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Chapter 33

Amino Acid Profiling from Fingerprints Using Amide Stationary-Phase UPLC-MS

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Abstract

UPLC-MS is a commonly used technique to first separate complex samples and subsequently quantify molecules of interest. Herein we describe the use of UPLC-MS using an amide stationary phase to quantify non-derivatized amino acids extracted from fingerprints. As detector either a triple-quadrupole MS/MS or a TOF-MS detector was used. This method allows for a simple and fast sample preparation, which facilitates the analysis of large amounts of samples.

Key words Amino acid analysis, UPLC-MS, HILIC, Mass spectrometry, Fingerprint, Amino acid profiling

1 Introduction

In recent decades, many investigations have been performed into the chemical composition of fingerprints. Common metabolites such as amino acids and fatty acids have been investigated. Gas chromatography coupled to mass spectrometry (GC-MS) is used in numerous studies to analyze amino acid profiles from fingerprints [1–3]. Most studies were based on relatively small datasets, but reported that the chemical composition of fingerprints might be influenced by certain donor traits, which would open the possibility to retrieve donor information from fingerprints. For example, amino acid profiles have been reported to be influenced by gender, age, and health [4, 5]. Besides GC-MS, several other analytical techniques have been used in studies into the amino acid profiles of fingerprints. High-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) using a MS as detector are techniques that have been successfully applied to separate and quantify amino acids retrieved from fingerprints [6, 7]. To be able to determine what the exact effect of certain donor factors on the chemical composition of a fingerprint is, larger datasets are needed, which requires a simple and fast sample preparation and analysis.

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In this chapter, we describe a method for the extraction and analysis of amino acids from fingerprints using ultrahigh-performance liquid chromatography (UHPLC or UPLC) with an amide stationary phase, circumventing the need for amino acid derivatization. Secondly, we describe the differences between the use of a tandem quadrupole (QqQ) MS/MS and a time-of-flight (TOF) MS; both detectors are frequently used in metabolomic profiling studies. The TOF-MS offers high resolution and mass accuracy, whereas the triple-quadrupole MS/MS provides structural information. The compared methods have been used for the analysis of the amino acid profiles from the fingerprints of 19 donors.

2 Materials

2.1 Chemicals

1. 21 Amino acids (Sigma-Aldrich, *see* **Note 1**).
2. 21 Isotopically labeled amino acid internal standards (CDN isotopes Pointe-Claire (Canada), Sigma-Aldrich Zwijndrecht (the Netherlands), Isotec Zwijndrecht (the Netherlands), and Cambridge Isotope Laboratories Tewksbury MA (USA), *see* **Note 2**).
3. Methanol (UPLC grade).
4. Acetonitrile (UPLC grade).
5. Ultrapure water (purified deionized water, to attain a sensitivity of 18 MΩ-cm at 25 °C).
6. Formic acid (UPLC grade).

2.2 Amino Acid Standard Stocks

1. MeOH containing 5% (v/v) formic acid: Add 50 mL of formic acid to a 1 L volumetric flask. Add methanol to 1 L.
2. Individual amino acid standard solutions of 100–500 mg/L, depending on their solubility, were prepared in MeOH containing 5% (v/v) formic acid using a volumetric flask and were stored at −20 °C (*see* **Notes 3** and **4**).
3. Individual amino acid internal standard solutions of 100–500 mg/L, depending on their solubility, were prepared in MeOH containing 5% (v/v) formic acid using a volumetric flask and were stored at −20 °C.
4. Master mixture of 2 mg/L of each of the 21 amino acids was prepared from the individual stocks using a volumetric flask and stored at −20 °C.
5. Internal standard mixture of 2 mg/L of each of the 21 amino acid internal standards was prepared using a volumetric flask from the individual internal standard stocks and stored at −20 °C.

2.3 Sample Preparation

1. 2.5 × 5 cm Aluminum foil sheets.
2. 15 mL Polypropylene conical tubes (Fisherbrand).
3. Vortex mixer (IKA vortex Genius 3).
4. Evaporator (Pierce model 18,780 Reacti-Vap).
5. 15 mL Tube racks polystyrene (Fisherbrand).
6. Pipettes with volumes of 10, 20, 100, 300, 1000, and 5000 μL (Eppendorf Research Plus).
7. Analytical balance (Sartorius BP2105).

