

# Chapter 2

## Combination of an AccQ-Tag-Ultra Performance Liquid Chromatographic Method with Tandem Mass Spectrometry for the Analysis of Amino Acids

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### Abstract

Amino acid analysis is a powerful tool in life sciences. Current analytical methods used for the detection and quantitation of low abundance amino acids in complex samples face intrinsic challenges such as insufficient sensitivity, selectivity, and throughput. This chapter describes a protocol that makes use of AccQ-Tag chemical derivatization combined with the exceptional chromatographic resolution of ultra performance liquid chromatography (UPLC), and the sensitivity and selectivity of tandem mass spectrometry (MS/MS). The method has been fully implemented and validated using different tandem quadrupole detectors, and thoroughly tested for a variety of samples such as *Plasmodium falciparum*, human red blood cells, and *Arabidopsis thaliana* extracts. Compared to currently available methods for amino acid analysis, the AccQ-Tag UPLC-MS/MS method presented here provides enhanced sensitivity and reproducibility, and offers excellent performance within a short analysis time and a broad dynamic range of analyte concentration. The focus of this chapter is the application of this improved protocol for the compositional amino acid analysis in *A. thaliana* leaf extracts using the Xevo TQ for mass spectrometric detection.

**Key words:** 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, AccQ-Tag, Amino acid analysis, UPLC-ESI-MS/MS

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### 1. Introduction

The importance of quantitative analysis of amino acids in life sciences and food industry cannot be emphasized enough. Stein et al. (1) initially introduced amino acid analysis in the late 1950s, and since then many analytical platforms for amino acids analysis have been developed. These approaches include the combination of different separation strategies, such as capillary electrophoresis, liquid chromatography, or gas chromatography, coupled with ultraviolet,

fluorescence, electrochemical, and mass spectrometry detection systems (2–8). In general, currently employed amino acid analyses can be divided into three major categories: (a) direct analysis of free amino acids, (b) separation of free amino acids by ion-exchange chromatography followed by postcolumn derivatization (e.g., with ninhydrin or *o*-phthalaldehyde), and (c) precolumn derivatization techniques (e.g., with phenyl isothiocyanate; 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole, or 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, AQC). Direct analysis methods are attractive due to high throughput and simplicity. Nevertheless, sensitivity of analysis can be compromised as a result of matrix interferences. On the other hand, precolumn derivatization techniques may be influenced by buffer salts in the samples and may also result in multiple derivatives of a given amino acid, which have to be considered in the result interpretation. Despite that, precolumn derivatization techniques are very sensitive and, unlike their postcolumn counterparts, usually require very small sample size per analysis and result in better throughput. In general, precolumn derivatization, combined with reversed-phase separation, offers greater efficiency, ease of use, and higher speed of analysis than the conventional ion-exchange techniques (9).

Among the instrumental methods based on precolumn derivatization for the analysis of free amino acids, high-performance liquid chromatography (HPLC) and ultra performance liquid chromatography (UPLC) combined with AccQ·Tag technology continue to gain acceptance and recognition for their improved limits of detection, superior efficiency and sensitivity, and reduced analysis time (4, 6–12). AccQ·Tag technology uses AQC which reacts with primary and secondary amines to form highly stable fluorescent urea derivatives. During the reaction the excess reagent is rapidly hydrolyzed to yield 6-aminoquinoline (AMQ), *N*-hydroxysuccinimide, and carbon dioxide. AccQ·Tag-derivatization followed by reverse-phase liquid chromatographic (RPLC) separation of the less-polar derivatives is readily amenable to mass spectrometry.

A method for amino acid analysis using precolumn AccQ·Tag derivatization followed by UPLC separation coupled to electrospray ionization tandem mass spectrometry (ESI-MS/MS) with multiple reaction monitoring (MRM) detection has been previously described by our research group using the Waters Acquity TQD system (9). This UPLC-MS/MS MRM quantitation method was further improved and implemented in the Waters Xevo TQ mass spectrometer for increased sensitivity in amino acid detection and quantitation (13). The latest method, herein described, resulted in improved detection limits ranging from 10.2 amol to 23.1 fmol (on column), as well as enhanced linear dynamic range. The application of this method to the analysis of amino acids in *Arabidopsis thaliana* is illustrated.

