

Chapter 2

Amino Acid Profiling for the Diagnosis of Inborn Errors of Metabolism

Monique Piraud, Séverine Ruet, Sylvie Boyer, Cécile Acquaviva, Pascale Clerc-Renaud, David Cheillan, and Christine Vianey-Saban

Abstract

The diagnosis of inherited metabolic disorders of amino acid (AA) metabolism is based on the qualitative and/or the quantitative analysis of AAs, mainly in blood and urine. For years, the most widespread technique in use was ion-exchange chromatography followed by post-column derivatization with ninhydrin, a method which is the basis of numerous automated AA analyzers with a throughput of about eight samples/day. The emergence of tandem mass spectrometry (MS/MS) coupled to liquid chromatography (LC) has made possible the measurement of many metabolites for the diagnosis of inborn errors of metabolism. The LC-MS/MS method described here allows the clinical diagnosis of AA disorders by analysis of underivatized AAs and derivative molecules in various biological samples prepared by methanol precipitation. AAs are separated by ion-pairing reversed-phase LC, using perfluorocarboxylic acid as an ion-pairing agent. Each AA is detected in MS/MS-positive ionization mode by its specific transition. The method allows the analysis of about 40 biological samples/day.

Key words: Amino acids, inborn errors of metabolism, tandem mass spectrometry, liquid chromatography.

1. Introduction

The diagnosis of inherited metabolic disorders of amino acid (AA) metabolism is based on the qualitative and/or the quantitative investigation of about 80 AAs or derivative molecules in biological fluids, mainly blood and urine, but also cerebrospinal fluid (CSF), leukocytes, and amniotic fluid (AF). For years, the most

widespread technique for this purpose was ion-exchange chromatography followed by post-column derivatization with ninhydrin, a method which is the basis of numerous automated AA analyzers with a throughput of about eight samples/day (1).

Tandem mass spectrometry (MS/MS) coupled to liquid chromatography (LC) is still a developing technology capable of replacing classical methods in clinical biology (2) for the diagnosis of inborn errors of metabolism (3–5). Indeed, LC-MS/MS has been applied to newborn screening and other numerous metabolic conditions (6).

An LC-MS/MS method has been developed allowing for the analysis of underivatized AAs in various biological samples for clinical diagnosis (7–9). The samples (plasma, urine, CSF, leukocytes, AF) are prepared by methanol precipitation. AAs are separated by ion-pairing reversed-phase liquid chromatography, using perfluorocarboxylic acid as an ion-pairing agent. Each AA is detected in MS/MS-positive ionization mode by its specific transition. The method allows a much higher throughput (about 40 biological samples/day) compared to traditional methods, and the technique can be adapted to other kinds of samples.

2. Materials

Reagents used for daily analysis of AAs are underlined. All of them are made ready for analysis.

2.1. Common Reagents

1. Acetonitrile.
2. HPLC-grade methanol.
3. Sterile deionized water.

2.2. LC

1. Solvent A: 0.5 mM Tridecafluoroheptanoic acid (TDFHA). Store at room temperature. For preparation of the stock solution, *see* **Note 1**.
2. Solvent B: Acetonitrile. Store at room temperature.
3. Octadecyl-bonded silica gel LC column: Modulo-cart QS Uptisphere, 120 Å, 3 µm BP2, 50 mm × 2 mm (Interchrom; Interchim, Montluçon, France) (*see* **Note 2**).
4. Pre-columns of the same stationary phase (Interchim): 10 mm × 2 mm.
5. On-line filters (Interchim): 2 µm.
6. Direct connectors for 10-mm pre-column (Interchim).
7. Two Series 200 micropumps (Perkin-Elmer, Norwalk, CT, USA) (*see* **Note 3**).

8. Series 200 autosampler (Perkin–Elmer) (*see Note 3*).
9. The temperature of the LC column is controlled with a Croco-Cil (Cluzeau Info Labo, Sainte-Foy-la-Grande, France).

2.3. MS/MS

1. API 2000 triple-quadrupole mass spectrometer (AB Sciex, Toronto, Canada) equipped with a TurboIonSpray source.
2. Balston generator (Parker Hannifin International Ltd, Milan, Italy) to produce nitrogen from compressed (8 bars) air for desolvation and collision.
3. Analyst 1.3.1 software (AB Sciex).

2.4. Amino Acids and AA Qualitative Standards

Beside the individual stock solution for each molecule, a solution containing 26 common AAs is prepared for daily checking of the chromatographic separation.

1. Investigated AAs and other derivative molecules and their manufacturers are listed in **Table 2.1**, with abbreviations used.
2. Stock solutions of each molecule (5 mM in water (*see Note 4*)). Store in aliquots at -20°C for a maximum of 1 year.
3. L-Cysteine-L-homocysteine disulfide (Cys-Hcy) is not commercially available (*see Note 5*).
4. Anhydrides of L-argininosuccinic acid (ASA anhydrides) are not commercially available (*see Note 6*).
5. 50 μM stock solution (26 AAs): Combine 1 mL of each 5 mM AA solution (*see list in Table 2.2*) and complete to 100 mL with water. Store in 1 mL aliquots at -20°C for a maximum of 1 year.
6. 25 μM solution (26 AAs): Dilute 1 mL of the 50 μM stock solution with 1 mL of 1.25 mM TDFHA in a 2-mL injection vial. This solution should be prepared every other week.
7. S-2-Aminoethylcysteine (S2AE, *see Note 7*) 1 mM stock solution in methanol: prepare in a glass flask with grinded cap by dissolving 20.1 mg of S2AE in 100 mL methanol. Store in a glass flask with grinded cap tightly closed at 4°C (maximum 6 months, *see Note 8*). Do not let the flask open longer than necessary when preparing the deproteinization reagent (*see Note 8*).

Table 2.1

List of amino acids and derivative molecules of biological interest for the diagnosis of inherited disorders of amino acids metabolism

Name	Abbreviation	MM	Manufacturer	Biological interest	Sample
Ethanolamine, HCl	EA	61	Calbiochem	Hypoxia	P,U
Glycine	Gly	75	Merck	Nonketotic hyperglycinemia, organic acidurias	P,U,CSF
L-Alanine	Ala	89	Calbiochem	Hyperlactacidemia	P,U
Sarcosine	Sar	89	Sigma	MAD (ETF or ETF-QO deficiency)	P,U
β -Alanine	β -Ala	89	Merck	Hyper- β -alaninemia	U
L- α -Amino- <i>n</i> -butyric acid	Abu	103	Calbiochem	Protein intake	P
N,N-Dimethylglycine	(Me) ₂ -Gly	103	Sigma	MAD (ETF or ETF-QO deficiency)	P,U
β -Aminoisobutyric acid	β -AIB	103	Sigma	Neoplasia, excessive tissue breakdown	U
γ -Aminobutyric acid	GABA	103	Calbiochem	CNS disorders, neurotransmitter disorders	P,U,CSF
L-Serine	Ser	105	Calbiochem	Vitamin B6 deficiency, B6 synthesis deficiency, serine deficiency disorders	P,U, CSF
Δ^1 -Pyrroline-5-carboxylic acid	PC	113	Sigma	Hyperprolinemia (type II)	U
L-Proline	Pro	115	Calbiochem	Hyperprolinemia (types I, II)	P,U
L-Valine	Val	117	Merck	Maple syrup urine disease	P,U
L-Homoserine	Hse	119	Sigma	Interference (neuroblastoma)	P, U
L-Threonine	Thr	119	Calbiochem	Hepatic failure	P,U
Taurine	Tau	125	Sigma	Sulfite oxidase deficiency	P,U
L-Pipecolic acid	Pip	129	Sigma	Peroxisomal disorders	P,U
Pyro-L-glutamic acid	pGlu	129	Fluka	Pyroglutamic aciduria	P,U
ϵ -Aminocaproic acid	eCap	131	Calbiochem	Interference (therapeutic agent)	
δ -Aminolevulinic acid	dALA	131	Sigma	Tyrosinemia type I, porphyrias	U
4-Hydroxy-L-proline	Hyp	131	Calbiochem	Hydroxyprolinemia	P,U
L-Leucine	Leu	131	Calbiochem	Maple syrup urine disease	P,U
L- <i>allo</i> -Isoleucine	<i>al</i> Ile	131	Sigma	Maple syrup urine disease	P,U

Table 2.1
(continued)