2.4 UPLC-MS(/MS)

1. 150 mm (length), 2.1 mm (internal diameter), 1.7 μm (particle size) ethylene-bridged hybrid (BEH) amide analytical column (Waters, USA).
2. 5 mm (length), 2.1 mm (internal diameter), 1.7 μm (particle size) ethylene-bridged hybrid (BEH) amide guard column (Waters, USA).
3. Acquity I-class UPLC autosampler and binary solvent pump (Waters, USA).
4. Clear glass 12 × 32 mm screw-neck total recovery vial, with cap and PTFE/silicone septum, 1 mL volume (Waters, USA).
5. Tandem quadrupole mass spectrometer (MicroMass Quattro Premier XE, Waters, USA) or Accurate mass TOF with a dual-electrospray source (ESI) (Agilent 6220, USA).

3 Methods

3.1 Calibration Series

1. Prepare a calibration series using the master mixture and internal standard mixture of 0, 100, 200, 400, 600, 1200, and 1600 μg/L of each amino acid with 400 μg/L of each internal standard on the day of the UPLC-MS analysis (total volume of 100 μL) and transfer to injection vials.
2. Put the vials in the sample manager of the Acquity I-class UPLC autosampler (set at 8 °C).

3.2 Amino Acid Fingerprint Extraction

1. Collect fingerprints on 2.5 × 5 cm pieces of aluminum foil (*see* **Notes 5** and **6**).
2. Transfer the aluminum foil to a 15 mL polypropylene conical tube.
3. Add 2 mL of MeOH containing 5% (v/v) formic acid and 20 μL of internal standard solution to the 15 mL polypropylene conical tube.
4. Vortex for 120 s.

5. Using a clean tweezers, remove the foil from the tube and evaporate under nitrogen flux using the evaporator.
6. Reconstitute in 100 μL MeOH containing 5% (v/v) formic acid by using the vortex mixer, briefly spin down, and transfer to an injection vial.
7. Put the vial in the sample manager of the Acquity I-class UPLC autosampler (set at 8 °C).

3.3 UPLC

1. Prepare solvent A: Acetonitrile containing 0.4% (v/v) formic acid: add about 900 mL acetonitrile to a 1 L cylinder. Add 4 mL of formic acid to the cylinder using a pipette. Add acetonitrile to the cylinder to the final volume of 1 L (*see* **Note 7**).
2. Prepare solvent B: Water containing 0.4% (v/v) formic acid: add about 900 mL ultrapure water to a 1 L cylinder. Add 4 mL of formic acid to the cylinder using a pipette. Add ultrapure water to the cylinder to the final volume of 1 L.
3. Assemble the guard and analytical column in the column oven of the Acquity I-class UPLC and set the oven temperature at 65 °C.
4. Purge and prime the UPLC system.
5. Set flow rate at 0.5 mL/min and condition the column at 95% solvent A and 5% solvent B for 25 min.
6. Program the UPLC gradient as follows: 95% solvent A for 3 min followed by a linear gradient from 5 to 50% solvent B in 19 min followed by 1 min of 50% B. Finally, recondition the column for 2 min with 95% solvent A.
7. Program the method to wash sample syringe with acetonitrile.
8. Generate the sample list including sample name, type, vial position, injection volume (2.5 μL), UPLC method, and MS method (in case of the MicroMass Quattro Premier XE, *see* Subheading 3.5). When using the TOF-MS, program the autosampler to generate a pulse (contact closure) from 0.01 to 0.2 min using a 9-pole cable (*see* **Notes 8** and **9**).
9. After analysis, store column with both ends capped at 25 °C.

3.4 TOF-MS Parameters

1. Operate the system in the positive ESI mode.
2. Acquire MS spectra from m/z 40 to 1200 at a rate of 1 spectrum per second, from 1 to 24 min.
3. Set the capillary voltage at 3.5 kV, the source gas temperature at 325 °C, the drying gas flow at 5 L/min, and the nebulizer pressure at 30 psig.