## 2. Materials

### 2.1. AccQ·Tag Ultra Amino Acid Derivatization

1. Four-block digital readout dryblock heater (VWR 13259-056 or similar).
2. Refrigerated benchtop shaker-incubator (Lab-Line MaxQ 4000, Barnstead or similar).
3. Limited volume polypropylene vial: 250  $\mu$ L.
4. Clear glass, screw-thread vials: 2 mL.
5. AccQ·Tag Ultra Reagent Powder (Waters Corporation): AQC derivatizing reagent, dry powder.
6. AccQ·Tag Ultra Borate Buffer (Waters Corporation): Borate derivatization buffer used to ensure optimum pH (8.8) for derivatization.
7. AccQ·Tag Ultra Reagent Diluent (Waters Corporation): Acetonitrile (LC-MS grade) for reagent reconstitution.
8. Derivatizing reagent solution: Tap AccQ·Tag Ultra Reagent Powder vial and add 1 mL of AccQ·Tag Ultra Reagent Diluent. Vortex. Heat on top of heating block at 55°C for up to 15 min and vortex occasionally to complete solubilization (see Note 1).
9. Methanol-water mixture, 50:50 v/v: In a clean glass container, mix 50 mL of LC-MS grade water and 50 mL of LC-MS grade methanol.
10. Stable-isotope-labeled reference compounds (internal standards): L-asparagine- $^{15}\text{-N}_2$ ; L-serine,2,3,3- $\text{-d}_3$ ; L-glutamine-2,3,3,4,4- $\text{-d}_5$ ; glycine- $\text{-d}_5$ ; D-L-alanine-2,3,3,3- $\text{-d}_4$ ; proline-2,5,5- $\text{-d}_3$ ; methionine-methyl- $\text{-d}_3$ ; tryptophan-2',4',5',6',7'- $\text{-d}_5$  (indole- $\text{-d}_5$ ); leucine- $\text{-d}_{10}$ ; valine- $\text{-d}_8$ ; L-histidine (ring 2- $^{13}\text{C}$ ); L-glutamic acid-2,4,4- $\text{-d}_3$ ; ornithine-3,3,4,4,5,5- $\text{-d}_6$ ; lysine-3,3,4,4,5,5,6,6- $\text{-d}_8$ ; phenyl- $\text{-d}_5$ -alanine (Cambridge Isotope Laboratories, Andover, MA, USA and CDN isotopes, Pointe-Claire, Quebec, Canada).
11. Internal standard stock solutions (1 mg/mL): Prepare 1 mg/mL solutions of individual internal standards in LC-MS grade water into an externally threaded cryotube vial. Store solutions at  $-80^\circ\text{C}$ .
12. Internal standard mixture (30  $\mu\text{g/mL}$ ): In a 25-mL volumetric flask, mix 750  $\mu\text{L}$  each from the stock solutions of internal standards. Make up to final volume with 50% v/v methanol-water mixture. Leave one aliquot for current use and store remaining aliquots at  $-80^\circ\text{C}$  in amber borosilicate glass vials with screw caps.
13. Internal standard mixture (8  $\mu\text{g/mL}$ ): Add 2,670  $\mu\text{L}$  of the 30  $\mu\text{g/mL}$  internal standard mixture into a 10-mL volumetric flask. Make up to final volume with 50% v/v methanol-water

mixture. Divide the solution into working aliquots. Leave one aliquot for current use and store remaining aliquots at  $-80^{\circ}\text{C}$  in amber borosilicate glass vials with screw caps.

14. Extraction buffer with internal standards ( $4\text{ }\mu\text{g/mL}$ ): Add  $6.7\text{ mL}$  of the  $30\text{ }\mu\text{g/mL}$  internal standard mixture into a  $50\text{-mL}$  volumetric flask. Make up to a final volume with  $50\%$  v/v methanol–water mixture. Divide the solution into working aliquots and store at  $-80^{\circ}\text{C}$  in amber borosilicate glass vials with screw caps (see Note 2).
15. Amino acid calibration stock solution ( $0.5\text{ }\mu\text{mol/mL}$  in  $0.2\text{N}$  lithium citrate buffer). Available from Sigma-Aldrich, Inc; Product No. A9906 (see Note 3).
16. Working calibration mixture ( $0.25\text{ }\mu\text{mol/mL}$  amino acids,  $4\text{ }\mu\text{g/mL}$  internal standards): Mix  $500\text{ }\mu\text{L}$  of amino acid stock solution ( $0.5\text{ }\mu\text{mol/mL}$ ) with  $500\text{ }\mu\text{L}$  of the  $8\text{ }\mu\text{g/mL}$  internal standard mixture in a  $2\text{-mL}$  clear glass vial.
17. Calibration curve standards: Prepare calibration curve standards ( $1\text{ mL}$  each) with concentration range from  $0.25\text{ }\mu\text{mol/mL}$  to  $476.8\text{ fmol/mL}$  by serial dilutions of the  $0.25\text{ }\mu\text{mol/mL}$  working calibration amino acid mixture spiked with isotopically labeled internal standards ( $4\text{ }\mu\text{g/mL}$ ). Use internal standard mixture ( $4\text{ }\mu\text{g/mL}$ ) to make up the final volume of each standard to  $1\text{ mL}$  and to keep a constant concentration of internal standards at  $4\text{ }\mu\text{g/mL}$  in all the concentration levels. Divide each solution into working aliquots. Leave one aliquot of each standard for current use and store remaining aliquots at  $-80^{\circ}\text{C}$ .

## **2.2. Direct Infusion ESI-MS/MS**

1. LC-MS grade water or ultrapure water prepared by purifying deionized water in a water treatment system ( $18.2\text{ M}\Omega\text{-cm}$ ).
2. L-Amino acids, kit of  $1\text{ g}$  each: L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-cystine, L-glutamic acid, L-glutamine, glycine, L-histidine, *trans*-4-hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine (Sigma-Aldrich, Co., St. Louis, MO; Cat. No. LAA-21).
3. Amino acid stock solutions ( $1\text{ mg/mL}$ ): Prepare  $1\text{ mg/mL}$  solutions of individual L-amino acids in LC-MS grade water.
4. Derivatized amino acid solutions for infusions ( $10\text{ }\mu\text{g/mL}$ ): Prepare  $100\text{ }\mu\text{g/mL}$  solution of each L-amino acid by appropriate dilution of its corresponding stock solution. Derivatize each amino acid using the AccQ-Tag derivatization kit. Final

amino acid concentration after derivatization is 10 µg/mL (see Note 4 and 5).

5. Waters Xevo TQ mass spectrometer with electrospray ionization (ESI) probe (or similar tandem quadrupole mass spectrometer).

