispersive SPE method using a mixture of C18 and strong cationic exchange bulk material for clean-up measured on an LC-MS/MS System (consisting of a Thermo Scientific CTC PAL autosampler, Thermo Scientific Accela 1259 pump, and Thermo Scientific TSQ Vantage ESI-MS/MS). Samples were prepared in 5 replicates and recovery was determined comparing the ratio of the monoisotopic m/z signal (= internal standards) and uniformly labeled analogue. For compounds marked with an asterisk "*", the signal of the internal standard was too low or saturated and therefore determination of recovery was based on comparison of U¹³C intensities instead.

Metabolite	Average [%]	SD [%]
3AMP (RPLC)	84.7	6.6
5AMP (RPLC)	92.5	3.7
5CMP (RPLC)*	84.7	6.6
5GMP (RPLC)	89.2	26.3
AKG (RPLC)*	87.3	34.8
Ala (HILIC)*	89.2	26.3
Arg (HILIC)	92.8	2.5
Asn (HILIC)	75.7	19.9
Asp (HILIC)	94.9	10.7
Cit (RPLC)	87.0	1.5
Fum (RPLC)*	97.3	15.8
Gln (HILIC)	94.9	5.9
Glu (HILIC)	91.9	9.3
His (HILIC)	91.4	2.8
Ile (RPLC)	97.9	7.5
Leu(RPLC)	94.6	12.8
Lys (HILIC)	90.2	2.1
Mal (RPLC)	92.2	17.3
Met (RPLC)	93.2	14.5
NAD ⁺ (RPLC)*	68.5	4.8
NADP (RPLC)*	52.5	5.5
Phe (RPLC)*	92.5	3.7
Pro (HILIC)*	116.5	13.7
Ser (HILIC)	93.0	13.1
Suc (RPLC)*	91.3	18.2
Thr (HILIC)	97.7	8.6
Trp (RPLC)	89.7	3.0
Tyr (HILIC)	88.2	8.0
Val (HILIC)	91.8	9.3
Val (RPLC)	97.0	5.7

Table S3: Recoveries of metabolites in CHO cell culture supernatants during pre-experiments for clean-up procedure given in percent. All values were estimated with single experiments and therefore have an estimated RSD of 20%. F: Filtration, SA: Solid phase extraction with strong cationic exchanger, C4: Solid phase extraction with C₄ material, C18: Solid phase extraction with C₁₈ material. Separation with “-“ marks sequential application of preparation steps. Due to low signal intensity of the ¹³C analogue, recovery values that are underlined are assessed by direct comparison of intensities of the monoisotopic mass. For chromatographic peaks showing a signal to noise ratio (S/N) of < 10, no values were reported.