4. Set the fragmentor, skimmer, and octapole 1 RF voltages at 160, 65, and 250 V, respectively.
5. Generate a sample list including sample name, type, and MS method. Make sure to set the run type under worklist run parameters to external start. Start the MS sample list before starting the UPLC sample list (*see* **Note 9**).
6. Process data using Mass Hunter Qualitative Analysis and Quantitative Analysis software and generate calibration curves for each amino acid by plotting the area ratio of amino acid and the respective internal standard on the y-axis and the amino acid concentration on the x-axis, using linear regression.
7. Calculate the amino acid concentration for the amino acids for every sample using the intercept and slope of the calibration curves and the area ratio of amino acid and internal standard in the samples.

3.5 MS/MS Parameters

1. Operate the system in the positive ESI mode.
2. Set ESI parameters to capillary voltage of 0.5 kV; source temperature of 130 °C; and desolvation gas temperature of 400 °C at a flow rate of 1000 L/h (N_2) and set the cone gas flow rate at 100 L/h (N_2).
3. Set the collision gas (argon) to a flow rate of 0.27 mL/min.
4. Enter the optimal multiple reaction monitoring (MRM) transitions, cone voltages, and collision energies for all analytes and internal standards (Table 1).
5. Process data using MassLynx and QuanLynx software and generate calibration curves for each amino acid by plotting the area ratio of amino acid and the respective internal standard on the y-axis and the amino acid concentration on the x-axis, using linear regression.
6. Calculate the amino acid concentration for the amino acids for every sample using the intercept and slope of the calibration curves and the area ratio of amino acid and internal standard in the samples. For a comparison between the MS and MS/MS quantification, *see* **Note 10** and Table 2.

4 Notes

1. For this study we used 19 proteinogenic L-amino acids (Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr), the L-cysteine dimer L-cystine (Cys-Cys), and hydroxy-L-proline (OH-Pro). All amino acids except glycine contain a chiral carbon atom. If important for

Table 1
MS/MS conditions (MicroMass Quattro Premier XE) for multiple reaction monitoring in ESI+

Compound	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
Cystine	240.90	119.70	0.040	10.00	18.00
Cystine	240.90	151.90	0.040	16.00	10.00
Cystine-D6	247.10	155.00	0.040	16.00	10.00
Ornithine	133.00	43.20	0.040	12.00	26.00
Ornithine	133.00	70.10	0.040	12.00	16.00
Ornithine	133.00	115.90	0.040	12.00	10.00
Ornithine-D6	139.20	76.20	0.040	12.00	14.00
Lysine	147.00	56.10	0.040	14.00	30.00
Lysine	147.00	84.00	0.040	14.00	14.00
Lysine	147.00	130.00	0.040	14.00	8.00
Lysine-D4	151.10	88.00	0.040	14.00	14.00
Arginine	175.01	115.90	0.040	20.00	14.00
Arginine	175.01	130.00	0.040	20.00	14.00
Arginine	175.01	157.90	0.040	20.00	12.00
Arginine-D7 15N4	186.00	78.00	0.040	30.00	22.00
Aspartic acid	134.00	74.00	0.040	12.00	12.00
Aspartic acid	134.00	88.00	0.040	12.00	10.00
Aspartic acid	134.00	116.00	0.040	12.00	8.00
Aspartic acid-D3	137.00	75.00	0.040	14.00	14.00
Histidine	156.00	82.90	0.040	16.00	20.00
Histidine	156.00	93.00	0.040	16.00	24.00
Histidine	156.00	110.00	0.040	16.00	12.00
Histidine-D3	159.00	113.00	0.040	16.00	12.00
Glutamic acid	148.00	83.90	0.040	20.00	16.00
Glutamic acid	148.00	101.90	0.040	20.00	12.00
Glutamic acid	148.00	129.90	0.040	20.00	8.00
Glutamic acid-D5	153.00	88.20	0.040	16.00	18.00
Asparagine	133.00	74.00	0.040	14.00	14.00
Asparagine	133.00	87.00	0.040	14.00	10.00
Asparagine	133.00	116.00	0.040	14.00	12.00
Asparagine-D3	135.90	90.00	0.040	14.00	10.00

(continued)

Table 1
(continued)