Name	Abbreviation	MM	Manufacturer	Biological interest	Sample
L-Isoleucine	Ile	131	Calbiochem	Maple syrup urine disease	P,U
L-Ornithine, HCl	Orn	132	Merck	Urea cycle disorders, HHH, hyperornithinemia	P,U
L-Asparagine	Asn	132	Fluka	Asparaginase treatment (leukemia)	P,U
L-Aspartic acid	Asp	133	Merck	Asparaginase treatment (leukemia)	P,U
L-Homocysteine	Hcy	135	Sigma	Homocystinuria, Cbl C/D deficiency	P,U
L-Glutamine	Gln	146	Merck	Hyperammonemia, urea cycle disorders	P,U
L-Lysine, HCl	Lys	146	Calbiochem	Hyperlysinemia, cystinuria-lysinuria	P,U
L-Glutamic acid	Glu	147	Calbiochem	Urea cycle disorders	P,U
L-Methionine	Met	149	Calbiochem	Homocystinuria, hypermethioninemia	P,U
L-Histidine, HCl	His	155	Calbiochem	Histidinemia	P,U
L- α -Aminoadipic acid	Aad	161	Sigma	α -Aminoadipic aciduria	P,U
5-Hydroxy-L-lysine, HCl	Hyl	162	Sigma	Hydroxylysinuria	U
N-Acetyl-L-cysteine	NAcCys	163	Merck	Interference (therapeutic agent)	P,U
L-Phenylalanine	Phe	165	Merck	Phenylketonuria, hyperphenylalaninemia	P,U
N ³ -Methyl-L-histidine	His-1Me	169	Calbiochem	Renal insufficiency	P,U
N ¹ -Methyl-L-histidine	His-3Me	169	Calbiochem	Renal insufficiency, denutrition, starvation	P,U
Glycyl-L-proline	Gly-Pro	171	Sigma	Iminodipeptiduria	U
L-Arginine	Arg	174	Merck	Urea cycle disorders	P,U
Formimino-L-glutamic acid	FIGLU	174	Sigma	FIGLU aciduria	U
N-Acetyl-L-ornithine	NAcOrn	174	Sigma	Interference (hyperornithinemia)	
L-Citrulline	Cit	175	Merck	Urea cycle disorders	P,U
L-Tyrosine ^o	Tyr	181	Calbiochem	Tyrosinemia type I or II	P,U
L-Homocitrulline	Hci	189	ICN	HHH	P,U
L-3-(3,4-Dihydroxyphenyl)-alanine ^o	Dopa	197	Sigma	Tyrosinemia, neurotransmitter disorders	U

Table 2.1
(continued)

Name	Abbreviation	MM	Manufacturer	Biological interest	Sample
S-Sulfo-L-cysteine	S-Cys	201	Sigma	Sulfite oxidase deficiency	P,U
N^G, N^G - Dimethylarginine, HCl	asym(Me) ₂ Arg	202	Sigma	Physiological	U
L-Tryptophan ^o	Trp	204	Calbiochem	Hartnup disease	P,U
L-Kynurenine	Kyn	208	Sigma	Vitamin B6 deficiency	U
5-Hydroxy-L-tryptophan	Hyt	220	Sigma	Therapeutic agent, neuro-transmitter disorders	U
L-Cystathionine ^o	Hcy(Ala)	222	Sigma	Cystathioninuria, neuroblastoma	P,U
3-Hydroxy-L-kynurenine	Hyk	224	Sigma	Vitamin B6 deficiency	U
L-Carnosine	Car	226	Calbiochem	Carnosinemia, physiological (meat)	U
L-Anserine	Ans	240	Sigma	Carnosinemia, physiological (meat)	U
L-Cystine	(Cys) ₂	240	Merck	Cystinuria, cystinuria–lysinuria, homocystinuria, sulfite oxidase deficiency Cystinosis	P,U L
L-Cysteine-L-homocysteine disulfide	Cys-Hcy	254	*	Homocystinuria, cystinuria	P,U
L-Homocystine	(Hcy) ₂	268	Sigma	Homocystinuria	P,U
L-Argininosuccinic anhydrides	ASA anh	272	*	Urea cycle disorders (AS aciduria)	P,U
L-Saccharopine	Sac	276	Sigma	Saccharopinuria	P,U
L-Argininosuccinic acid	ASA	290	Sigma	Urea cycle disorders (AS aciduria)	P,U
Glutathione (reduced form)	GSH	307	Sigma	γ GT deficiency, GSH synthase deficiency	P,U
β -Aspartyl-glucosamine	GlcNAc-Asn	335	Sigma	Aspartylglucosaminuria	U

Table 2.1
(continued)

Name	Abbreviation	MM	Manufacturer	Biological interest	Sample
S-Adenosyl-L-homocysteine	Ad-Hcy	384	Sigma	Homocystinuria, hypermethioninemia	P,U
S-Adenosyl-L-methionine	Ad-Met	398	Sigma	Homocystinuria, hypermethioninemia	P,U
Glutathione (oxidized form)	GSSG	612	Sigma	γ GT deficiency, GSH synthase deficiency	P,U

*: for preparation, *see* text. °: molecules for which 2 drops of 37% HCl are added per 10 mL of 5 mM stock solution for dissolution. MM, molecular mass; MAD, multiple acyl-CoA dehydrogenase deficiency; ETF, electron transfer flavoprotein; ETF-QO, ETF-ubiquinone oxidoreductase; CNS, central nervous system; Cbl, cobalamine; HHH, hyperammonemia, hyperornithinemia, homocitrullinuria; P, plasma; U, urine; CSF, cerebrospinal fluid; L, leukocytes. Reproduced with permission from ref. (7).

2.5. Stable Isotope-Labeled AAs (Internal Standards, AAs*)

1. Stable isotope-labeled standards for MS/MS are listed in **Table 2.3**, along with their abbreviations (*see* **Note 9**).
2. Stock solutions of each molecule (25 mM for Gly*, 15 mM for Gln*, 10 mM for Ala* and Pro*; 5 mM for others) in water (*see* **Note 4**). Store in aliquots at -20°C for a maximum of 1 year.
3. Working solutions: 17 AAs* at 200 μM (except Pro* and Ala* at 400 μM , Gln* at 600 μM , and Gly* at 1 mM). In a 50-mL flask, add 2 mL of each AA* stock solution. Complete to 50 mL with water, mix, and store in aliquots at -20°C for a maximum of 1 year.

2.6. AA Quantification Standards

Two quantitative standards are used daily for quantification: one is prepared from a commercially available AA standard supplemented in order to obtain a standard with all the most commonly measured physiological AAs for the diagnosis of inborn errors of metabolism (100 and 50 μM AAs standards) and one is home-made and contains all the molecules to be quantified that are not present in the other standard (supplemental AAs standard).

1. "Amino acid standards, physiological acidics, neutrals and basics for calibrating amino acid analyzers" (Sigma-Aldrich) containing several AAs at 500 μM .
2. AA additional stock solution: Gln 6.25 mM, Gly 5 mM, Asn 1.25 mM. Store in single-use aliquots at -20°C for a maximum of 6 months.

Table 2.2

List of unlabeled AAs and derivative molecules of biological interest for the diagnosis of inherited disorders of amino acid metabolism. Corresponding specific transitions used for the identification of AAs in MS/MS positive ionization mode are given with their specific declustering potential (DP, in V) and collision energy (CE, in eV). Concentrations (in μM) of analytes in the 100 μM AA standard solution and in the supplemental standard solution are indicated, as well as AAs present in the 25 μM solution (26 AAs). Transitions are monitored either in period 1 (0–3.5 min of the chromatogram), in period 2 (3.5–9 min), and/or in period 3 (9–20 min). #: transition 132 > 86 is common to Hyp, Leu, Ile, and dALA; transition 175 > 130 is common to Arg and FIGLU; RT: retention time; *In italics*: molecules for which quantitation could not be validated. Reproduced with permission from ref. (9)

	Abbreviation	Monitored transition	DP	CE	AA*	26 AAs	Expected RT	Conc. in		Monitoring period
								μM AA standard	Supplemental AA standard (in μM)	
Ethanolamine	EA	62 > 44	20	16	Leu*		8.8	100		3
Glycine	Gly	76 > 30	10	16	Gly*	x	1.8	500		1
L-Alanine	Ala	90 > 44	20	20	Ala*	x	2.4	100		1
Sarcosine	Sar	90 > 44	20	20	Ala*		1.6	100		1
<i>β-Alanine</i>	<i>β-Ala</i>	<i>90 > 72</i>	<i>20</i>	<i>12</i>	<i>Val*</i>		<i>8.0</i>	<i>100</i>		2–3
L- α -Amino- <i>n</i> -butyric acid	Abu	104 > 58	20	18	Pro*	x	3.7	100		1–2
N,N-Dimethylglycine	(Me) ₂ Gly	104 > 58	20	18	Gly*		1.3		200	1
β -Aminoisobutyric acid (1)	β -AIB	104 > 86	20	14	Lys*		9.5	100	200	3
γ -Aminobutyric acid	GABA	104 > 86	20	14	Lys*		9.5	100		3
		104 > 87	20	14						3

Table 2.2
(continued)