### **2.3. Metabolite Extraction from Arabidopsis Leaf Tissue**

1. Dry ice.
2. LC-MS grade methanol.
3. LC-MS grade water or ultrapure water (18.2 MΩ·cm).
4. Mixer Mill MM 300 and Mixer Mill Adapter Set (QIAGEN).
5. Bench top refrigerated microcentrifuge (Beckman Coulter, Inc., Brea, CA or similar).
6. Ultrasonic cleaner (Branson, model 3510R-MT or similar).
7. Microcentrifuge tubes with screw caps and O-rings: 1.5 mL.
8. Stainless steel beads: 2.3 mm.
9. Limited volume polypropylene vial: 250 µL.
10. Polypropylene caps with Red PTFE/White Silicone/Red PTFE septa.
11. Methanol–water mixture, 50:50 v/v.
12. Extraction buffer with internal standards (4 µg/mL).

### **2.4. UPLC-MS/MS Analysis**

1. Waters Acquity UPLC system equipped with a binary solvent manager, an autosampler, a column heater, and interfaced to a Waters Xevo TQ mass spectrometer by means of an electrospray ionization probe (or similar UPLC system integrated with tandem quadrupole mass spectrometer).
2. AccQ·Tag Ultra column, 2.1 × 100 mm, 1.7 µm particles (Waters Corp.).
3. Acetonitrile (LC/MS grade).
4. LC-MS grade water or ultrapure water (18.2 MΩ·cm).
5. AccQ·Tag Ultra concentrate solvent A (Waters Corp.).
6. AccQ·Tag Ultra solvent B (Waters Corp.).
7. Eluent A: 10:90 v/v AccQ·Tag Ultra concentrate solvent A/water.
8. Eluent B: 100% AccQ·Tag Ultra solvent B.
9. Strong wash solvent: 70% acetonitrile in LC/MS water.
10. Weak wash solvent: 10% acetonitrile in LC/MS water.
11. Collision gas: dry, high purity argon (99.997%).
12. API gas: dry, oil-free nitrogen (with a purity of at least 95%).

### 3. Methods

#### **3.1. Direct Infusion ESI-MS/MS and UPLC-MS/MS Analysis**

1. Using the IntelliStart software, or any other available instrument specific software, determine the MRM transitions for each amino acid adduct by direct infusion of the derivatized L-amino acid solutions. Set the infusion flow rate to 20  $\mu\text{L}/\text{min}$ , the desolvation temperature to 350°C, and the desolvation gas flow rate to 600 L/h. Select the option to automatically create a MS tune file which will be populated with the optimized cone voltages and collision energies for all transitions of each amino acid. The MRM transitions, cone voltages, and collision energies for a selected group of amino acids are listed in Table 1 (see Notes 6 and 7).
2. Create an LC method with the following gradient: 0–0.54 min (99.9% A), 5.74 min (90.0% A), 7.74 min (78.8% A), 8.04–8.64 min (40.4% A), 8.73–10 min (99.9% A) (see Note 8).
3. Perform a UPLC-MS/MS analysis of a representative sample or a mixture of amino acids using the MS tune file created earlier to determine the retention times of each targeted amino acid (column flow rate = 0.7 mL/min, column temperature = 55°C, autosampler temperature = 25°C, injection volume = 1  $\mu\text{L}$ , desolvation temperature = 600°C, desolvation gas flow rate = 1,000 L/h, collision gas (argon) flow rate = 0.15 mL/min, dwell time = 0.01 s).
4. After determination of the retention times, proceed to the adjustment of the time window per MRM function. Divide the 10 min run time into time segments such that the minimum number of MRM transitions is observed per time window (see Note 9). If an Acquity UPLC system coupled to a Xevo TQ mass spectrometer is used, the optimized MRM method described in Table 1 can be used for the analysis of amino acids.

#### **3.2. Metabolite Extraction from Arabidopsis Leaf Tissue**

1. Transfer 5 mg of freeze-dried plant leaf tissue to a 1.5-mL microcentrifuge tube with screw caps/O-rings. Keep plant leaf tissue on dry ice before and after weighing.
2. Add two 2.3 mm stainless steel beads to the microcentrifuge tube.
3. Add 125  $\mu\text{L}$  of extraction buffer (4  $\mu\text{g}/\text{mL}$ ).
4. Grind samples two times in the mixer mill and rotate adapter set 180° between repetitions (30 cycles/s, 30 s per repetition).
5. Incubate sample on dry ice for 5 min.
6. Sonicate samples for 1 min in ultrasonic cleaner.