Compound	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
Serine	105.80	60.00	0.040	20.00	10.00
Serine	105.80	70.00	0.040	20.00	12.00
Serine	105.80	87.90	0.040	20.00	12.00
Serine-D3	109.10	91.00	0.040	20.00	12.00
Threonine	120.00	56.20	0.040	16.00	14.00
Threonine	120.00	74.10	0.040	16.00	10.00
Threonine	120.00	102.00	0.040	16.00	8.00
Threonine-D2	122.10	76.10	0.040	16.00	10.00
Hydroxyproline	132.00	41.30	0.040	20.00	22.00
Hydroxyproline	132.00	68.00	0.040	20.00	18.00
Hydroxyproline	132.00	86.00	0.040	20.00	14.00
Hydroxyproline-D3	135.10	71.10	0.040	20.00	18.00
Alanine	90.00	44.20	0.040	16.00	8.00
Alanine	90.00	62.10	0.040	16.00	4.00
Alanine	90.00	72.30	0.040	16.00	16.00
Alanine-D4 13C3 15N 1	98.00	51.00	0.040	18.00	10.00
Tyrosine	182.00	91.00	0.040	14.00	26.00
Tyrosine	182.00	136.00	0.040	14.00	12.00
Tyrosine	182.00	165.00	0.040	14.00	10.00
Tyrosine-D2	184.00	167.00	0.040	14.00	10.00
Glutamine	147.00	56.20	0.040	14.00	26.00
Glutamine	147.00	84.00	0.040	14.00	16.00
Glutamine	147.00	129.90	0.040	14.00	10.00
Glutamine-D5	152.10	135.00	0.040	14.00	10.00
Valine	118.00	55.20	0.040	14.00	20.00
Valine	118.00	72.00	0.040	14.00	10.00
Valine-D8	126.00	80.00	0.040	16.00	10.00
Proline	116.00	43.00	0.040	22.00	22.00
Proline	116.00	70.10	0.040	22.00	12.00
Proline-13C5 15N1	121.90	75.00	0.040	22.00	12.00

(continued)

Table 1
(continued)

Compound	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
Methionine	150.00	56.20	0.040	16.00	16.00
Methionine	150.00	104.00	0.040	16.00	10.00
Methionine	150.00	133.00	0.040	16.00	10.00
Methionine-D3	153.10	107.10	0.040	16.00	10.00
Tryptophan	205.10	118.00	0.040	14.00	28.00
Tryptophan	205.10	145.90	0.040	14.00	14.00
Tryptophan	205.10	188.10	0.040	14.00	8.00
Tryptophan-D5	210.20	150.10	0.040	18.00	18.00
Isoleucine	132.00	44.30	0.040	16.00	22.00
Isoleucine	132.00	69.10	0.040	16.00	16.00
Isoleucine	132.00	86.10	0.040	16.00	10.00
Isoleucine-13C6 15N1	139.00	92.00	0.040	18.00	10.00
Phenylalanine	166.00	77.00	0.040	14.00	34.00
Phenylalanine	166.00	103.00	0.040	14.00	28.00
Phenylalanine	166.00	120.00	0.040	14.00	14.00
Phenylalanine-D5	171.10	125.00	0.040	14.00	14.00
Leucine	132.00	44.00	0.040	16.00	16.00
Leucine	132.00	86.00	0.040	16.00	10.00
Leucine-D10	142.20	96.00	0.040	16.00	10.00

your analysis, be sure to use the enantiomer of interest (for biological purposes usually the L-enantiomer).

2. Ideally, internal standards for mass spectrometric analyses have identical physical and chemical properties except their mass. Hence, the best internal standards in this case are isotopically labeled amino acids, usually containing ^{2}H, ^{13}C, or ^{15}N (or a combination of these). When selecting isotopically labeled internal standards, make sure that the mass difference between the analyte of interest and your internal standard is at least 2 Dalton, to avoid overlapping.
3. Consider any hydrates and hydrochlorides which might be present in the amino acids when calculating the amount to weigh.

