Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Identification and quantification of amino acids and related compounds based on Differential Mobility Spectrometry

Francis Berthias,[a] Yali Wang,[a] Eskander Alhajji,[a] Bernard Rieul,[a] Fathi Moussa,[a] Jean-François Benoist,[b,c] and Philippe Maître*[a]

Table S1. Composition of the standard mixture consisting of 33 compounds amino acids and related compounds. The list of isotopically labeled amino acids and related compounds used for quantification is also given, as well as their final concentration (µM) in the samples.

Standards		Isotopically stable internal standards (IS)		
Compound	$[M+H]^+$ *(m/z)*	Compound	$[M+H]^+$ *(m/z)*	Concentration (µM)
Glycine	76	Glycine-$^{13}C_2$	78	27
α-Alanine	90	α-Alanine-$^{13}C_2$	92	20
β-Alanine	90			
α-Aminobutyric Acid	104			
β -Aminoisobutyric Acid	104			
γ-Aminobutyric acid	104			
Serine	106	Serine-d_3	109	13
Proline	116	Proline-d_7	123	20
Valine	118	Valine-d_8	126	20
Threonine	120	Threonine-$^{13}C_1$, d_2	123	7
Taurine	126	Taurine-d_4	130	7
Pipecolic acid	130	Pipecolic acid-d_9	139	1
Hydroxyproline	132			

Table S1. Continued.

Isoleucine	132	Isoleucine-$^{13}C_6$	138	2
Leucine	132	Leucine-d_3	135	13
Alloisoleucine	132	Alloisoleucine-d_{10}	142	13
Ornithine	133	Ornithine-d_6	139	7
Asparagine	133	Asparagine-$^{15}N_2$	135	7
Aspartic Acid	134			
Lysine	147	Lysine-d_4	151	13
Glutamine	147	Glutamine-d_5	152	67
Glutamic Acid	148			
Methionine	150	Methionine-d_3	153	8
Histidine	156	Histidine-$^{13}C_1$	157	13
2-Aminoadipic Acid	162			
Phenylalanine	166	Phenylaline-d_5	171	7
Arginine	175	Arginine-$^{15}N_2$	177	7
Citrulline	176	Citrulline-$^{13}C_1$	177	1
Tyrosine	182	Tyrosine-$^{13}C_9$, $^{15}N_1$	192	7
Sulfocysteine	202			
Tryptophan	205	Tryptophan-d_5	210	27
Cystathionine	223			
Cystine	241			

Table S2: Concentration (μM) of amino acids and related compounds of interest in plasma determined using the LC-MRM protocol (see text).

M	$[M+H]^+$ *m/z*	P1	P2	P3	P4
glycine	76	235 ± 11	165 ± 7	259 ± 12	534 ± 24
α-alanine	90	323 ± 19	247 ± 15	431 ± 26	1109 ± 66
serine	106	132 ± 9	130 ± 9	111 ± 8	209 ± 15
proline	116	212 ± 10	138 ± 7	147 ± 7	456 ± 22
valine	118	244 ± 12	216 ± 11	186 ± 9	207 ± 10
threonine	120	131 ± 8	159 ± 10	103 ± 6	412 ± 25
taurine	126	125 ± 11	62 ± 6	58 ± 5	395 ± 36
pipecolic acid	130	2 ± 0.2	1 ± 0.1	1 ± 0.1	8 ± 1
isoleucine	132	69 ± 6	68 ± 6	46 ± 4	41 ± 4
leucine	132	140 ± 7	120 ± 6	97 ± 5	113 ± 6
alloisoleucine	132	1 ± 0.1	0 ± 0.1	1 ± 0.1	1 ± 0
asparagine	133	47 ± 3	44 ± 3	47 ± 3	17 ± 1
ornithine	133	75 ± 5	50 ± 3	68 ± 4	115 ± 7
glutamine	147	556 ± 26	458 ± 22	652 ± 31	672 ± 32
lysine	147	158 ± 10	153 ± 9	164 ± 10	397 ± 24
methionine	150	28 ± 2	23 ± 1	23 ± 1	52 ± 3
histidine	156	76 ± 3	77 ± 4	78 ± 4	93 ± 4
phenylalanine	166	85 ± 5	423 ± 24	49 ± 3	95 ± 6
arginine	175	72 ± 3	44 ± 2	69 ± 3	59 ± 3
citrulline	176	23 ± 2	17 ± 1	28 ± 2	25 ± 2
tyrosine	182	67 ± 4	32 ± 2	436 ± 24	184 ± 11
tryptophan	205	61 ± 5	75 ± 6	69 ± 5	41 ± 3