**Table 1**  
**UPLC-MS/MS conditions for the determination of AccQ·Tag-amino acid derivatives<sup>a</sup>**

Compound number	Amino acid/ labeled amino acid	Parent ion <sup>b</sup> ( <i>m/z</i> )	Daughter ion <sup>b</sup> ( <i>m/z</i> )	Cone voltage (V)	Collision energy (eV)	<i>R</i> <sub>t</sub> (min)	Time window (min)	Internal standard
1	Hydroxyproline	302.11	171.01	24	21	1.49	1.03–2.02	32
2	Histidine	326.21	171.01	18	17	1.60	1.13–2.16	3
3	L-Histidine (ring 2- <sup>13</sup> C)	327.21	171.01	18	17	1.64	1.13–2.16	
4	Asparagine	303.13	171.01	24	21	1.84	1.60–2.36	5
5	L-Asparagine- <sup>15</sup> -N <sub>2</sub>	305.27	171.01	24	21	1.87	1.39–2.39	
6	3-Methyl-histidine	340.21	171.01	24	21	1.97	1.50–2.10	3
7	Taurine	296.11	171.01	18	15	2.13	1.66–2.66	11
8	1-Methyl-histidine	340.21	171.01	24	21	2.20	2.08–2.50	3
9	L-Serine-2,3,3- <sup>3</sup> -d <sub>3</sub>	279.11	171.01	25	19	2.48	2.04–3.05	
10	Serine	276.11	171.01	25	19	2.51	2.04–3.05	9
11	L-Glutamine-2,3,3,4,4-d <sub>5</sub>	322.15	171.01	22	24	2.64	2.16–3.16	
12	Glutamine	317.21	171.01	22	24	2.67	2.16–3.16	11
13	Carnosine	397.21	171.01	24	21	2.74	2.27–3.27	3
14	Arginine	345.21	171.01	27	31	2.77	2.29–3.29	40
15	Glycine-d <sub>5</sub>	248.25	171.01	27	21	2.88	2.42–3.42	
16	Glycine	246.08	171.01	27	21	2.88	2.39–3.39	15
17	Homoserine	290.12	171.01	24	21	3.02	2.40–3.40	9

(continued)

Table 1  
(continued)

Compound number	Amino acid/stable isotope labeled amino acid	Parent ion <sup>b</sup> ( <i>m/z</i> )	Daughter ion <sup>b</sup> ( <i>m/z</i> )	Cone voltage (V)	Collision energy (ev)	<i>R</i> <sub>t</sub> (min)	Time window (min)	Internal standard
18	Ethanolamine	232.09	171.01	24	21	3.04	2.58–3.58	15
19	Aspartic acid	304.11	171.01	27	23	3.24	2.76–3.76	21
20	Sarcosine	260.17	171.01	25	21	3.68	3.30–3.90	15
21	L-Glutamic acid-2,4,4-d <sub>3</sub>	321.11	171.01	27	21	3.82	3.33–4.33	
22	Glutamic acid	318.11	171.01	27	21	3.84	3.35–4.34	21
23	Citrulline	346.21	171.01	22	24	3.87	3.39–4.38	11
24	B-Alanine	260.17	171.01	25	21	4.08	3.88–4.45	15
25	Threonine	290.11	171.01	25	21	4.30	3.81–4.80	9
26	D-L-Alanine-2,3,3,3-d <sub>4</sub>	264.1	171.01	25	21	4.72	4.22–5.22	
27	L-Alanine	260.17	171.01	25	21	4.74	4.44–5.26	26
28	γ-Amino- <i>n</i> -butyric acid	274.11	171.01	25	21	4.92	4.43–5.25	15
29	α-Amino adipic acid	332.33	171.01	25	21	5.13	4.63–5.64	21
30	Creatinine	284.11	171.01	24	21	5.34	5.00–5.84	32
31	β-Aminoisobutyric acid	274.11	171.01	25	21	5.38	5.24–5.89	15
32	Proline-2,5,5-d <sub>3</sub>	289.32	171.01	25	21	5.38	4.88–5.88	
33	Proline	286.16	171.01	25	21	5.39	4.91–5.91	32
35	δ-Hydroxylysine	503.21	171.01	28	21	5.62, 5.73	5.12–6.12	47
36	α-Amino- <i>n</i> -butyric acid	274.11	171.01	25	21	5.99	5.75–6.75	26
38	Cystathionine	563.6	171.01	28	21	6.10, 6.24	5.60–6.60	40



40	Ornithine-3,3,4,4,5,5-d <sub>6</sub>	479.22	171.01	16	18	6.11	5.62–6.62	
42	Ornithine	473.22	171.01	16	18	6.14	5.65–6.65	40
44	Cystine	581.64	171.01	28	21	6.45	5.96–6.96	40
47	Lysine-3,3,4,4,5,5,6,6-d <sub>8</sub>	495.58	171.01	18	18	6.52	6.03–7.03	
49	Lysine	487.21	171.01	18	18	6.55	6.05–7.05	47
50	Tyrosine	352.21	171.01	24	21	6.61	6.12–7.12	61
51	Methionine-methyl-d <sub>3</sub>	323.13	171.01	27	21	6.75	6.25–7.25	
52	Methionine	320.21	171.01	27	21	6.77	6.27–7.27	51
53	Valine-d <sub>8</sub>	296.21	171.01	28	21	6.88	6.39–7.39	
54	Valine	288.21	171.01	28	21	6.91	6.43–7.43	53
56	Homocystine	609.36	171.01	28	21	7.54	7.04–8.02	40
58	Leucine	302.21	171.01	28	21	7.67	7.18–8.18	60
59	Isoleucine	302.21	171.01	28	21	7.75	7.26–8.26	60
60	Leucine-d <sub>10</sub>	312.24	171.01	28	21	7.78	7.13–8.13	
61	Phenyl-d <sub>5</sub> -alanine	341.21	171.01	28	21	7.84	7.34–8.34	
62	Phenylalanine	336.21	171.01	28	21	7.86	7.37–8.36	61
63	Tryptophan-2',4',5',6',7'- d <sub>5</sub> (indole-d <sub>5</sub> )	380.21	171.01	28	21	7.94	7.44–8.44	
64	Tryptophan	375.21	171.01	28	21	7.96	7.44–8.44	63

<sup>a</sup> Conditions are specific for the Waters Acquity UPLC – Xevo TQ MS system

<sup>b</sup> (M + H)<sup>+</sup>-adduct

7. Centrifuge sample at 13,000 rpm/17,900×*g* and 4°C for 5 min.
8. Transfer the supernatant to a limited volume vial. Be careful not to transfer any debris.
9. Re-extract the sample with 125 µL extraction buffer.
10. Perform second round of extraction (steps 4–7) and combine the extracts. Store the extract at –80°C if it is not derivatized immediately.