Table 2
Linearity (R^2), limit of detection (LOD), limit of quantification (LOQ), and intraday reproducibility of the 21 amino acids on both the triple-quadrupole and the time-of-flight MS

	Triple-quadrupole MS/MS				TOF-MS			
Compound	R^2	LOD (ng/fingerprint)	LOQ (ng/fingerprint)	Intraday reproducibility (RSD)	R^2	LOD (ng/fingerprint)	LOQ (ng/fingerprint)	Intraday reproducibility (RSD)
L-Alanine	0.992	1.04	3.46	6.63	0.997	0.89	2.97	9.53
L-Arginine	0.996	0.17	0.58	13.4	0.993	0.33	1.11	7.30
L-Asparagine	0.998	0.06	0.19	13.9	0.994	0.35	1.15	7.26
L-Aspartic acid	0.995	0.38	1.28	13.2	0.999	0.75	2.49	6.13
L-Cystine	0.993	0.71	2.35	12.8	0.998	1.96	6.54	13.6
L-Glutamic acid	0.993	0.16	0.55	7.98	0.994	0.21	0.70	7.86
L-Glutamine	0.998	0.08	0.27	6.78	0.999	0.57	1.90	8.79
L-Histidine	0.999	0.58	1.95	9.04	0.994	0.67	2.24	9.33
Hydroxy-L-proline	0.999	0.11	0.37	9.20	0.991	0.10	0.34	8.19
L-Iso/leucine	0.997	0.05	0.17	4.64	0.988	0.04	0.15	10.7
L-Lysine	0.995	0.58	1.92	13.2	0.987	1.45	4.84	9.47
L-Methionine	0.982	0.23	0.77	11.2	0.964	0.63	2.09	5.77
L-Ornithine	0.997	0.19	0.64	10.9	0.997	0.74	2.48	6.97
L-Phenylalanine	0.981	0.32	1.08	7.77	0.992	0.05	0.18	6.98

(continued)

Table 2
(continued)

Compound	Triple-quadrupole MS/MS				TOF-MS			
	R^2	LOD (ng/fingerprint)	LOQ (ng/fingerprint)	Intraday reproducibility (RSD)	R^2	LOD (ng/fingerprint)	LOQ (ng/fingerprint)	Intraday reproducibility (RSD)
L-Proline	0.992	0.03	0.11	13.6	0.992	0.04	0.14	6.57
L-Serine	0.992	0.11	0.38	6.12	0.992	0.34	1.14	7.40
L-Threonine	0.992	0.07	0.24	9.15	0.998	0.17	0.58	5.79
L-Tryptophan	0.995	0.11	0.38	5.94	0.995	0.04	0.14	8.48
L-Tyrosine	0.999	0.78	2.58	5.53	0.991	0.09	0.29	7.54
L-Valine	0.991	0.03	0.12	9.93	0.992	0.11	0.35	10.8

4. The most convenient way is to dissolve the amino acids after filling the volumetric flask to about 75%. After completely dissolving the amino acids, add until final volume.
5. Aluminum foil was chosen as substrate as it is nonporous and flexible and hence it is convenient in the sample preparation process. Other substrates such as glass slides might also be used. Extraction is then performed in a beaker using an ultrasonic bath. If substrates are nonporous, extraction generally is simple and fast.
6. Guidelines on how to collect fingerprints (e.g., the number of donors, whether to use groomed or natural fingerprints, the substrate, and deposition pressure) are described by the International Fingerprint Research Group [8].
7. Prepare fresh UPLC solvents at least weekly.
8. Run blanks (MeOH containing 5% (v/v) formic acid) between the calibration series and samples to minimize carryover.
9. When using the UPLC-TOF-MS setup, it is necessary to generate two sample lists, one using the Waters software to specify the sample position, injection volume, and UPLC method. In the UPLC method, program the contact closure at the start of the UPLC run (using a 9-pole cable as contact closure cable). Generate a worklist in the Mass Hunter Acquisition Software, specifying sample name, sample type, and MS method. Set under the worklist run parameters, and run type to external start. Start the MS worklist, wait for the instrument to reach pre-run state, and then start the UPLC sample list. The MS will automatically start recording spectra after the UPLC has injected a sample.
10. Table 2 provides comparison of limits of detection, quantification, intraday reproducibility, and linearity of the 21 amino acids on both the triple-quadrupole and the time-of-flight MS. LOD and LOQ were estimated based on S/N ratios (3:1 and 10:1). Intraday reproducibility is based on the relative standard deviation of peak areas from isotopically labeled amino acids from five different standards.

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