	Abbreviation	Monitored transition	DP	CE	AA*	26 AAs	Expected RT	Conc. in 100 μ M AA standard (in μ M)	Conc. in Supplemental AA standard (in μ M)	Monitoring period
L-Serine	Ser	106 > 60	10	14	Ser*	x	1.6	100		1
Δ 1-Pyrroline-5-carboxylic acid	PC	114 > 68	25	20	Val*		2.0		200	1
L-Proline	Pro	116 > 70	20	20	Pro*	x	2.3	100		1
L-Valine	Val	118 > 72	25	16	Val*	x	6.0	100		2
L-Homoserine	Hse	120 > 44	20	32			1.9			1
		120 > 74	20	32						1
L-Threonine	Thr	120 > 74	20	32	Ala*	x	1.9	100		1
<i>Taurine</i>	<i>Tau</i>	126 > 108	40	16	Ala*		0.77	100		1
L-Pipecolic acid	Pip	130 > 84	30	20	Val*		5.2		200	2
<i>Pyroglutamic acid</i>	<i>pGlu</i>	130 > 84	30	20	Glu*		1.1			1
ϵ -Aminocaproic acid	eCap	132 > 79	30	24			11.2			3
		132 > 114	20	16						
δ -Aminolevulinic acid	dALA	132 > 114	20	16	Leu*		9.6		200	3
4-Hydroxy-L-proline	Hyp	132 > 68	30	30	Asp*	x	1.3	100		1
L-Leucine	Leu	132 > 43	30	36	Leu*	x	10.4	100		3
L- <i>allo</i> -isoleucine	<i>alle</i>	132 > 69	25	26	Leu*	x	9.2		200	2-3
L-Isoleucine	Ile	132 > 69	25	26	Leu*	x	9.8	100		2-3
		132 > 86# (2)	25	16						

Table 2.2
(continued)

Abbreviation	Monitored transition	DP	CE	AA*	26 AAs	Expected RT	Conc. in		Monitoring period
							100 μ M AA standard (in μ M)	Supplemental AA standard (in μ M)	
L-Ornithine	Orn	133 > 70	10	22	Orn*	x	12.1	100	3
L-Asparagine	Asn	133 > 74	25	18	Asp*	x	1.5	100	1
L-Aspartic acid	Asp	134 > 74	25	18	Asp*	x	1.3	100	1
L-Homocysteine	Hcy	136 > 90	10	16			4.3		2
L-Glutamine	Gln	147 > 84	20	24	Gln*	x	1.74	500	1
L-Lysine	Lys	147 > 84	20	24	Lys*	x	12.6	100	3
		147 > 67	20	36					3
L-Glutamic acid	Glu	148 > 84	15	24	Glu*	x	2.0	100	1
L-Methionine	Met	150 > 104	10	14	Met*	x	6.5	100	2
L-Histidine	His	156 > 110	20	18	Lys*	x	12.0	100	3
L- α -Aminoadipic acid	Aad	162 > 98	20	24	Pro*		3.9	100	1–2
5-Hydroxy-L-lysine	Hyl	163 > 128	5	18	Lys*		12.4		3
L-Phenylalanine	Phe	166 > 120	30	20	Phe*	x	11.2	100	3
N ¹ -Methylhistidine	His-3Me	170 > 124	30	20	Lys*	x	12.0	100	3
N ³ -Methylhistidine	His-1Me	170 > 126	30	20	Lys*	x	11.9	100	3
Glycyl-L-proline	Gly-Pro	173 > 116	15	16	Leu*		9.7	200	2–3
L-Arginine	Arg	175 > 70	20	32	Arg*	x	12.7	100	3
Formimino-L-glutamic acid	FIGLU	175 > 84	25	32	Glu*		3.5	200	2
		175 > 130#	20	22					2

Table 2.2
(continued)

	Abbreviation	Monitored transition	DP	CE	AA*	26 AAs	Expected RT	Conc. in 100 μ M AA standard (in μ M)	Conc. in Supplemental AA standard (in μ M)	Monitoring period
L-Citrulline	Cit	176 > 159	10	14	Ala*, Pro*	x	2.6	100		1–2
L-Tyrosine	Tyr	182 > 165	25	12	Tyr*	x	8.5	100		2–3
L-Homocitrulline	Hci	190 > 173	20	16	Val*		4.5	200		2
L-3-(3,4-Dihydroxyphenyl)-alanine	Dopa	198 > 152	10	20	Met*		7.0	200		2
S-Sulfo-L-cysteine	S-Cys	202 > 120	15	18	Ala*		0.6	200		1
N _G ,N _G -Dimethylarginine	asym(Me) ₂ Arg	203 > 70	20	42	Lys*		12.8	200		3
L-Tryptophan	Trp	205 > 188	10	18	Lys*		12.4	200		3
L-Kynurenine	Kyn	209 > 192	20	14	Lys*		11.9	200		3
5-Hydroxy-L-tryptophan	Hyt	221 > 204	20	10			10.4			3
L-Cystathionine	Hcy(Ala)	223 > 88	15	40	Ala*		3.5	100		1–2
3-Hydroxy-L-kynurenine	Hylk	225 > 208	20	14	Lys*		10.0	200		3
L-Carnosine	Car	227 > 110	30	30	Lys*		14.0	100		3
L-Anserine	Ans	241 > 109	30	35	Lys*		13.8	100		3

Table 2.2
(continued)

Abbreviation	Monitored transition	DP	CE	AA*	26 AAs	Expected RT	Conc. in μM AA standard (in μM)	Conc. in Supplemental AA standard (in μM)	Monitoring period
L-Cystine	(Cys) ₂	241 > 74	25	40	(Cys) ₂ [*]	x	2.1	100	1
L-Cysteine-L-homocysteine disulfide	Cys-Hcy	255 > 134	100	18	(Cys) ₂ [*]		5.0		2
L-Homocystine	(Hcy) ₂	269 > 136	10	12	(Hcy) ₂ [*]		11.8	100	3
L-Argininosuccinic anhydrides	ASA anb	273 > 70	20	55	Lys [*]		12.4		3
L-Saccharopine	Sac	277 > 84	25	36	Val [*]		5.3	200	2
L-Argininosuccinic acid	ASA	291 > 70	30	48	Lys [*]		11.5		3
Glutathione (reduced form)	GSH	308 > 179	25	18	Val [*]		4.1		2
β -Aspartylglucosamine	GlcNAc-Asn	336 > 126	15	24	Asp [*]		1.6	200	1
S-Adenosyl-L-homocysteine	Ad-Hcy	385 > 88	30	64	Lys [*]		12.7	200	3
S-Adenosyl-L-methionine	Ad-Met	399 > 250	15	20	Lys [*]		15.3	200	3
Glutathione (oxidized form)	GSSG	613 > 355	20	30	Val [*]		8.7		2
S-2-Aminoethylcysteine	S2AE	165 > 120	8	18	Lys [*]		11.1		

(1) BAIB is not measurable when GABA is detectable.
(2) Common transition to Leu, Ile, alle, dALA, and Hyp. It cannot be used for Ile quantification when dALA is present.

Table 2.3
List of the 17 stable isotope-labeled standards (AA*)

Abbreviation	Monitored transition	DP	CE	Manufacturer	Stock solution (mM)
Glycine (2,2-D ₂)	78 > 32	10	16	CIL	25
DL-Alanine (2,3,3,3-D ₄)	94 > 48	20	20	CIL	10
DL-Serine (2,3,3- D ₃)	109 > 63	10	14	CIL	5
DL-Proline (2,3,3,4,4,5,5-D ₇)	123 > 77	20	20	CIL	10
DL-Valine-D ₈	126 > 80	25	16	CIL	5
L-Leucine (5,5,5-D ₃)	135 > 89	25	16	Euriso-top	5
	135 > 46	30	36		5
L-Ornithine (5,5-D ₂), 2HCl	135 > 72	10	22	CIL	5
L-Aspartic acid (2,3,3-D ₃)	137 > 75	25	18	Euriso-top	5
DL-Glutamic acid (2,4,4-D ₃)	151 > 87	15	24	CIL	5
DL-Lysine (4,4,5,5-D ₄), 2HCl	151 > 88	20	24	Euriso-top	5
L-Glutamine (2,3,3,4,4-D ₅)	152 > 88	20	24	CIL	15
L-Methionine (methyl-D ₃)	153 > 107	10	14	Euriso-top	5
L-Phenylalanine (ring-D ₅)	171 > 125	30	20	CIL	5
L-Arginine (guanido- ¹⁵ N ₂), HCl	177 > 70	20	32	CIL	5
L-Tyrosine (ring 3,5-D ₂)	184 > 167	25	12	CIL	5
DL-Cystine (3,3,3',3'-D ₄)	(Cys) ₂ * 245 > 74	25	40	CIL	5
DL-Homocystine (3,3,3',3',4,4,4',4'-D ₈)	(Hcy) ₂ * 277 > 140	10	12	CIL	5

Corresponding specific transitions used for their identification in MS/MS positive ionization mode are given with their specific declustering potential (DP, in V) and collision energy (CE, in eV). CIL, Cambridge Isotope Laboratories. Reproduced with permission from ref. (8).

3. AA diluted solution: Gln 625 μM , Gly 500 μM , Asn 125 μM . Add 1 mL AA additional stock solution to 9 mL of water. Use this solution within the day of preparation.
4. 100 μM AA standard (*see* list in **Table 2.2**): all AAs at 100 μM (except Gly and Gln at 500 μM). Add 300 μL of the Sigma standard (“amino acid standards, physiological acidics, neutrals, and basics for calibrating amino acid analyzers”) to 1200 μL of AA diluted solution. Store in aliquots at -20°C for a maximum of 1 month. When thawed, store at 4°C for a maximum of 1 week.
5. 50 μM AA standard: Dilute the 100 μM AA solution. Prepare weekly and use for a maximum of 1 week with storage at 4°C .
6. Supplemental AA standard (*see* list in **Table 2.2**): several molecules at 200 μM , which are not present in the 100 μM standard. Combine 1 mL of each 5 mM stock solution and complete to 25 mL. Store in 1 mL aliquots at -20°C for a maximum of 2 years. When thawed, store at 4°C for a maximum of 1 week.
7. 0 μM standard is a water blank.