### **3.3. AccQ-Tag Ultra Amino Acid Derivatization**

1. Place 70 µL of AccQ-Tag Ultra Borate Buffer in a 250-µL reduced volume polypropylene vial using a micropipette.
2. Add 10 µL of amino acid standard or sample extract and mix.
3. Add 20 µL of the derivatizing reagent solution, vortex immediately after addition.
4. Let the solution stand for 1 min at room temperature.
5. Heat the vial in a heating block or an incubator for 10 min at 55°C.
6. Remove samples from heating block and place in instrument for analysis (see Note 10).

### **3.4. Sample Analysis and Data Analysis**

1. Start the UPLC-ESI-MS/MS system and equilibrate to initial conditions of the method (flow rate = 0.7 mL/min, 99.9% of eluent A, 0.1% of eluent B, column temperature = 55°C, sample temperature = 25°C).
2. Create a sample analysis sequence with a blank as the first run followed by quality control standard (a solution containing derivatized internal standards) followed by experimental samples.
3. Insert appropriate number of blanks between the experimental samples or calibration standards to avoid carryover. The sequence should end with a quality control sample followed by a blank sample. Representative total ion chromatograms obtained by UPLC-ESI-MS/MS analysis of amino acids in *A. thaliana* leaf extract are shown in Fig. 1.
4. Perform the UPLC-ESI-MS/MS analysis with the chromatographic and MS parameters specified in Table 1.
5. For data analysis use instrument specific software. If the Acquity UPLC – Xevo TQ MS system is being used, conduct the data

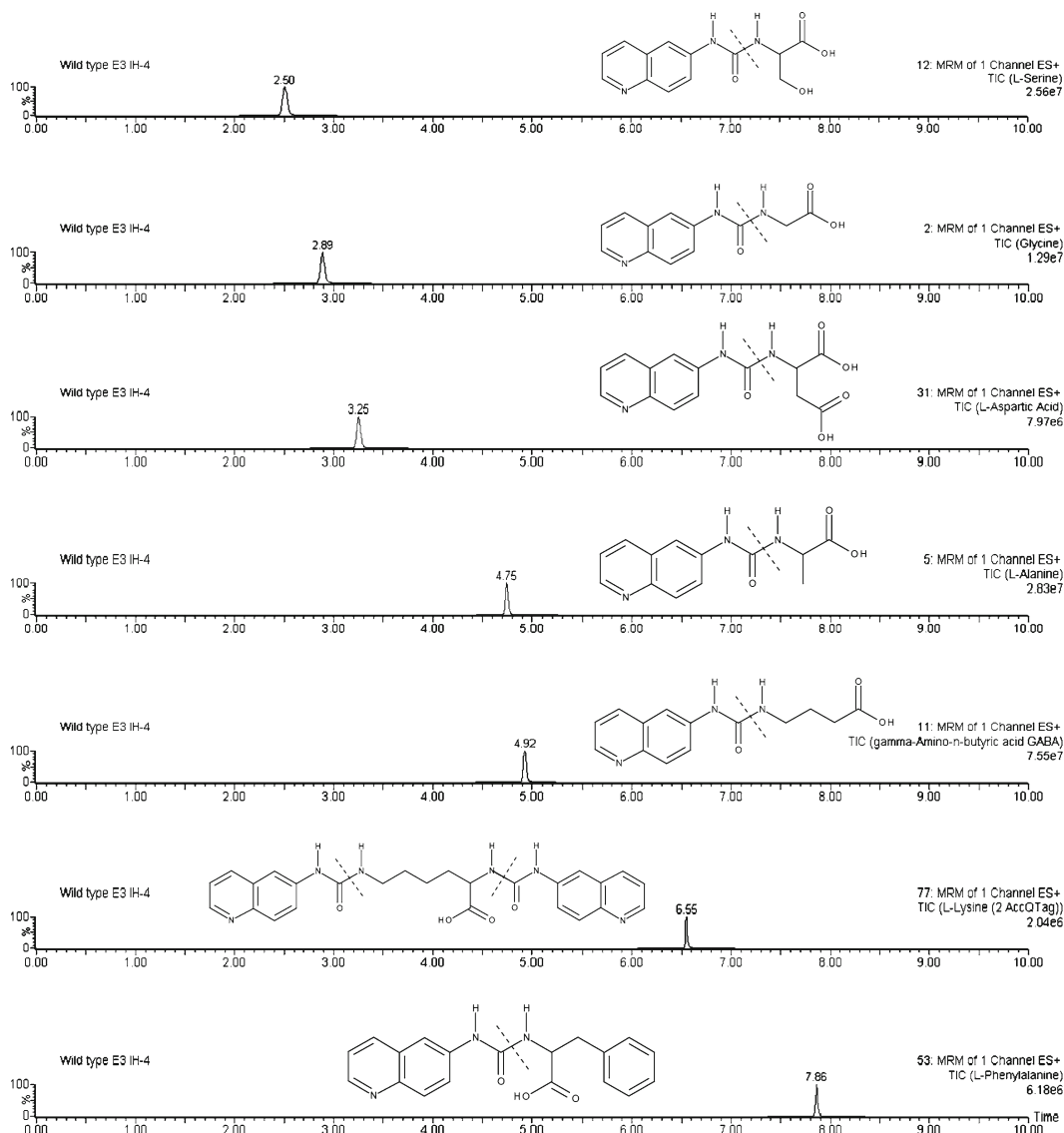


Fig. 1. Total ion chromatograms of selected AccQ-Tag-derivatized amino acids analyzed in wild-type Arabidopsis extracts by UPLC-ESI-MS/MS. Chemical structures show fragmentation site upon MS/MS.

