

## LC-MS/MS analysis of amino acids and amino metabolites

Targeted profiling of amino acids and amino metabolites was conducted at the Mayo Clinic by LC-MS as previously described<sup>53,54</sup>. Briefly, either 20  $\mu$ L of plasma samples or 5 mg of tissue homogenates were spiked with an internal standard solution consisting of isotopically labeled amino acids (U-<sup>13</sup>C<sub>4</sub> L-aspartic acid, U-<sup>13</sup>C<sub>3</sub> alanine, U-<sup>13</sup>C<sub>4</sub> L-threonine, U-<sup>13</sup>C L-proline, U-<sup>13</sup>C<sub>6</sub> tyrosine, U-<sup>13</sup>C<sub>5</sub> valine, U-<sup>13</sup>C<sub>6</sub> leucine, U-<sup>13</sup>C<sub>6</sub> phenylalanine, U-<sup>13</sup>C<sub>3</sub> serine, U-<sup>13</sup>C<sub>5</sub> glutamine, U-<sup>13</sup>C<sub>2</sub>glycine, U-<sup>13</sup>C<sub>5</sub> glutamate, U-<sup>13</sup>C<sub>6</sub>, <sup>15</sup>N<sub>2</sub> lysine, U-<sup>13</sup>C<sub>5</sub>, <sup>15</sup>N methionine, 1,1U-<sup>13</sup>C<sub>2</sub> homocysteine, U-<sup>13</sup>C<sub>6</sub> arginine, U-<sup>13</sup>C<sub>5</sub> ornithine, <sup>13</sup>C<sub>4</sub> asparagine, <sup>13</sup>C<sub>2</sub> ethanolamine, d3 sarcosine, d6 4-aminobutyric acid) . The supernatant was immediately derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate using a MassTrak kit (Waters). A 10-point calibration standard curve underwent a similar derivatization procedure after the addition of internal standards. Both derivatized standards and samples were analyzed on a Quantum Ultra triple quadrupole mass spectrometer (ThermoFischer) coupled with an Acquity liquid chromatography system (Waters). The LC gradient is shown in Table 6. Buffer A was 0.1% formic acid, 1% Acetonitrile, 99% Water and Buffer B was 1% water, 99% Acetonitrile. Data acquisition was conducted by using selected ion monitoring (SRM) in positive ion mode using transitions shown in Table 7. Concentrations of 42 analytes of each unknown were calculated against their respective calibration curves.

Table 6. LC-MS gradient program

Time	%A	%B	Flow (mL/min)
initial	99.9	0.1	0.400
1.00	99.9	0.1	0.400
2.00	99.9	0.1	0.400
5.50	98.1	1.9	0.400
6.50	98.0	2.0	0.400
10.00	97.5	2.4	0.400
12.00	96.0	4.0	0.400
20.00	88.0	12.0	0.400
27.00	86.5	13.5	0.400
31.00	80.0	20.0	0.400
32.00	2.0	98.0	0.500
35.00	2.0	98.0	0.500
35.50	99.9	0.1	0.400
39.00	99.9	0.1	0.400

Table 7. SRM transitions

Compound	Abbrev.	Prec ion scan 8-8-13	Prod Ion	I.S.
Ammonia_188	ammon	188	171	u-arg