2.7. Materials for Preparation of Standards and Samples

1. 2-mL injection vials with screw-top caps (Interchim).
2. 9-mm silicone/PTFE slit screw caps (Interchim).
3. Microtest tube rack (Brand, Wertheim, Germany).
4. Microtubes PP 1.5 mL with attached PP safety cap (Sarstedt France, Marnay, France).
5. Deproteinization reagent: 20 μM S2AE in methanol (*see Note 7*). Dilute the S2AE stock solution 1:49 with methanol in a 50-mL glass flask with grinded cap. Store tightly closed at 4°C for a maximum of 1 month. This reagent can be aliquoted to small glass flasks with grinded caps. Open only when used and close immediately after use (*see Note 8*).
6. 1.25 mM TDFHA working solution (for preparation of the stock solution, *see Note 1*)
7. Plasma: Sample blood with heparin, then centrifuge 10 min at $5000\times g$ at 4°C . Transfer plasma (200 μL minimum) in a tube. Deproteinize immediately or store at -80°C .
8. Urine: Determine creatinine concentration for each urine sample with any classical creatinine determination method for this purpose (e.g., Jaffé reaction) and freeze one aliquot at -20°C for AA determination (1 mL minimum).
9. Cerebrospinal fluid (CSF) and amniotic fluid (AF): Freeze the sample (300 μL minimum) at -20°C immediately.

10. Leukocytes: Isolate the leukocytes pellet from 15–20 mL of blood as soon as possible (maximum 48 h) after sampling with citric acid–citrate–dextrose as anticlotting agent, according to usual procedures currently in use in the laboratory for leukocytes isolation. For preparation of the leukocyte mixture and analysis, *see* **Note 10**.

2.8. Quality Control Samples

1. External QAP (Quality Assurance Program): ERNDIM amino acid scheme (www.erndimqa.nl).
2. External QAP: ERNDIM cystine in white blood cell scheme (www.erndimqa.nl).
3. Internal QAP: SKML control material amino acids (www.erndimqa.nl).

3. Methods

Samples for AA analysis are first deproteinized with methanol containing a known quantity of S2AE for control of the correct dilution/precipitation of the sample. Ion-pairing reversed-phase liquid chromatography is used for separation of AAs, and the use of volatile mobile phases (ACN, TDFHA, water) allows MS/MS detection, particularly with the TurboIonSpray source adapted to evaporation of water containing mobile phases. This chromatographic system must be used in stable conditions as described (with respect to rinsing periods) in order to obtain repeatability of the amino acid retention times and of the quantification results.

As necessary for MS/MS quantitative analysis, 17 stable isotope-labeled AAs are used as IS. Quantification of the 17 corresponding AAs has been validated in these conditions. Validation of quantification has been obtained for many other AAs but not all. Several AAs for which quantitative validation has not been obtained are described anyway as qualitative markers useful for the diagnosis of some inherited disorders of AA metabolism.

3.1. Sample Preparation

1. Push 1.5-mL microtubes firmly onto the microtube rack.
2. AA standards (0, 50, and 100 μ M, AA Suppl. STD 200 μ M), plasma, CSF, AF, and quality control samples are used undiluted. For leukocytes, *see* **Note 10**.
3. Do not dilute urine samples if the creatinine concentration is below 4 mM. If the creatinine concentration exceeds 4 mM, then dilute urine with water as follows:
 - a. 4–8 mM creatinine, dilute 1:1.
 - b. 8–12 mM creatinine, dilute 1:2.

- c. 12–16 mM creatinine, dilute 1:3.
 - d. 16–20 mM creatinine, dilute 1:4.
 - e. ≥ 20 mM creatinine, dilute 1:9.
4. Mix 150 μL of each sample (standards, plasma, CSF, AF, controls or adequately diluted urine) with 600 μL of deproteinization reagent, close the cap, and mix thoroughly for 2 min.
 5. Store the samples at room temperature for 5 min.
 6. Centrifuge the samples at $17,500\times g$ for 5 min at $+4^{\circ}\text{C}$, then transfer the supernatants to new 1.5-mL microtubes, and store at -80°C until used.
 7. Prior to analysis, thaw and homogenize the deproteinized samples and standards.
 8. Combine 200 μL of each supernatant with 40 μL of AA* working solution and 160 μL of 1.25 mM TDFHA in an injection vial and mix.

3.2. Liquid Chromatography

1. Assemble the column and the pre-column using the connectors and add a pre-filter.
2. Maintain the column at 26°C .
3. Before the first use, rinse the column with ACN at 200 $\mu\text{L}/\text{min}$ for 2 h.
4. Separations are carried out at a flow rate of 200 $\mu\text{L}/\text{min}$.
5. Inject 5 μL of sample or standard.
6. Gradient elution is as follows: from 0 to 15% B in 1 min; maintain 15% B for 5 min; from 15 to 25% B in 3 min; maintain 25% B for 6 min; from 25 to 0% B in 1 min; maintain 0% B for 15 min to re-equilibrate the column before a new injection.
7. The sample throughput of the method is 2 samples/h. The LC effluent is directed to the mass spectrometer for the first 20 min of the separation, after which it can be directed to waste.

3.3. Tandem Mass Spectrometry

1. Set the ionization source to positive ionization mode.
2. Set the TurboIonSpray source to 450°C .
3. Set the curtain gas (CUR) to 20.
4. Set the collision gas to 2.
5. Set the ion source nebulization gas (GS1) to 25 (arbitrary units are given by the manufacturer).
6. Set the auxiliary gas (GS2) to 40.
7. Set Q1 and Q3 to unit resolution.
8. Set the ion spray voltage (IS) to 5000 V.

9. Set the focusing potential (FP) to 200 V.
10. Set the entrance potential (EP) to -10 V.
11. Specific transitions optimized for each unlabeled (**Table 2.2**) or labeled (**Table 2.3**) compound are listed (*see Note 11*) with their specific declustering potential (DP, in V) and collision energy (CE, in eV) parameters (*see Note 12*).
12. For each sample, acquisition of data is performed during the first 20 min of the gradient analysis, according to the chosen transitions in positive ionization mode and specific parameters (**Tables 2.2** and **2.3**). During the re-equilibration time, the flow can be diverted to waste.
13. The predicted retention time for each molecule is indicated (*see Table 2.2*).
14. For qualitative analysis (25 μ M solution (26 AAs)), the acquisition method contains all the involved transitions in a single period with a dwell time of 25 ms each.
15. For quantification using the Analyst 1.3.1 software, the acquisition time is divided into three periods (period 1 is from 0 to 3.5 min; period 2 is from 3.5 to 9 min; period 3 is from 9 to 20 min), during which only the relevant molecules are managed with their own transition (for the corresponding period, *see Table 2.2*). For each transition, the dwell time is 25 ms, except for Gly and Gly* (100 ms).
16. Some molecules are monitored over two periods in case there is a slight shift in RT (*see Table 2.2*).

3.4. LC-MS/MS Analysis

1. After rinsing the LC column, inject samples in the following order (*see Note 13*):
 - a. One 0.5 mM TDFHA sample (not usable).
 - b. One 25 μ M solution (26 AAs) (for checking the quality of the analysis).
 - c. 12–14 standards or samples (for quantification).
 - d. Rinse the column on line 1 h with ACN at 200 μ L/min.
2. The same sequence (from a to d) can be repeated two more times. Modifying or avoiding the rinsing periods may alter the chromatogram and cause shift in retention times.
3. The 0 and 100 μ M AA standards are analyzed in each sequence.
4. The 50 μ M and supplemental AA standards are analyzed at least two times every 24 h.
5. After each use (maximum 24 h, three sequences, 36–42 samples), rinse the column (out-line, reverse flow) with methanol/water (85:15) for 16 h at 100 μ L/min.