Table S3. Composition and concentrations of the calibration solutions. Final concentrations (μM) of the targeted compounds in the six spiked P1 plasma solution.

Compound	$[M+H]^+$ *m/z*	C0	C1	C2	C3	C4	C5
Glycine	76	12	14	15	29	47	187
α-Alanine	90	16	18	20	34	51	191
Serine	106	7	8	10	24	42	182
Proline	116	11	12	14	28	46	186
Valine	118	12	14	16	30	47	187
Threonine	120	7	8	10	24	42	182
Taurine	126	6	7	8	15	24	94
Pipecolic acid	130	<1	2	4	18	35	175
Isoleucine	132	3	5	7	21	38	178
Leucine	132	7	9	11	25	42	182
Alloisoleucine	132	<1	2	4	18	35	175
Asparagine	133	2	4	6	20	37	177
Ornithine	133	4	6	7	21	39	179
Glutamine	147	28	31	35	63	98	378
Lysine	147	8	10	11	25	43	183
Methionine	150	1	3	5	19	36	176
Histidine	156	4	6	7	21	39	179
Phenylalanine	166	4	6	8	22	39	179
Arginine	175	4	5	7	21	39	179
Citrulline	176	1	3	5	19	36	176
Tyrosine	182	3	5	7	21	38	178
Tryptophan	205	3	5	7	21	38	178

Table S4. Compensation voltage values (in V), and collisional cross section (CCS) values (in Å^2) taken from from (a) ref. Zheng et al.[1], (b) ref. Barran[2], (c) ref. Paglia et al.[3], and (d) ref. Davidson et al.[4]

Name	$[M+H]^+$ m/z	Ion Type	CV (All)	CCS[(a)]	CCS[(b)]	CCS[(c)]	CCS [(d)]
Glycine	76	A	-10.7				117.4
Sar	90	A	-10.8				
β-Ala	90	A	-8.2				
α-Ala	90	A	-7.9				117.1
α-ABA	104		-6.2				
β-ABA	104		-5.7				
γ-ABA	104		-5.7				
Dimethylglycine	104		-1.2	124		116	
L-Serine	106	A	-6.6				121.1
L-Proline	116	A	-5.3	126	125	118	121.8
L-Valine	118	B	-3.7	134	121		124.8
L-Threonine	120	A	-4.8			122	123.5
Cysteine	122	A	-4.8	150		113	124.6
Trans-4-Hydroxy-proline	132		-4	130		122	
Cis-4-hydroxy-proline	132		-3.6				
L-Leucine	132	B	-3	135	124	128	130.9
L-Allo-Isoleucine	132		-2.9				
L-Isoleucine	132	B	-2.8	137		129	129.5
L-Asparagine	133	A	-4.1	132		118	124.7
L-Ornithine	133		-1.3	130		121	
L-Aspartic Acid	134	A	-4.4			122	128
unassigned	134		-2.6				
unassigned	135		-2.7				
Glutamine	147	B	-2.7	133		124	127
L-Lysine	147	B	-0.3	132		127	127.6
L-Glutamic Acid	148	B	-2.9			123	128.2
L-Methionine	150	B	-2.3	134		127	129.4
L-Histidine	156	B	-1.5	132		127	128.5
L-Phenylalanine	166	B	-1.4		140	135	137.2
1-Methylhistidine	170		-0.2			129	
3-Methyl-L-histidine	170		0.4		133		
L-Arginine	175	C	0.9		136	133	132.8
L-Citrulline	176		-0.7		137	132	
L-Tyrosine	182	B	-1.4		146	139	141.9
L-Tryptophan	205	C	0.2	154		145	146.5

Table S5. Limit of detection (LOD) values in units of μM derived using linear regression method for 13 metabolites of interest.

