MSU MSMC Protocol 2a

Free amino acid analysis - UPLC/MS/MS method

(last edited 5/13/20)

Sample prepared using protocol *MSU_MSMC_002 Free Amino Acid extraction*

The only instrument in the facility that this method can be run on is the <u>Quattro Micro</u>. No other instrument should ever have 10 mM perfluoroheptanoic acid (PFHA) in water as a mobile phase.

A template Masslynx project (Amino Acids.PRO) is located on the Quattro Micro and should be used to create new users' projects for amino acid analyses.

<u>Sample List Preparation</u>: Samples should be analyzed by first analyzing 1-2 reagent blanks, calibration standards in order of increasing concentration, then sample extracts, usually organized in random order (the RANDBETWEEN function in Microsoft Excel can be used for this purpose). It is recommended that pooled quality control samples or reference standard samples be analyzed after approximately 10 "real samples".

<u>Protocol for preparing mobile phase solvent A (10 mM PFHA in water):</u> Weigh 3.64 g of perfluoroheptanoic acid (PFHA, Aldrich part # 342041-25G), into a 50-mL polypropylene Falcon centrifuge tube (***do not use glass vessels with PFHA except for the final solvent bottle***), and dissolve in 20 mL of Milli-Q water, gently vortexing to ensure complete dissolution. Transfer the liquid to a 1 L glass solvent bottle. Repeat addition of 20 mL water two more times and combine these washes in the same 1 L bottle. Add 940 mL of Milli-Q water to make 1 L of 10 mM aqueous PFHA. Solvent B is acetonitrile (no additives).

<u>HPLC column:</u> Acquity UPLC HSS T3, 2.1 x 100 mm, 1.7 μm particle size. (Waters part # 186003539). Use with 0.2 μm pre-column filter [(Waters part # 205000343 (Kit, Acquity col. In-line Filter)]

<u>Mobile phase solvent:</u> A) 10 mM PFHA in Water, B) Acetonitrile

<u>LC gradient (linear gradient, slope setting = 6):</u>

Time (min)	Flow rate (mL/min)	%A	%B
0.00	0.300	100	0
1.00	0.300	100	0
8.00	0.300	35	65

8.01	0.300	10	90
9.00	0.300	10	90
9.01	0.300	100	0
13.00	0.300	100	0

Column Temp: 40°C

Autosampler Temp: 10°C

Injection volume: 10 µL

Tune Page parameters: (For Quattro Micro) (AMS2.ipr)

Ionization method: electrospray ionization; standard ESI probe

- Ionization method: electrospray ionization; standard ESI probe
- Capillary voltage: 1.0 kV (Positive ion mode)
- Source Temp: 120°C
- Desolvation Temp: 350°C
- Desolvation gas: 800 L/hr
- Cone gas: 40L/hr

MS/MS methods:

There are two MS method files that can be used (and modified as necessary). For the 20 standard amino acids only, use the method '13min_Amino acids_MRM'. To include several other related metabolites (listed in the MRM table below), select the 'GLBRC_amino acid method 26 Jun 2019'.

The MS method can include settings to divert flow to waste and it is suggested to use the following settings to reduce the amount of PFHA building up in the source.

Solvent Delay:

- Enable divert valve. Set flow to waste from 0-0.5 min and from 9-13 min.

List of MRM channels

Positive ion mode (Function 1) (Retention time: 0.00 - 4.50 min)					
Compound	**Parent ion (*m/z*)**	**Daughter ion (*m/z*)**	**Cone voltage**	**Collision voltage**	**Approx. retention time (min)**
Glycine	76	30	17	8	2.05
[$^{13}C_2$,^{15}N]-Glycine	79	32	17	8	2.05