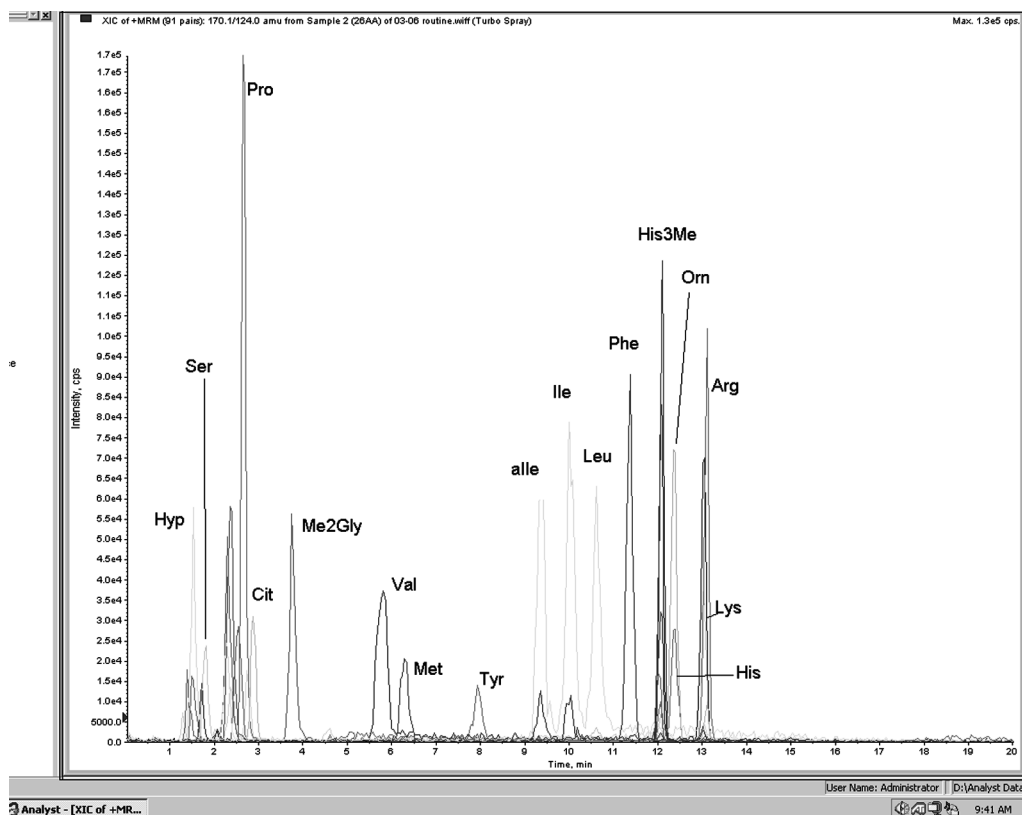


Fig. 2.1. Overall chromatogram obtained with the 26 AA sample, with an MS/MS method integrating the transitions of each molecule during all the analysis time (20 min). Column: Uptisphere 50 mm $\times$ 2 mm ID 3 μ m. Elution gradient: solvent A: 0.5 mM TDFHA in water and solvent B: acetonitrile; from 0 to 15% B in 1 min, 1–6 min 15% B maintained, 6–9 min linear gradient to 25% B, 9–15 min 25% B maintained, 15–16 min gradient back to 0% B, then 16–31 min 0% B to equilibrate the column before a new injection. Injection volume, 5 μ L; concentration of molecules in mixtures, 25 μ M each.

6. Check the quality of the chromatography based on the 25 μ M solution (26 AAs) (*see Fig. 2.1*).
7. Identify each AA based on its transition and its retention time.
8. The overall aspect of the chromatogram and the elution order of the AA must remain the same. The observed retention time for each AA must correspond to the expected value (*Table 2.2* and *Fig. 2.2*; *see Note 14*).
9. Visually inspect the separation of the critical pairs of AAs (*see Fig. 2.2*), i.e., mainly Glu and Gln (*see Fig. 2.2c*), Asp and Asn (*see Fig. 2.2d*), *α*Ile, Ile, and Leu (*see Fig. 2.2g*; *see Note 15*).
10. Verify the sensitivity of the system daily by comparing peak intensities of AAs with those of the previous days.

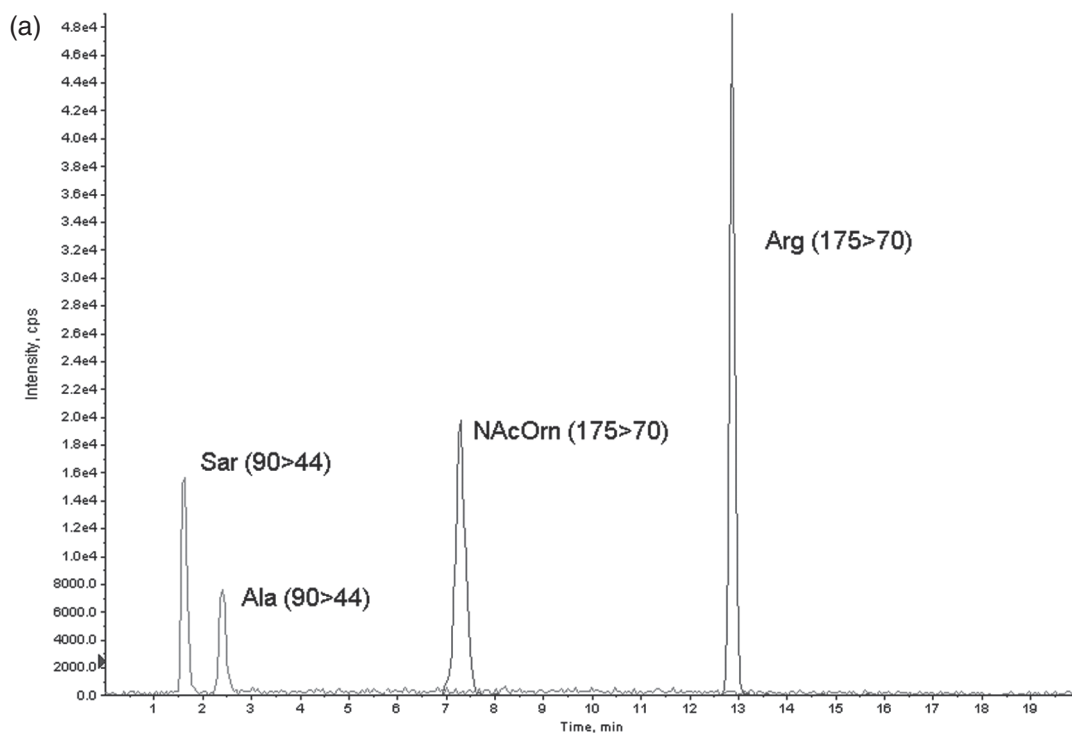


Fig. 2.2. (continued)

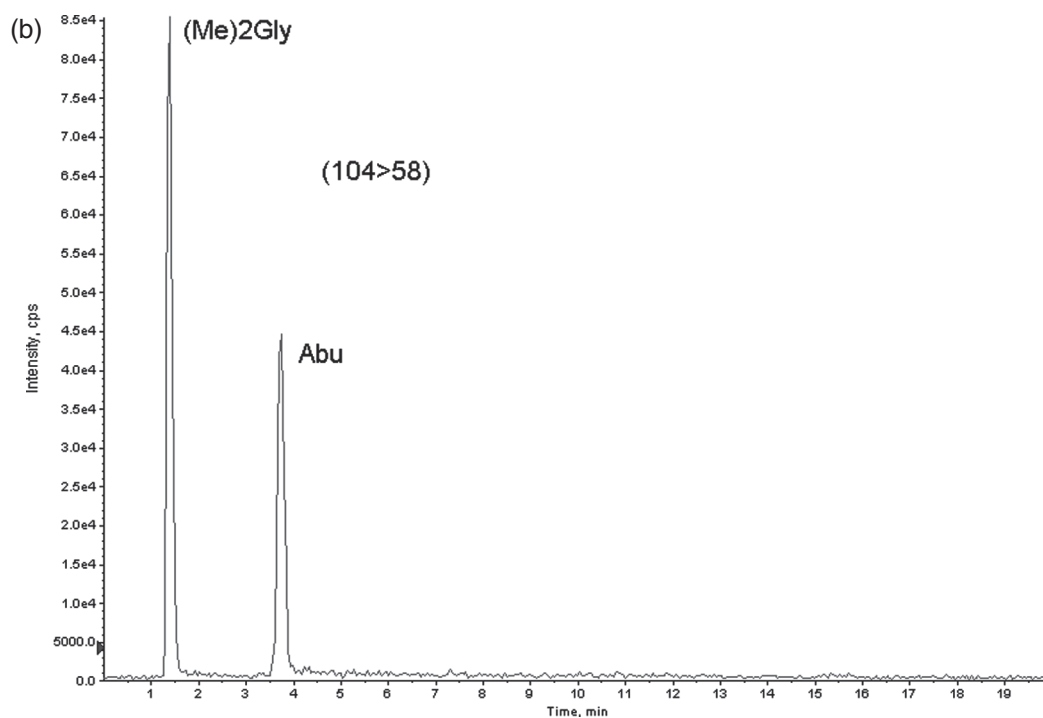


Fig. 2.2. (continued)