Histidine_163	his	164	171	u-arg
Hydroxyproline_302	hyPro	302	171	u-arg
1-Methylhistidine_340	1MH	340	171	u-arg
3-Methylhistidine_340	3MH	340	171	u-arg
Asparagine_303	Asn	303	171	u-asn
<b>U-<sup>13</sup>C<sub>4</sub>-Asparagine_307</b>	<b>u-Asn</b>	<b>307</b>	171	
Phosphoethanolamine_312	PEA	312	171	u-arg
Arginine_345	arg	345	171	u-arg
<b>U-<sup>13</sup>C<sub>6</sub>-Arginine_351</b>	<b>u-arg</b>	<b>351</b>	171	
Carnosine_199	carn	199	171	u-gly
Taurine_296	tau	296	171	u-gly
Anserine_206	ans	206	171	U- <sup>13</sup> C <sub>3</sub> -ser
Serine_276	ser	276	171	U- <sup>13</sup> C <sub>3</sub> -ser
<b>U-<sup>13</sup>C<sub>3</sub>-Serine_279</b>	<b>u-ser</b>	<b>279</b>	171	-
Glutamine_317	gln	317	171	u-gln
<b>U-<sup>13</sup>C<sub>5</sub>-Glutamine_322</b>	<b>u-gln</b>	<b>322</b>	171	-
Ethanolamine_232	EA	232	171	u-EA
<b>U-<sup>13</sup>C<sub>2</sub>-Ethanolamine_234</b>	<b>u-EA</b>	<b>234</b>	171	
Glycine_246	gly	246	171	u-gly
<b>U-<sup>13</sup>C<sub>2</sub>-Glycine_249</b>	<b>u-gly</b>	<b>249</b>	171	-
Aspartic Acid_304	asp	304	171	u-asp
<b>U-<sup>13</sup>C<sub>4</sub>-Aspartic acid_308</b>	<b>u-asp</b>	<b>308</b>	171	-
Sarcosine_260	sar	260	171	d3-sar
<b>d3-Sarcosine_263</b>	<b>d3-sar</b>	<b>263</b>	171	
Citrulline_346	cit	346	171	u-glu
Glutamic Acid_318	glu	318	171	u-glu
<b>U-<sup>13</sup>C<sub>5</sub>-glutamic acid_323</b>	<b>u-glu</b>	<b>323</b>	171	-
beta-Alanine_260	b-ala	260	171	u-glu
Threonine_290	thr	290	171	u-thr
<b>U-<sup>13</sup>C<sub>4</sub>-threonine_294</b>	<b>u-thr</b>	<b>294</b>	171	-
Alanine_260	ala	260	171	u-ala
U- <sup>13</sup> C <sub>3</sub> -alanine_263	<b>u-ala</b>	<b>263</b>	171	-
gamma-Amino-N-butyric-acid_274	gaba	274	171	d6-gaba
<b>d6-gamma-Amino-N-butyric-acid_280</b>	<b>d6-gaba</b>	<b>280</b>	171	
alpha-Aminoadipic-acid_332	aada	332	171	u-ala
beta-Aminoisobutyric-acid_274	baib	274	171	u-ala
Proline_286	pro	286	171	u-pro
<b>u-pro_291</b>	<b>u-pro</b>	<b>291</b>	171	-
Hydroxylysine 1_252	HL 1	252	171	u-pro
Hydroxylysine 2_252	HL 2	252	171	u-pro
alpha-Amino-N-butyric-acid_274	aaba	274	171	u-val
Ornithine_237	orn	237	171	u-orn
<b>U<sup>13</sup>C<sub>5</sub>-Ornithine_239</b>	<b>u-orn</b>	<b>239</b>	171	-
Cystathionine 1_282	cyst1	282	171	u- <sup>15</sup> N <sub>2</sub> -Lys

dervitizing agent_315	deriv agent	315	171	u-tyr
Cystathionine 2_282	cyst2	282	171	u- <sup>15</sup> N <sub>2</sub> -Lys
Lysine_244	lys	244	171	u- <sup>15</sup> N <sub>2</sub> -Lys
<b>U<sup>13</sup>-C<sub>6</sub>, <sup>15</sup>N<sub>2</sub>-Lysine_248</b>	<b>u-<sup>15</sup>N<sub>2</sub>-Lys</b>	<b>248</b>	171	-
Cystine_291	cys	291	171	u-tyr
Tyrosine_352	tyr	352	171	u-tyr
<b>U<sup>13</sup>-C<sub>6</sub>-Tyrosine_358</b>	<b>u-tyr</b>	<b>358</b>	171	-
Methionine_320	met	320	171	u- <sup>15</sup> N <sub>2</sub> -met
<b>U<sup>13</sup>-C<sub>5</sub>, <sup>15</sup>N<sub>2</sub>-met_326</b>	<b>u-<sup>15</sup>N<sub>2</sub>-met</b>	<b>326</b>	171	-
Valine_288	val	288	171	u-val
<b>U<sup>13</sup>C<sub>5</sub>-Valine_293</b>	<b>u-val</b>	<b>293</b>	171	-
Isoleucine_302	ileu	302	171	u-leu
allo-Isoleucine_302	ailou	302	171	u-leu
Homocystine_306	hcys	305	171	<sup>13</sup> C <sub>2</sub> -hys
<b>1,1-<sup>13</sup>C<sub>2</sub>-Homocystine_308</b>	<b>1,1-<sup>13</sup>C<sub>2</sub>-h</b>	<b>306</b>	171	-
Leucine_302	leu	302	171	u-leu
<b>U<sup>13</sup>C<sub>6</sub>-Leucine_302</b>	<b>u-leu</b>	<b>308</b>	171	-
Phenylalanine_335	phe	335	171	u-phe
<b>U<sup>13</sup>C<sub>6</sub>-Phenylalanine_342</b>	<b>u-phe</b>	<b>342</b>	171	-
Tryptophan_375	trp	375	171	u-phe