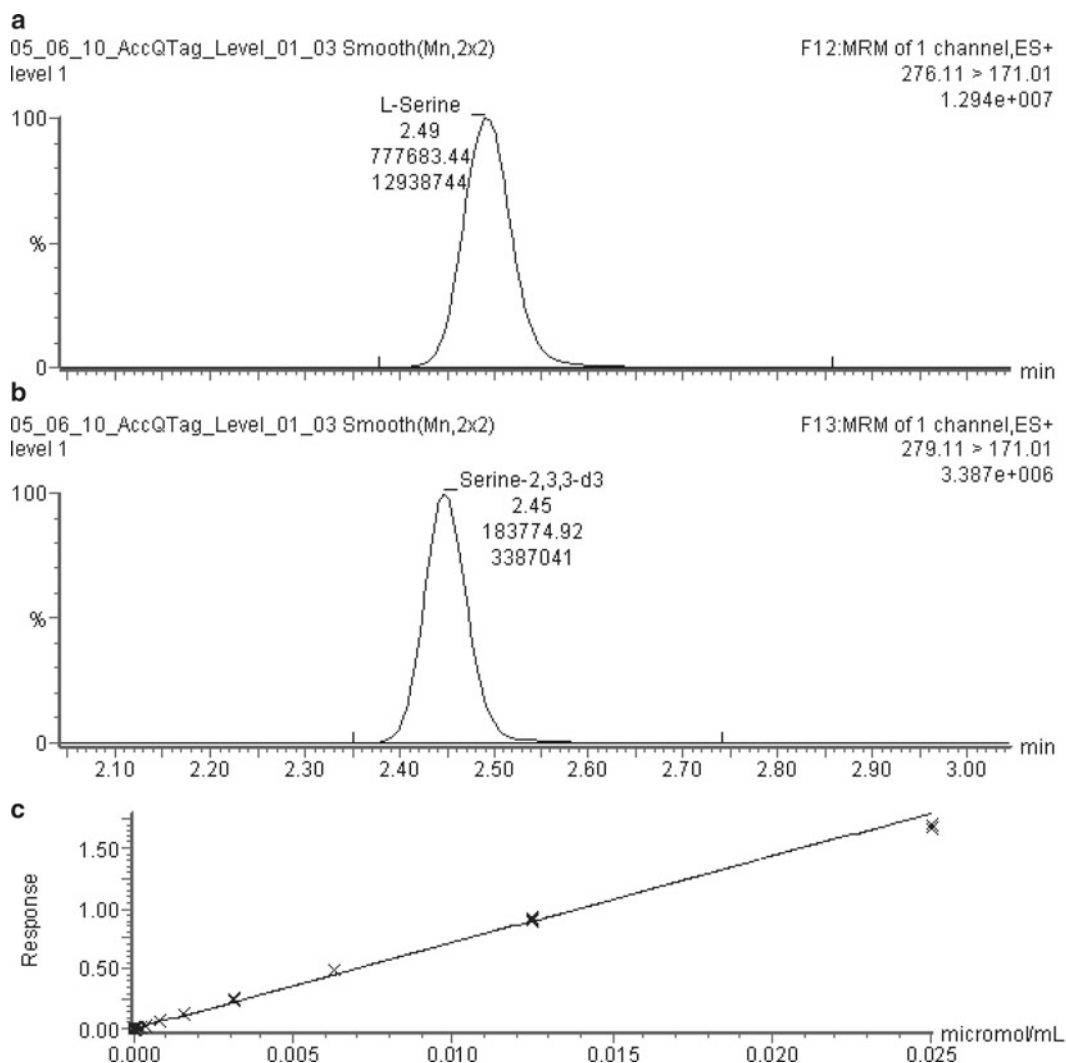


Fig. 2. Chromatogram (a) and internal calibration curve (c) for serine (AQC-derivatized serine at 0.025  $\mu\text{mol/mL}$ ; internal standard (b), serine-2,3,3-d<sub>3</sub>, at 0.4  $\mu\text{g/mL}$ ).

analysis (calibration curves and amino acid quantitation) with the TargetLynx software (see Note 11). A typical chromatogram window for serine and its internal standard (serine-2,3,3-d<sub>3</sub>), and the corresponding internal calibration curve is depicted in Fig. 2. A comparison of detection limits and dynamic ranges obtained by AccQ·Tag-UPLC-ESI-MS/MS amino acid analysis using the Xevo TQ and TQD mass spectrometric detectors is given in Table 2.