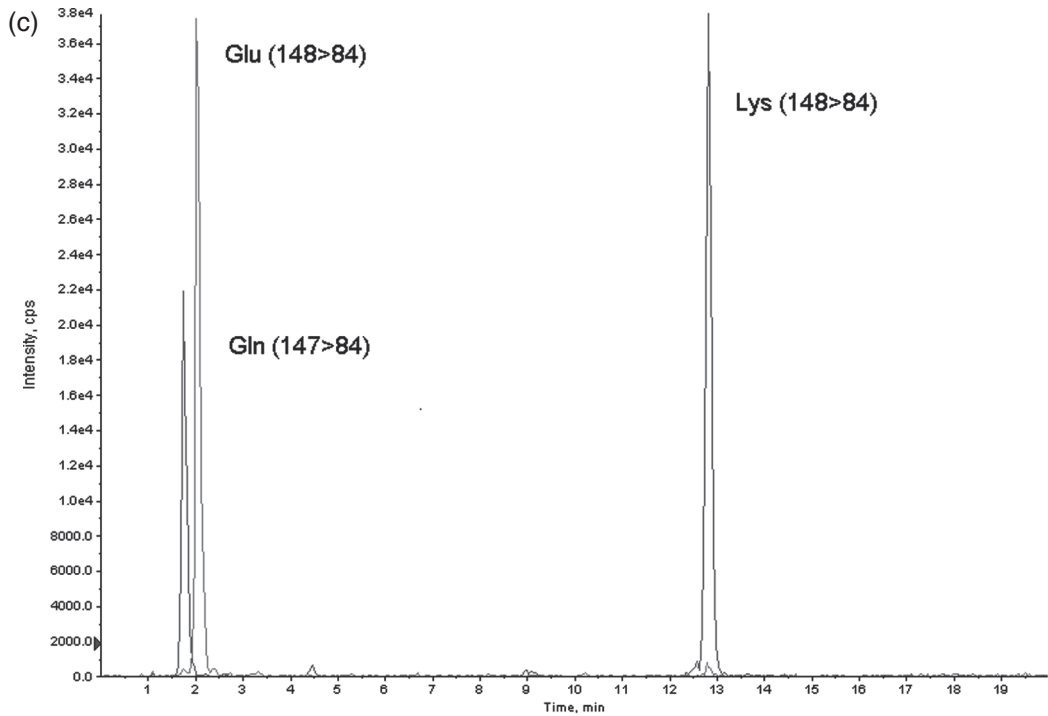


Fig. 2.2. (continued)

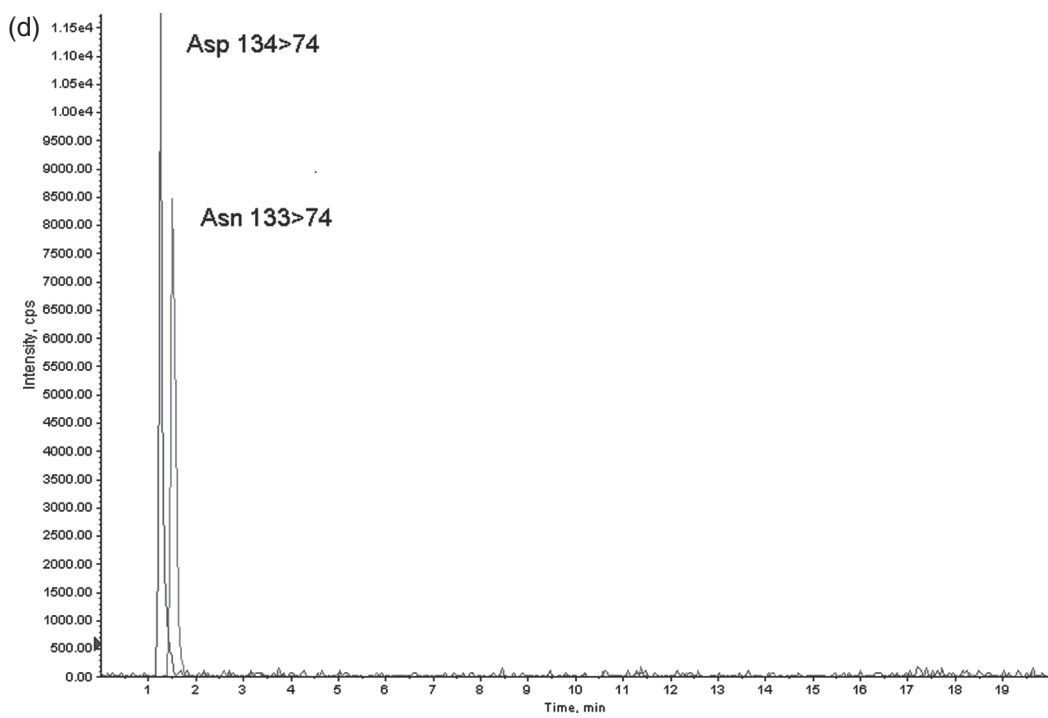


Fig. 2.2. (continued)

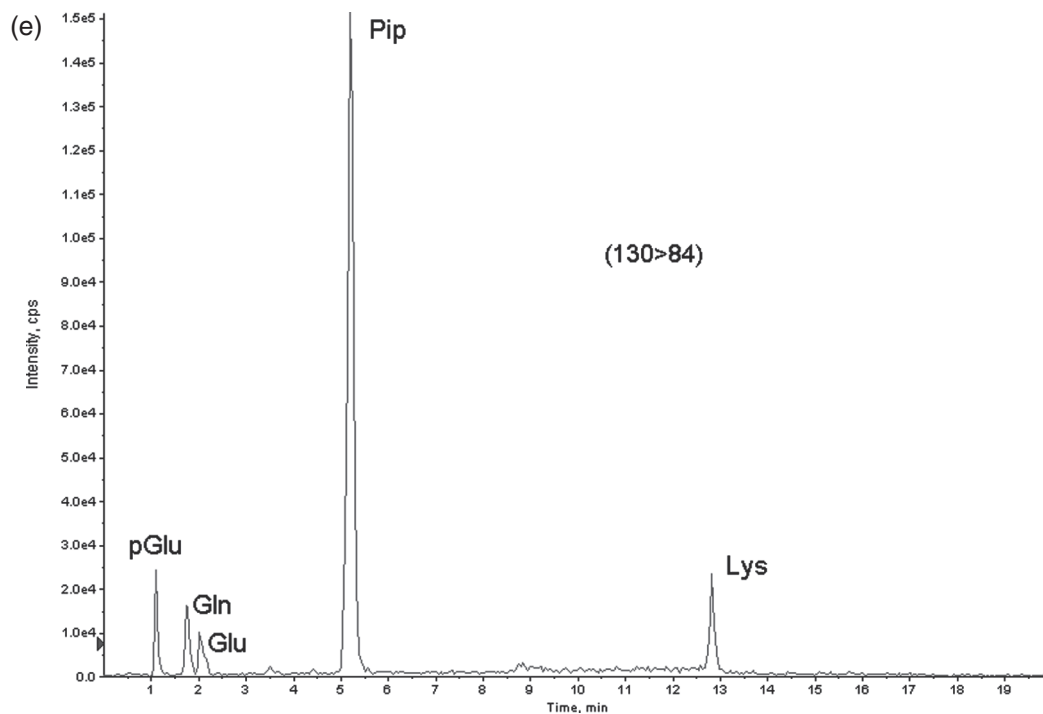


Fig. 2.2. (continued)

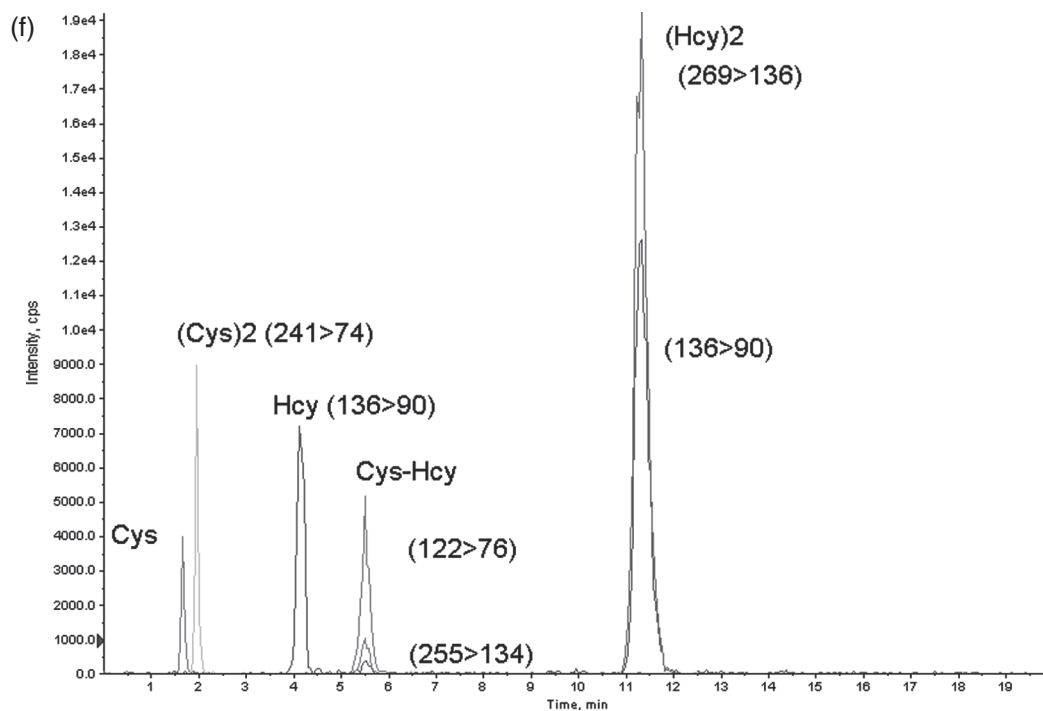


Fig. 2.2. (continued)

