Amino acid analysis by UHPLC-MS/MS

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Abstract

A method allowing the simultaneous quantitation of 21 amino acids in dry leaf tissue using liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) in multiple reaction monitoring (MRM) mode.

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Before start

Samples, standards and solvents need to be freshly prepared.

Only use water and solvents of UHPLC-MS/MS grade.

Materials

- Heptaflurobutyric acid HPLC Grade 53104 by Thermo Fisher Scientific
- Formic acid, LC-MS grade 28905 by Thermo Fisher Scientific
- Acetonitrile, LC-MS grade 51101 by Thermo Fisher Scientific
- Water, LC-MS grade 51140 by Thermo Fisher Scientific
- ✓ LAA-21 200-157-7 by Contributed by users

Protocol

Sample and standard preparation

Step 1.
Free amino acid extraction is performed from 5 mg of dried leaf tissue in 1 ml of water.

Step 2.

Cystine is added to the samples at 2 mg L^{-1} as an internal standard.

Step 3.
After homogenization by vortexing, samples are incubated for 10 min at room temperature, centrifuged at 10.000 xg for 10 min, and supernatants filtered through 0.45 µm pore membrane

filters.

Step 4.

Standards are prepared in water by spiking the 21 amino acids at concentrations from 0.25 to 10 mg L^{-1}.

Quantitation of amino acids by multiple reaction monitoring

Step 5.
The amino acid analysis is carried out on an Agilent 1290 Infinity UHPLC System coupled to an Agilent 6490 triple quadrupole mass spectrometer through an Agilent Jet Stream ion source in positive ionization mode.

Step 6.
Separation of analytes is performed on an Agilent Zorbax Extend-C18 column (2.1 × 50 mm, 1.8 µm), which is maintained at 25ºC during the analysis.

Step 7.
Mobile phase consists in solvent A (0.05% formic acid and 0.03% heptafluorobutyric acid (HFBA) in water) and solvent B (0.05% formic acid and 0.03% HFBA in acetonitrile).

Step 8.
Gradient employed: 0 min 0% B, 2.5 min 0% B, 5.5 min 40% B, 5.60 min 90% B, 6 min 90% B; at a constant flow rate of 0.4 mL.min-1.

Step 9.
For glutamate quantification a specific chromatographic method is needed consisting in: solvent A (0.5% formic acid and 0.3% HFBA in water) and solvent B (0.5% formic acid and 0.3% HFBA in acetonitrile) using the gradient 0 min 0% B, 2.5 min 0% B, 3 min 40% B, 3.5 min 90% B, 4 min 90% B; at a constant flow rate of 0.4 mL/min.

Step 10.
In all cases the injection volume is 1 µL.

Step 11.

Multiple reaction monitoring (MRM) analysis mode is used to monitor the transitions from precursor ions to dominant product ions.

Step 12.

The optimized source parameters are: gas curtain temperature 275ºC, gas flow 11 L min^{-1}, cell acceleration voltage 2 V, nebulizer pressure 50 psi, capillary voltage 3000 V and dwell time 10 ms.

Step 13.

The specific MRM transitions used for quantitation of each amino acid and the optimized MRM parameters, such as fragmentor voltage and collision energy, are summarized in the following table:

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	CE (V)
Alanine	0.521	90,09	**44,05** 42	380 380	10 10
Arginine	1.538	175,2	**70** 60,06	380 380	16 25

Asparagine	0.424	133,1	87,1 **74,1**	380 380	4 10
Aspartate	0.442	134,1	88 **74**	380 380	5 10
Cisteine	0.509	122	76 **59**	380 380	25 25
Cystine	0.484	241	**151,9** 195	380 380	4 4
Glycine	0.447	76,07	51,7 **29,9**	380 380	4 30
Glutamate	0.8	148,1	130,1 **84,1**	380 380	4 15
Glutamine	0.450	147,1	130,1 **84,1**	380 380	15 5
Hydroxyproline	0.434	132	**85,9** 68	380 380	4 4
Histidine	0.822	156,1	**109,9** 93,04	380 380	25 25
Isoleucine	4.224	132,2	**69,1** 56,9	380 380	4 20
Leucine	4.289	132,2	**86,1** 54,9	380 380	4 20
Lysine	0.9	146,9	130,08 **83,9**	380 380	25 25
Methionine	2.5	150,2	104,05 **56,05**	380 380	25 25
Phenylalanine	4.5	166,2	**119,9** 103,06	380 380	25 25
Proline	0.641	116,1	**70** 68,05	380 380	25 25
Serine	0.436	106,1	88,04 **60,05**	380 380	15 25
Threonine	0.491	120,1	74,01 **56,01**	380 380	20 20
Tryptophan	4.843	205,2	**188** 146	380 380	4 16
Tyrosine	4.089	182	**136** 91	380 380	10 20
Valine	2.110	118,1	72 **55,06**	380 380	25 25