**Table 2**

**Comparison of detection limits and dynamic ranges for AccQ-Tag-derivatized amino acids analyzed by Waters Acquity UPLC system interfaced with the Waters TQD MS and the Waters Xevo TQ MS**

Amino acid	UPLC-ESI-MS/MS			
	Xevo TQ		TQD	
	Detection limit (M)	Dynamic range	Detection limit (M)	Dynamic range
Hydroxy-L-proline	$4.86 \times 10^{-11}$	100,000	$1.65 \times 10^{-9}$	100
Histidine	$2.31 \times 10^{-8}$	1,000	$1.33 \times 10^{-8}$	100
3-Methyl-histidine	$2.16 \times 10^{-11}$	1,000	$1.21 \times 10^{-8}$	1,000
Taurine	$1.09 \times 10^{-11}$	10,000	$3.95 \times 10^{-9}$	100
1-Methyl-histidine	$1.02 \times 10^{-11}$	10,000	$1.80 \times 10^{-8}$	100
Serine	$1.10 \times 10^{-8}$	10,000	$1.65 \times 10^{-9}$	1,000
Carnosine	$4.13 \times 10^{-11}$	1,000	$5.88 \times 10^{-9}$	1,000
Arginine	$2.41 \times 10^{-10}$	10,000	$1.19 \times 10^{-8}$	100
Glycine	$3.20 \times 10^{-9}$	10,000	$1.65 \times 10^{-9}$	10,000
Ethanolamine	$3.02 \times 10^{-9}$	10,000	$1.65 \times 10^{-9}$	1,000
Aspartic acid	$3.17 \times 10^{-9}$	10,000	$3.49 \times 10^{-9}$	100
Sarcosine	$4.83 \times 10^{-10}$	10,000	$1.65 \times 10^{-9}$	1,000
Glutamic acid	$4.64 \times 10^{-9}$	10,000	$5.40 \times 10^{-9}$	100
Citrulline	$2.87 \times 10^{-10}$	10,000	$3.98 \times 10^{-9}$	100
$\beta$ -Alanine	$1.43 \times 10^{-9}$	10,000	$2.27 \times 10^{-9}$	1,000
Threonine	$1.30 \times 10^{-9}$	10,000	$2.63 \times 10^{-9}$	1,000
L-Alanine	$1.06 \times 10^{-9}$	10,000	$1.85 \times 10^{-9}$	1,000
$\gamma$ -Amino- <i>n</i> -butyric acid	$2.69 \times 10^{-9}$	10,000	$1.65 \times 10^{-9}$	1,000
$\alpha$ -Amino adipic acid	$9.27 \times 10^{-11}$	10,000	$1.79 \times 10^{-9}$	1,000
$\beta$ -Aminoisobutyric acid	$9.13 \times 10^{-11}$	100,000	$1.65 \times 10^{-9}$	1,000
Proline	$1.55 \times 10^{-9}$	10,000	$2.83 \times 10^{-9}$	1,000
$\alpha$ -Amino- <i>n</i> -butyric acid	$1.43 \times 10^{-9}$	100,000	$1.71 \times 10^{-9}$	1,000
Tyrosine	$8.05 \times 10^{-11}$	10,000	$3.85 \times 10^{-9}$	1,000
Methionine	$1.60 \times 10^{-9}$	100,000	$1.65 \times 10^{-9}$	1,000
Valine	$8.24 \times 10^{-10}$	10,000	$1.65 \times 10^{-9}$	1,000

(continued)

**Table 2**  
(continued)

Amino acid	UPLC-ESI-MS/MS			
	Xevo TQ		TQD	
	Detection limit (M)	Dynamic range	Detection limit (M)	Dynamic range
Leucine	$2.74 \times 10^{-10}$	10,000	$1.65 \times 10^{-9}$	1,000
Isoleucine	$1.20 \times 10^{-10}$	10,000	$1.65 \times 10^{-9}$	1,000
Phenylalanine	$6.28 \times 10^{-10}$	10,000	$4.01 \times 10^{-9}$	10,000
Tryptophan	$6.18 \times 10^{-10}$	10,000	$7.57 \times 10^{-9}$	1,000

#### 4. Notes

1. Protect any unused reconstituted reagent solution from light and atmospheric water by wrapping the vial with aluminum foil, further sealing the cap with Parafilm, and storing the vial in a desiccator. Under these conditions, the reconstituted reagent last for up to 1 week.
2. The extraction buffer containing the labeled internal standards at 4  $\mu\text{g/mL}$  should be defrosted only once for the extraction of amino acids.
3. The amino acid stock solution from Sigma-Aldrich contains physiological acidic, neutral and basic amino acids:  $\beta$ -alanine, L-alanine, L- $\alpha$ -aminoadipic acid, L- $\alpha$ -amino-*n*-butyric acid, D,L- $\beta$ -aminoisobutyric acid, L-arginine, L-aspartic acid, L-carnosine, L-citrulline, creatinine, L-cystathionine, L-cystine, ethanolamine, L-glutamic acid, glycine, L-histidine, L-homocystine,  $\delta$ -hydroxylysine, hydroxyl-L-proline, L-isoleucine, L-leucine, L-lysine, L-methionine, 1-methyl-L-histidine, 3-methyl-L-histidine, L-ornithine, L-phenylalanine, L-proline, L-sarcosine, L-serine, taurine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.
4. Signal suppression is problematic during direct infusion of AccQ-Tag-derivatized amino acids into the Xevo TQ mass spectrometer when the borate buffer is used for optimum pH adjustment of the reaction solutions. This problem is attributed to the nonvolatile nature of the borate buffer. When this is the case, an alternative buffer for the derivatization protocol must be identified. A 50-mM ammonium acetate buffer (pH 9.3) demonstrated to be suitable for this purpose and was used for direct infusion of derivatized amino acid standards into the Xevo TQ mass spectrometer.