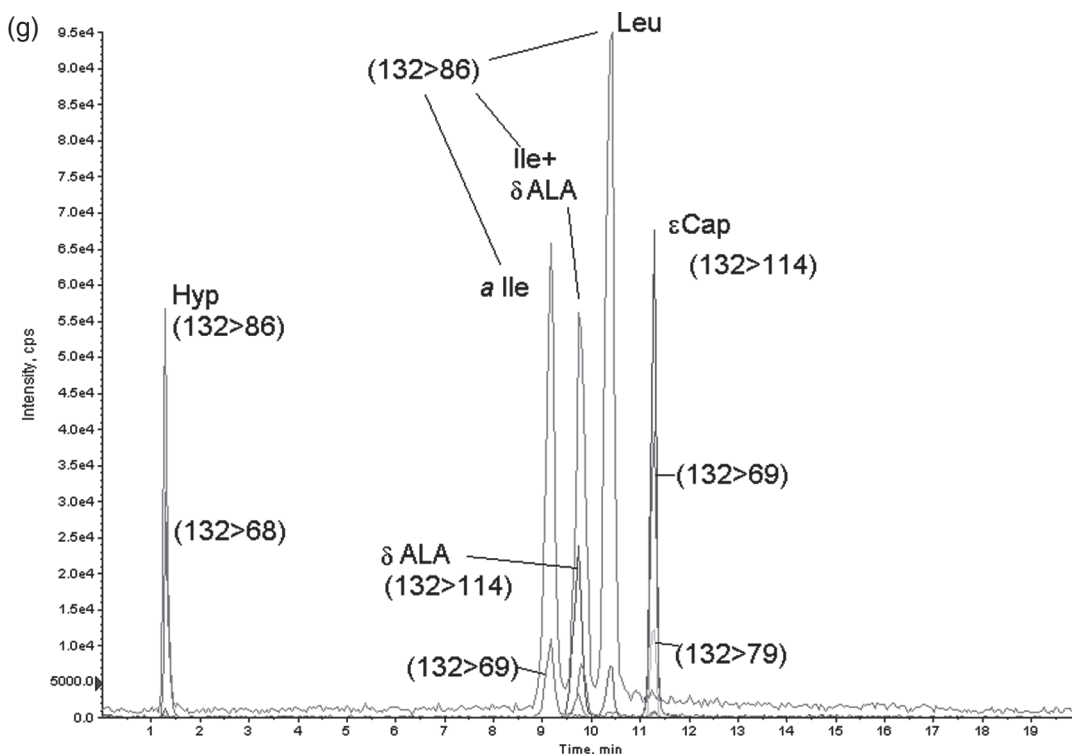


Fig. 2.2. Extracted ion current (XIC) chromatograms of the amino acid pairs that have similar MS/MS characteristics. Column: Uptisphere 50 mm $\times$ 2 mm ID, 3 μ m. Elution gradient: solvent A: 0.5 mM TDFHA in water and solvent B: acetonitrile; from 0 to 15% B in 1 min, 1–6 min 15% B maintained, 6–9 min linear gradient to 25% B, 9–15 min 25% B maintained, 15–16 min gradient back to 0% B, then 16–31 min 0% B to equilibrate the column before a new injection. Injection volume, 5 μ L; concentration of molecules in mixtures, 25 μ M each. (a) Ala, Sar (90 > 44); *N*-acetyl-L-ornithine (NAcOrn), Arg (175 > 70). (b) Abu, (Me)₂Gly (104 > 58). (c) Gln (147 > 84), Glu, Lys (148 > 84). (d) Asn (133 > 74), Asp (134 > 74). (e) Pip, pGlu (130 > 84). (f) Cys (122 > 76), Hcy (136 > 90), (Cys)₂ (241 > 74), (Hcy)₂ (269 > 136), Cys-Hcy (255 > 134). (g) Isobar molecules giving a parent ion at m/z 132: Leu, Ile, alle, dALA, Hyp, and ϵ Cap. Reproduced with permission from ref. (8).

3.5. Quantification

1. Perform quantification of AA after acquisition of all the samples in the three sequences using Analyst 1.3.1 software.
2. Use the corresponding AAs* as internal standards (*see* Table 2.2, Note 16) (peak area ratio), and the 0 and 100 μ M AA standards (or 200 μ M supplemental AA standard) as external standards according to a linear calibration curve.
3. The 50 μ M AA standard is used as a control sample.
4. The retention times of AAs and AAs* are noted, and the AA retention time/AA* retention time ratio (ratio of retention times) is calculated.
5. Confirm the quality of the peak integrations manually (*see* Note 17).
6. Quantitative validation has been obtained for most AAs measured by this method (*see* Note 17). However, quantita-

tive validation could not be obtained for some of them (**Table 2.2**). Therefore, their analysis is only qualitative (*see Note 18*).

7. In several cases, qualitative identification of the disease marker is sufficient for a diagnosis (e.g., ASA, pGlu, FIGLU, S-Cys, Cys-Hcy). Using the stable isotope-labeled IS analogues of the compounds when available will improve the results.
8. Direct quantification of Cys-Hcy and ASA is not possible because of the lack of quantitative standards (*see Notes 19 and 20*).
9. Validation of the series is achieved if quantitative results match with the calculated consensus values of the internal control SKML amino acids.

4. Notes

1. Preparation of the 13.73 mM Tridecafluoroheptanoic acid (TDFHA) stock solution: Prepare by dissolving 5 g of TDFHA in 1 L water and store at room temperature (maximum 6 months).
2. Other ultrapure octadecyl-bonded silica gel columns of the same characteristics could be used with no or slight modifications to the gradient shape, provided that the separation of critical pairs of AAs is obtained (*see 7, Section 3.4, Fig. 2.2 and Notes 14 and 15*).
3. Any other HPLC pumps/autosampler can be used for solvent delivery and automated sample introduction, if adapted to the given flow/sample introduction volume.
4. When necessary, stock solutions are acidified with 2 drops of 37% HCl per 10 mL, in order to enhance the solubility of the compound (*see Table 2.1*).
5. A qualitative standard solution containing Cys-Hcy can be obtained by heating a solution containing 0.5 M Cys and 0.5 M Hcy at 50°C for 2 h.
6. A 0.5 mM ASA anhydrides solution can be obtained by heating a 0.5 M argininosuccinic acid solution at 100°C for 60 min.
7. S2AE is used as an internal standard for the control of the correct dilution/precipitation of samples.

8. Methanol is an efficient reagent for protein precipitation. Its use limits ion suppression that can occur with other precipitating reagents.
This solution is used as a standard for deproteinization. Methanol is highly volatile. If evaporation occurs, the concentration may be altered. If the solution stays open longer than necessary, then it will be best to discard the flask and use a new one.
9. The use of stable isotope-labeled ISs of AAs to be quantified corrects the ion suppression phenomenon occurring when several molecules co-elute, assuming that the signal modification present in each sample is the same for the analyte and its corresponding IS. Due to their cost, the number of AAs* has been limited to 17, and they have been chosen according to the necessity of a precise quantification of the AAs for the diagnosis of metabolic disorders. For the remaining AAs, one of the 17 nonhomologous AAs* has been used as a surrogate IS after careful validation of their quantification in these conditions. In some cases, the quantification could not be validated, but the method can be used for qualitative analysis of the marker. If necessary, other AAs* can be easily added to the method (e.g., Cit*), in order to improve quantification results and/or to extend the linear dynamic range of the measurement.
10. Preparation and analysis of leukocyte samples for cystine measurements are slightly modified when compared to those for other samples, in order to enhance the sensitivity of the method for this purpose. The method is modified as follows:
 - a. A leukocyte mixture is prepared by mixing the leukocyte pellet with 250 μ L water and sonicating according to usual procedures currently in use in the laboratory for this purpose.
 - b. Protein concentration is determined.
 - c. The 10, 5, and 2.5 μ M AA standards for cystine measurement in leukocytes are prepared by diluting adequately the 100 μ M AA standard (*see* Step 4 of **Section 2.6**) in water.
 - d. Leukocytes extracts, standards, and controls (ERNDIM external QAP for cystine in white blood cells and SKML internal QAP) are immediately deproteinized in 1.5-mL microtubes, as described in Step 4 of **Section 3.1**, with the exception that 200 μ L of leukocyte extracts, standard (0, 2.5, 5, and 10 μ M), or controls are mixed with 600 μ L of deproteinization reagent.