Compound	$[M+H]^+$ *m/z*	R^2 value[(a)]	Linear Range (μM)	LOD[(b)] (μM)
Serine	106	0.995-0.999	7-182	3.9
Threonine	120	0.998-1.000	7-182	2.0
Asparagine	133	0.926-0.998	2-177	8.1
Glutamine	147	0.938-0.994	28-378	8.9
Lysine	147	0.868-1.000	8-183	6.5
Methionine	150	0.999-1.000	1-176	1.0
Histidine	156	0.973-0.996	4-179	2.8
Phenylalanine	166	0.979-0.998	4-179	7.3
Arginine	175	0.935-1.000	4-179	1.8
Citrulline	176	0.863-0.950	1-36	5.6
Tyrosine	182	0.978-1.000	3-178	2.7
Tryptophan	205	0.998-1.000	3-178	2.1

(a) Range of R^2 values determined for each linear regression; (b) LOD values, defined as 3*SD(intercept)/slope using the linear regression method, are the mean values of each linear regression.

Table S6. Day-to-day reproducibility of the peak positions (CV in Volt) of a selected set of AAs and related compounds. Confidence intervals have been calculated using a significance level of 0.05 for a set of 51 measurements performed over 6 days on 3 different plasma samples, spiked or not with AAs and related compounds. Electric field (in $kV.m^{-1}$) and reduced electric field (in Td) are also given.

Name	<CV> (V)	Electric field ($kV.m^{-1}$)	Reduced electric field (Td)	Confidence interval (V)
Ala-AA	-7.5	10.7	0.50	0.3
Ser-AA	-6.6	9.5	0.44	0.2
Pro-AA	-5.3	7.6	0.35	0.1
Val-AA	-3.7	5.4	0.25	0.1
Thr-AA	-4.9	6.9	0.32	0.2
Asn-AA	-4.1	5.8	0.27	0.1
Gln-AA	-2.7	3.9	0.18	0.1
Lys-AA	-0.3	0.4	0.02	0.1
Met-AA	-2.3	3.3	0.15	0.1
His-AA	-1.5	2.2	0.10	0.1
Phe-AA	-1.4	1.9	0.09	0.1
Arg-AA	0.9	1.4	0.06	0.0
Cit-AA	-0.7	1.0	0.05	0.1
Tyr-AA	-1.4	2.0	0.09	0.1
Trp-AA	0.2	0.2	0.01	0.1
Orn-AA	-1.4	1.9	0.09	0.1