5. It is worth mentioning that signal suppression due to the nature of the borate buffer is only observed under direct infusion conditions in the Xevo TQ mass spectrometer. Such phenomenon is absent during UPLC-MS/MS analysis of AccQ·Tag-derivatized amino acids due to the dilution of the sample with the mobile phase. Although the ammonium acetate buffer is suitable for the AccQ·Tag derivatization, its use is limited to the derivatization of amino acids for infusion experiments in our protocol. The borate buffer is preferred for the UPLC-MS/MS analysis of AccQ·Tag-derivatized amino acids because larger peak areas are obtained with this buffer compared to those observed with ammonium acetate. Lower yields of amino acid derivatives are suspected to be produced when the ammonium acetate buffer is used.
6. The MRM transition used for each targeted derivatized amino acid in the final MS method corresponded to the intense parent-daughter transition  $m/z$   $(M+H)^+ > 171$ . The  $m/z$  171 diagnostic ion results from the collision-induced cleavage at the ureide bond of the AccQ·Tag adduct of each amino acid.
7. Since creating MRM-MS methods is costly and time consuming, the optimal cone voltage and collision energy parameters for each MRM transition were found only for a selected number of AccQ·Tag -amino acid adducts. Representative compounds from each amino acid group (i.e., polar, nonpolar, acidic, basic, and neutral) were selected. The average values of cone voltages and collision energies observed for the evaluated amino acids were applied for the remaining targeted compounds through all the experiments. In addition, the cone voltages and collision energies assigned to the stable isotope-labeled reference compounds corresponded to those observed for their corresponding non-labeled amino acid homologs.
8. This gradient is specific for the AccQ·Tag Ultra column,  $2.1 \times 100$  mm,  $1.7 \mu\text{m}$  particles. Optimum resolution of AccQ·Tag-derivatized amino acids is obtained with this column when operated under the original gradient conditions described by Waters. If a different column with different specifications is to be used, the gradient must be empirically modified to attain optimum separation of the analytes.
9. The adjustment of the time window and the number of MRM transitions monitored per function are optimized during the MS method development in order to obtain as many scans as possible within a MS peak and increase the reliability of the quantitation.
10. According to the manufacturer, derivatized amino acid samples are stable at room temperature up to 1 week.
11. A quantitation method is necessary in order to perform this step. In the TargetLynx method editor, enter the name of each

compound of the analysis and the parameters that describe their quantitation (acquisition function number, transition, retention time, internal reference, integration parameters, etc.). Once the entire quantitation method is built, set up a pre-acquired sample list of a set of calibration standards for quantitation (specify sample types and concentrations). Process the standards using the quantitation method. Review the integration, the calibration curves and make the necessary adjustments until acceptable results are obtained for the range of linear response. Save the data set and export it as a calibration curve file. Proceed to the analysis of the unknown samples specifying the quantitation method and calibration curve file. The concentration of amino acids in the unknown samples will be calculated based on the response indicated by the calibration curves. Report the final concentration of amino acids in the unknown samples as  $\mu\text{mol}/\text{mg}$  dry weight of Arabidopsis tissue.

## References

1. Stein WH et al (1957) Observation on the amino acid composition of human hemoglobins. *Biochim Biophys Acta* 24: 640–642
2. Ullmer R, Plematl A, and Rizzi A (2006) Derivatization by 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate for enhancing the ionization yield of small peptides and glycopeptides in matrix-assisted laser desorption/ionization and electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom* 20: 1469–1479
3. Badiou S et al (2004) Determination of plasma amino acids by fluorescent derivatization and reversed-phase liquid chromatographic separation. *Clin Lab* 50:153–158
4. Bernal JL et al (2005) A comparative study of several HPLC methods for determining free amino acid profiles in honey. *J Sep Sci* 28:1039–1047
5. Cohen SA (2000) Amino acid analysis using precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. *Methods Mol Biol* 159:39–47
6. Cohen SA (2003) Amino acid analysis using pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. Analysis of hydrolyzed proteins and electroblotted samples. *Methods Mol Biol* 211:143–154
7. Hou S et al (2009) Determination of soil amino acids by high performance liquid chromatography-electro spray ionization-mass spectrometry derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. *Talanta* 80:440–447
8. Callahan DL et al (2007) Relationships of nicotianamine and other amino acids with nickel, zinc and iron in *Thlaspi* hyperaccumulators. *New Phytol* 176: 836–848
9. Armenta JM et al (2010) Sensitive and rapid method for amino acid quantitation in malaria biological samples using AccQ.Tag ultra performance liquid chromatography-electrospray ionization-MS/MS with multiple reaction monitoring. *Anal Chem* 82: 548–558
10. Bosch L, Alegria A, and Farre R (2006) Application of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) reagent to the RP-HPLC determination of amino acids in infant foods. *J Chromatogr B Analyt Technol Biomed Life Sci* 831: 176–183
11. Pappa-Louisi A et al (2007) Optimization of separation and detection of 6-aminoquinolyl derivatives of amino acids by using reversed-phase liquid chromatography with on line UV, fluorescence and electrochemical detection. *Anal Chim Acta* 593: 92–97
12. Martínez-Girón AB et al (2009) Development of an in-capillary derivatization method by CE for the determination of chiral amino acids in dietary supplements and wines. *Electrophoresis* 30: 696–704
13. Cohen SA, and Michaud DP (1993) Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Anal Biochem* 211: 279–287





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