- e. After deproteinization, the supernatant is kept frozen at -80°C .
 - f. Samples for injection are prepared by mixing 200 μL of each supernatant with 50 μL of AA* working solution in an injection vial.
 - g. 5 μL is injected.
 - h. The injection sequence is similar to that described in Step 1 of **Section 3.4**, with the exception that adequate standards and control samples are used. As leukocyte samples are usually very few, these samples can be included in another sample series, provided the leukocyte standards are also included in the same series.
 - i. Quantification (*see Section 3.5*) involves only the corresponding transitions for Cys and Cys* and can be done with a specific quantification method.
11. Transitions are pairs of $[\text{M}+\text{H}]^{+}$ precursor ion > fragment ion obtained before (precursor) and after (fragment) fragmentation of the AA and specific of it, e.g., $132 > 86$ corresponds to the precursor ion ($m/z = 132$) and fragment ion ($m/z = 86$) produced by leucine. For each AA, the most sensitive/specific transition has been chosen. In some cases, the monitoring of two transitions for one AA is necessary to ensure the confident identification/quantification of the AA to be measured. Every molecule that can give the same fragmentation as another is a potential interference when these two molecules are present in the same mixture. This interference phenomenon is mainly due either to isobaric molecules, arising from in-source, collision-induced fragmentation, or to natural isotopic contributions (i.e., mainly 1.1% ^{13}C and 4.5% ^{34}S). Interference occurring between molecules analyzed in this method is reported in (7), but most of them have been separated from the AA of interest in the described chromatographic system.
 12. Specific transitions have been determined with the API 2000 triple-quadrupole mass spectrometer. However, these transitions can be used in any triple-quadrupole mass spectrometer. DP and CE reported here are specific to the API 2000. The use of another triple-quadrupole mass spectrometer will necessitate the preliminary study of the specific parameters of the new apparatus for each AA. Various parameters (e.g., dwell time) for the data processing should be adapted to the software. Contact the manufacturer for any questions concerning this technology transfer.
 13. This method uses ion-pairing, reversed-phase liquid chromatography, a technique which must be run in strictly standardized conditions. Repeatability of the retention time is obtained only when the system is equilibrated with a few

gradients. Frequent rinsing is necessary in order to limit the fouling of the column (by samples and ion-pairing agent) and thus the peak drift. In a series, the shape of the gradient must not be modified nor the duration of the L re-equilibration time. The first sample injected after rinsing the column cannot be used for analysis. The second sample (25 μ M solution (26 AAs) is used to check the quality of the chromatography in the sequence. No more than three sequences must be analyzed in a series. When the column has to be maintained on-line for more than 24 h, it is possible to rinse the column on-line (10 h with 100 μ L/min ACN) and use it again for a 24-h period (three sequences, 36–42 samples).

14. Retention times can be slightly different if the conditions of the chromatography are slightly different than those described (HPLC pumps, column, etc.). Calculating the ratios of the retention time of the analyte to that of the IS is helpful. For example, in instances of slight shifts of the retention times after several runs or on some samples, this ratio has been found to be very stable and allows correct identification of the peaks (*see Note 15*).
15. Gln/Glu (*see Fig. 2.2c*) and Asn/Asp (*see Fig. 2.2d*) present interference due to the ^{13}C isotope contribution and must present as separated peaks. Asp and Glu may be overestimated if they are not baseline separated from Asn and Gln, respectively (mainly Glu, due to the important concentration of Gln in biological fluids). *n*Ile, Ile and Leu share a common $132 > 86$ transition and must give three clear peaks with baseline separation (*see Figs. 2.1 and 2.2g*). dALA coelutes with Ile. Thus, the common $132 > 86$ transition cannot be used for the measurement of Ile when dALA is detectable. GABA and β -AIB cannot be separated by this method. β -AIB is measurable only if GABA is undetectable in the sample. Hse and Thr cannot be separated by this method. Thr is measurable only if Hse is undetectable in the sample. However, in our experience, no Hse has been detected among more than 10,000 analyzed biological samples.
16. When the homologous labeled AA is not available, another AA*, as close as possible to the target AA in terms of retention time and structure, is used (*see Table 2.2*).
17. For every series, the following parameters must be systematically checked:
 - a. Each peak of each AA must be correctly integrated by the software. If not, manually correct the integration.
 - b. The AA retention time/AA* retention time ratio must be 100% for each AA for which the corresponding AA* is

present. For other molecules, the AA retention time/IS retention time ratio is the same in the standards and in the samples.

- c. The surface of the S2AE peak should be stable among all the standards and samples. If not, then repeat the preparation for injection and analysis.
- d. The identification of Leu, Ile, and *n*Ile peaks must be correct: Leu/Leu* retention time ratio is 100%; Ile/Leu* retention time ratio is about 95%, and *n*Ile/Leu* retention time ratio is about 90%. All of these can be quantified using the 132 > 86 common transition (135 > 89 for Leu*) except for Ile when dALA is present. The 132 > 43 specific transition can be used for Leu quantification (135 > 46 for Leu*). The 132 > 69 specific transition can be used for Ile and *n*Ile quantification (135 > 46 for Leu*).
- e. Hyp can be quantified either with the common 132 > 86 or with the 132 > 68 specific transition.
- f. The Asn/Asp* retention time ratio must be about 123%.
- g. The Gln/Gln* retention time ratio must be 100%.
- h. The Gln/Glu retention time ratio must be about 88%. Because of the prominent concentration of Gln in biological fluids (mainly plasma), Glu may be present as a double peak, with one peak at 100% of Glu* (corresponding to Glu) and one peak at 88% of Glu* (corresponding to Gln interference). Check that only the Glu part (as well as the Glu* part) is integrated for Glu quantification.
- i. The Sar/Ala* retention time ratio must be about 68%.
- j. The 76 > 30 transition used for Gly is the most sensitive of those characteristic for this AA, but it is not as sensitive relative to transitions for other AA. However, Gly concentrations are elevated in biological fluids, allowing for its accurate quantification. In CSF, the concentration of Gly is reduced, but elevated concentrations found in nonketotic hyperglycinemia can be detected.
- k. The His-3Me/Lys* retention time ratio is about 93%. An interfering peak is present at 88%, which may not be considered as His-3Me.
- l. The basic AA Arg, His, Lys, Orn elute late in the chromatographic system; a small interfering peak is present in the blank (0 μ M AA standard) which must be taken into account in blanks for their measurement.

18. Quantitative validation, including the determination of the limit of quantification (LOQ), linear dynamic range, intra-day and inter-day precision, and accuracy (correlation and/or recovery experiments) have been obtained for all the AAs for which the corresponding AAs* are available (8). It has also been obtained for many AAs for which the corresponding AAs* are not used (9) (*see Table 2.2*). LOQs given in (7) are those determined with the API 2000. LOQs are highly instrument dependent, and a linearity limit has been found up to 3 mM for AAs for which the corresponding AAs* are used in the method. For other AAs for which AAs* were not used, the linearity limit was found up to 1 mM (9). For some AAs, the quantitative validation could not be obtained (9) (*Table 2.2*).
19. Cys-Hcy can be qualitatively evaluated by the presence/absence of the peak. A semi-quantitative estimation is possible using (Cys)₂ as external standard and (Cys)₂* as IS for the management of patients affected with homocystinuria.
20. The dual form of ASA (acid and anhydrides) is the cause of poor quantitative results for ASA and ASA anhydride by this method. Complete transformation of ASA to ASA anhydride is obtained by heating standards and samples at 100°C for 60 min. Total ASA anhydride can thus be measured and allow for the management of patients affected with argininosuccinic aciduria.

Acknowledgments

The authors would like to thank Luc Anselmini for reading over this manuscript. The authors thank the journal Rapid Communication in Mass Spectrometry and Wiley Publishing for authorization to reproduce tables and figures from refs.(7–9).

References

1. Moore, S., Spackman, D. H., Stein, W. H. (1958) Automatic recording apparatus for use in the chromatography of amino acids. *Anal Chem* 30, 1190–1206.
2. Vogeser, M., Seger, C. (2008) A decade of HPLC-MS/MS in the routine clinical laboratory – goals for further developments. *Clin Biochem* 9, 649–662.
3. Dooley, K. C. (2003) Tandem mass spectrometry in the clinical chemistry laboratory. *Clin Biochem* 36, 471–481.
4. Rashed, M. S. (2001) Clinical applications of tandem mass spectrometry: ten years of diagnosis and screening for inherited metabolic diseases. *J Chromatogr B Biomed Sci Appl* 758, 27–48.

5. Gelb, M. H., Turecek, F., Scott, C. R., Chamoles, N. A. (2006) Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders. *J Inherit Metab Dis* 29, 397–404.
6. Dietzen, D. J., Rinaldo, P., Whitley, R. J., Rhead, W. J., Hannon, W. H., Garg, U. C., et al. (2009) National academy of clinical biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry executive summary. *Clin Chem* 55, 1615–1626.
7. Piraud, M., Vianey-Saban, C., Petritis, K., Elfakir, C., Steghens, J. P., Morla, A., Bouchu, D. (2003) ESI-MS/MS analysis of underivatized amino acids: a new tool for the diagnosis of inherited disorders of amino acid metabolism. Fragmentation study of 79 molecules of biological interest in positive and negative ionisation mode. *Rapid Commun Mass Spectrom* 17, 1297–1311.
8. Piraud, M., Vianey-Saban, C., Petritis, K., Elfakir, C., Steghens, J. P., Bouchu, D. (2005) Ion-pairing reversed-phase liquid chromatography/electrospray ionization mass spectrometric analysis of 76 underivatized amino acids of biological interest: a new tool for the diagnosis of inherited disorders of amino acid metabolism. *Rapid Commun Mass Spectrom* 19, 1587–1602.
9. Piraud, M., Vianey-Saban, C., Bourdin, C., Acquaviva-Bourdain, C., Boyer, S., Elfakir, C., Bouchu, D. (2005) A new reversed-phase liquid chromatographic/tandem mass spectrometric method for analysis of underivatized amino acids: evaluation for the diagnosis and the management of inherited disorders of amino acid metabolism. *Rapid Commun Mass Spectrom* 19, 3287–3297.



<http://www.springer.com/978-1-61737-984-0>

Metabolic Profiling

Methods and Protocols

Metz, Th.O. (Ed.)

2011, XI, 391 p., Hardcover

ISBN: 978-1-61737-984-0

A product of Humana Press