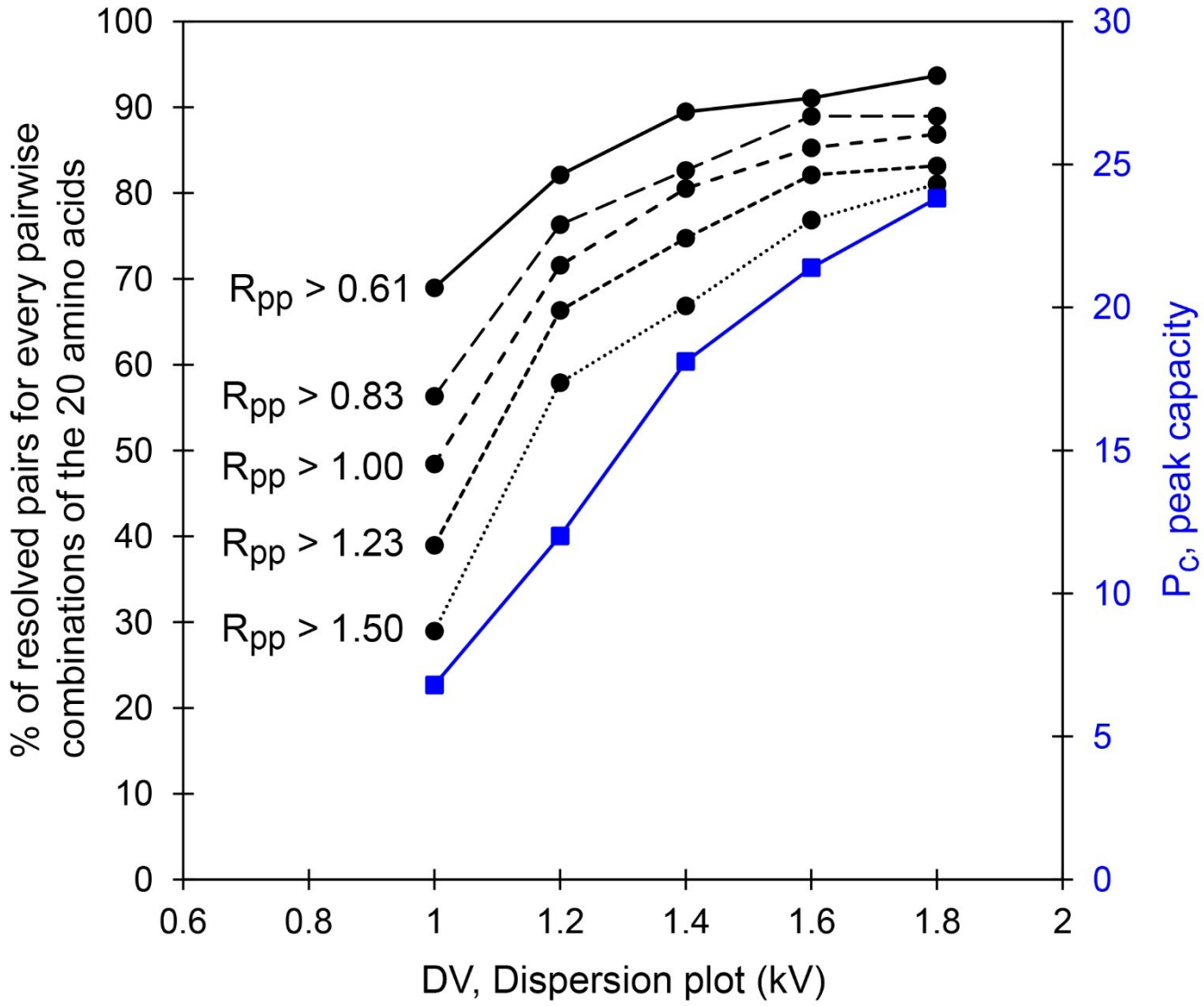

Fig. S1 Fraction of resolved pairs for every pairwise combination of the 20 protonated AAs (black lines, circle marks, left scale) and peak capacity (blue line, square marks, right scale) as a function of the dispersion voltage (DV). The different black lines correspond to five threshold values, Rpp greater than 0.61, 0.83, 1, 1.23 and 1.5 which represent 10 % of separation, a half-height separation, 75 %, 90 % of separation and baseline separation, respectively.

References

1. X. Y. Zheng, N. A. Aly, Y. X. Zhou, K. T. Dupuis, A. Bilbao, V. L. Paurus, D. J. Orton, R. Wilson, S. H. Payne, R. D. Smith and E. S. Baker, *Chem. Sci.*, 2017, **8**, 7724-7736.
2. P. Barran, *Analyst*, 2018, **143**.
3. G. Paglia and G. Astarita, *Nature Protocols*, 2017, **12**, 797-813.
4. K. L. Davidson and M. F. Bush, *Anal. Chem.*, 2017, **89**, 2017-2023.