Meta- bolites	F-SA-C18			F-C4		F-C4 (H ₂ O)		F-C4 (pH 4.5)		F-C4 (pH 8)
	F	SA	C18	F	C4	F	C4	F	C4	
PEP	88.9	77.8	22.2	87.6	70.3	100.0	135.0	104.9	109.9	88.0
Pyr	100.0	69.8	11.1	100.0	80.0	100.0	71.4	101.6	100.0	99.0
Cit	100.0	71.4	1.7	92.9	68.1	100.0	97.5	102.0	103.4	81.6
Iso-Cit	100.0	50.0	8.3	68.8	68.9	100.0	95.2	96.2	103.9	110.0
Aco	100.0	73.6	0.5	90.0	76.5	100.0	108.9	94.7	93.3	84.0
Akg	111.1	56.0	7.5	88.9	61.1	100.0	87.5	117.3	115.4	88.9
Suc	100.0	62.5	4.9	90.9	66.3	100.0	117.9	107.0	108.9	83.5
Fum	91.7	64.7	4.1	85.8	69.2	96.7	99.7	115.4	106.9	91.6
Mal	105.6	73.3	14.6	97.5	68.1	100.0	100.6	103.5	99.7	92.7
Met	<u>100.0</u>	<u>2.0</u>	<u>4.0</u>	82.2	48.9	97.2	90.0	105.2	87.3	88.9
Val	<u>100.0</u>	<u>0.0</u>	<u>0.0</u>	68.2	49.4	117.5	107.7	89.7	97.1	89.0
Ile	133.3	0.0	0.0	114.1	72.6	83.3	109.7	< S/N 10		80.0
Leu	100.0	0.0	0.0	123.5	93.1	83.3	105.0	<u>126.0</u>	<u>145.6</u>	111.1
Phe	100.0	0.0	0.0	90.2	38.6	100.0	71.3	114.3	86.4	79.9
Tyr	111.8	0.0	0.0	92.7	54.6	100.0	83.1	87.7	73.4	84.2
Trp	100.0	0.0	0.0	92.9	7.8	100.0	49.1	113.3	46.3	37.0
Ri-Fl	<u>100.0</u>	<u>0.0</u>	<u>0.0</u>	<u>92.9</u>	<u>0.0</u>	<u>107.1</u>	<u>0.0</u>	< S/N 10		<u>0.0</u>
NAD	100.0	0.0	0.0	78.3	28.2	104.6	74.2	60.9	49.2	66.2
NADP	177.8	28.0	0.4	100.0	57.7	100.0	85.9	98.5	28.7	75.0
3AMP	<u>107.7</u>	<u>0.0</u>	<u>0.0</u>	<u>97.0</u>	<u>6.7</u>	<u>100.2</u>	<u>82.7</u>	< S/N 10		<u>42.0</u>
5AMP	<u>100.0</u>	<u>0.0</u>	<u>0.0</u>	88.6	31.6	< S/N 10		< S/N 10		69.1
5CMP	<u>100.0</u>	<u>26.7</u>	<u>7.1</u>	<u>96.5</u>	<u>74.3</u>	<u>99.9</u>	<u>114.5</u>	<u>99.5</u>	<u>123.7</u>	<u>96.0</u>
5GMP	<u>100.0</u>	<u>10.0</u>	<u>0.0</u>	<u>97.4</u>	<u>68.3</u>	< S/N 10		<u>79.0</u>	<u>94.1</u>	102.7
5UMP	<u>100.0</u>	<u>67.5</u>	<u>6.0</u>	<u>94.5</u>	<u>66.8</u>	<u>102.9</u>	<u>114.0</u>	<u>108.7%</u>	<u>79.6</u>	82.7

Table S4: Assessment of recovery in % of target analytes in a CHO cell supernatants employing the described on-line clean-up procedure, consisting of filtration and solid phase extraction with C₄ material. *n*=5, if not stated otherwise. For isoleucine and leucine, the monoisotopic *m/z* signal (=internal standard) was only present in low abundance, therefor in addition to calculated ratios of internal standard (IS) to target mass, also recovery based only on comparison of intensities is shown (indicated as “without IS”)

Metabolites	Average [%]	SD [%]
PEP	83.6	5.8
Pyr	83.3	5.1
Cit	85.8	6.8
I-Cit	89.1	5.9
Aco	87.2	19.8
AKG (n=4)	92.5	5.7
Suc	84.6	3.3
Fum	90.5	3.6
Mal (n=4)	92.6	5.4
Ri-FI	-	-
NAD	65.0	10.6
NADP	86.7	4.3
3AMP	45.0	13.7
5AMP	78.2	17.8
5CMP	92.2	7.4
5GMP	85.5	5.6
5UMP	92.2	5.6
Ala RPLC	125.6	37.4
Arg HILIC	97.6	21.4
Asn RPLC	128.2	19.5
Asp RPLC	90.4	6.4
Gln HILIC	116.7	7.7
Glu HILIC	120.7	8.0
His HILIC	106.3	8.3
Lys HILIC	103.2	12.5
Pro HILIC	107.5	8.0
Ser HILIC	138.7	25.2
Thr HILIC	114.2	11.3
Met RPLC	94.3	8.3
Val HILIC	114.7	12.8
Ile RPLC	156.4	33.3
Ile without IS	109.5	5.5
Leu RPLC	94.7	32.5
Leu without IS	61.0	3.0
Phe RPLC	100.7	27.4
Tyr HILIC	91.3	7.6
Trp RPLC	45.5	8.8