Alanine	90.1	44	35	17	3.54
[$^{13}C_3$,^{15}N]-Alanine	94.1	47.1	17	8	3.54
Serine	106.1	60	30	10	1.79
[$^{13}C_3$,^{15}N]-Serine	110.1	63	19	10	1.79
Homoserine	120	74	16	10	2.44
Threonine	120.1	74	19	8	2.46
[$^{13}C_4$,^{15}N]-Threonine	125.1	78.1	19	8	2.46
Cysteine	122	76	18	15	2.32
[$^{13}C_3$,^{15}N]-Cysteine	126	79	18	15	2.32
Asparagine	133.1	74	35	14	1.79
Aspartic Acid	134.1	74	35	10	1.42
[$^{13}C_4$,^{15}N]-Aspartic Acid	139.1	77	19	11	1.42
O-Acetylserine	148	106	16	10	2.93
Glutamine	147.1	84	35	14	2.24
Glutamic Acid	148.1	84	34	14	2.01
[$^{13}C_5$,^{15}N]-Glutamine	154.1	89.1	17	14	2.24
N-Acetyl-L-cysteine	164.08	121.83	16	10	1.28
Glutathione (GSH)	308.2	179.1	22	10	3.29

Positive ion mode (Function 2) (Retention time: 4.50 - 6.50 min)					
Compound	**Parent ion (*m/z*)**	**Daughter ion (*m/z*)**	**Cone voltage**	**Collision voltage**	**Approx. retention time (min)**
N,*N*-Dimethylglycine	104	58	16	10	4.22
Choline	104	61	35	16	6.43
GABA	104.07	87	35	10	5.86
Glycine Betaine	118.1	59	35	16	4.98
Proline	116	70	35	10	5.13
[$^{13}C_5$,^{15}N]-Proline	122.1	75.1	35	10	5.13
Valine	118.1	72	35	9	6.05
[$^{13}C_5$,^{15}N]-Valine	124.1	77.1	35	9	6.05
Homocysteine	136.1	90	22	10	4.48
Trigonelline	138.1	92	28	22	5.66
Methionine	150.1	104	19	9	5.89
[$^{13}C_5$,^{15}N]-Methionine	156.1	109.1	19	9	5.89
N-acetyl-ornithine	175.21	115	16	10	5.69

Citrulline	176	70	16	28	4.63
Tyrosine	182.1	136.1	20	12	5.44
[$^{13}C_9$,^{15}N]-Tyrosine	192.1	145.1	20	12	5.44
Succinyl homoserine	220	102	16	16	4.72

Positive ion mode (Function 3) (Retention time: 6.50 - 13.00 min)					
Compound	**Parent ion (*m/z*)**	**Daughter ion (*m/z*)**	**Cone voltage**	**Collision voltage**	**Approx. retention time (min)**
Agmatine	131	72	46	16	8.00
Isoleucine and Leucine	132.1	86	35	9	6.76 and 6.90
[$^{13}C_5$,^{15}N]-Leucine	139.1	92	35	9	6.90
Ornithine	133	70	16	16	7.63
Lysine	147.1	84	19	14	7.70
[$^{13}C_6$,$^{15}N_2$]-Lysine	155.1	90.1	19	14	7.70
Histidine	156.1	110	20	12	7.73
[$^{13}C_6$,$^{15}N_3$]-Histidine	165.1	118.1	20	12	7.73
Phenylalanine	166.1	120	20	10	6.98
[$^{13}C_9$,^{15}N]-Phenylalanine	176.1	129.1	20	10	6.98
Arginine	175.1	70	24	18	7.85
[$^{13}C_6$,$^{15}N_4$]-Arginine	185.1	75	24	18	7.85
Tryptophan	205.1	146	19	14	7.04
[$^{13}C_{11}$,$^{15}N_2$]-Tryptophan	218.1	156	19	14	7.04
Cystathionine	223	134	16	13	7.37
Cystine	241.1	74	16	28	7.17
Argininosuccinate	291.24	69.84	22	34	7.57
GSSG	613.4	355	22	16	6.90

Notes:

- Retention time windows are for the basic 13min_Amino acids_MRM method that measures only the 20 standard amino acids. These are different for the more comprehensive method containing MRM channels for all compounds.
- Retention times are more susceptible to drift with ion-pairing chromatography especially if the PFHA mobile phase is a slightly different concentration.
- Dwell times should be adjusted based on number of channels in each function and the abundance of the compound in the samples being analyzed.