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Article

Profiling of Amino Acids in Urine Samples of Patients Suffering from Inflammatory Bowel Disease by Capillary Electrophoresis-Mass Spectrometry

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(Supplementary Material)

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Table 1. Normalized concentrations of amino acids in urine samples from healthy volunteers measured by the CE-MS/MS method.

	Vol.1	Vol.2	Vol.3	Vol.4	Vol.5	Vol.6	Vol.7	Vol.8	Vol.9	Vol.10
Glycine	162.73	178.04	82.32	45.83	54.29	231.49	153.38	279.00	123.21	162.30
Alanine	42.75	48.51	16.63	9.79	15.56	34.37	35.88	32.62	26.54	21.38
Serine	64.55	78.23	26.44	17.13	26.50	50.56	38.79	48.83	30.89	29.13
Proline	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Valine	4.42	4.91	2.98	2.08	3.35	3.45	2.63	6.07	3.01	3.78
Threonine	28.68	31.54	12.61	6.46	11.52	16.55	17.70	26.24	18.35	8.42
Cysteine	7.85	8.14	9.54	7.62	6.07	7.36	10.88	18.08	8.78	9.24
Isoleucine	1.07	1.34	0.93	0.71	1.17	1.20	0.82	2.49	1.08	1.38
Leucine	3.24	3.81	2.28	1.65	2.34	2.64	1.67	6.09	2.13	2.55
Asparagine	30.81	31.75	10.54	7.88	17.57	26.59	14.71	29.01	21.97	10.00
Aspartic acid	0.46	0.39	0.39	0.11	0.79	1.69	0.68	1.60	0.53	4.39
Glutamine	247.54	302.56	53.70	56.19	164.98	274.05	145.86	207.35	107.15	101.84
Lysine	14.33	13.83	5.37	3.56	7.31	15.11	8.84	11.73	5.64	4.24
Glutamic acid	1.01	0.95	0.82	0.67	0.99	1.48	0.83	2.52	0.61	3.98
Methionine	0.85	1.28	0.56	0.36	0.69	0.88	0.44	1.90	0.60	<LLOQ
Histidine	110.00	139.75	56.77	38.63	69.51	79.19	81.49	158.28	97.80	37.96
Phenylalanine	5.57	6.94	4.65	3.10	3.55	3.87	5.41	12.52	3.85	3.42
Arginine	9.65	13.21	20.15	11.00	7.41	8.38	10.40	33.02	31.39	8.57
Tyrosine	10.80	13.85	6.75	3.80	5.10	4.68	6.45	14.42	5.45	4.44
Tryptophan	5.13	7.14	4.74	2.85	3.25	2.54	4.69	9.33	4.03	3.32

The amino acid concentrations are expressed as μM/mM of the creatinine.

Table 2. Normalized concentrations of amino acids in urine samples from IBD patients undergoing thiopurine treatment measured by the CE-MS/MS method.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Glycine	50.87	1231.43	N.D.	102.65	49.46	110.19	65.02	179.44	74.49	34.08	54.55	239.36	368.62
Alanine	23.94	208.33	N.D.	29.62	6.44	26.35	12.00	26.29	30.18	11.97	26.27	58.94	44.88
Serine	18.75	235.26	N.D.	24.23	26.08	39.39	16.33	41.43	24.85	11.51	17.86	62.04	57.26
Proline	<LLOQ	<LLOQ	N.D.	<LLOQ	<LLOQ	<LLOQ	1.39	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Valine	2.61	32.04	N.D.	3.21	1.58	2.57	1.54	3.50	2.95	1.79	3.51	3.52	3.01
Threonine	20.67	99.95	N.D.	15.26	14.27	12.84	5.17	17.67	20.05	3.15	9.15	28.21	26.18
Cysteine	5.58	42.74	N.D.	4.95	4.41	10.51	3.96	10.54	6.26	5.20	9.91	12.44	7.66
Isoleucine	0.79	6.28	N.D.	0.85	0.55	1.38	0.70	1.99	1.67	0.79	1.62	1.43	1.05
Leucine	1.53	27.27	N.D.	1.62	2.05	2.25	1.33	2.73	2.28	1.25	2.83	3.36	2.06
Asparagine	9.69	177.85	N.D.	8.87	11.27	16.74	5.38	16.61	18.82	8.72	7.24	22.15	21.37
Aspartic acid	<LLOQ	<LLOQ	N.D.	<LLOQ	<LLOQ	<LLOQ	0.60	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Glutamine	99.43	1061.55	N.D.	105.90	108.22	135.08	47.58	175.43	103.69	49.35	110.65	250.09	312.21
Lysine	8.66	78.19	N.D.	3.57	6.67	6.70	2.70	12.41	3.92	3.07	4.92	26.85	6.49
Glutamic acid	0.96	<LLOQ	N.D.	0.71	0.76	2.65	0.28	2.18	1.01	1.00	4.02	1.91	1.89
Methionine	<LLOQ	<LLOQ	N.D.	0.88	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0.47	0.42	<LLOQ	<LLOQ	1.05
Histidine	67.41	547.89	N.D.	54.95	55.43	65.44	32.83	82.09	49.95	19.68	33.24	127.50	78.73
Phenylalanine	3.17	36.18	N.D.	5.11	2.47	5.23	1.63	4.21	3.94	1.90	4.58	5.03	4.83
Arginine	6.34	59.70	N.D.	4.81	3.86	3.82	6.79	11.65	6.59	10.24	10.21	7.45	14.27
Tyrosine	1.78	49.85	N.D.	8.32	3.52	7.56	2.62	6.75	5.03	2.69	4.62	4.12	10.18
Tryptophan	5.52	34.10	N.D.	7.16	3.17	5.05	2.41	4.16	4.29	2.04	2.81	8.52	4.62

N.D. – not determined (the concentration of creatinine was under the lowest level of the analytical measurement range, i.e. < 250 μM)

The amino acid concentrations are expressed as μM/mM of the creatinine

Table 3. Normalized concentrations of amino acids in urine samples from IBD patients undergoing thiopurine treatment measured by the UHPLC-MS method.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Glycine	50.69	1199.24	N.D.	99.00	47.03	98.71	67.30	154.97	85.83	38.26	53.72	261.18	350.27
Alanine	22.07	175.95	N.D.	26.64	7.17	28.92	12.35	29.26	27.96	8.69	16.64	58.03	50.43
Serine	23.36	224.81	N.D.	25.86	19.10	33.33	13.98	40.34	22.12	13.18	14.25	69.44	60.27
Proline	<LLOQ	<LLOQ	N.D.	<LLOQ	<LLOQ	<LLOQ	1.03	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Valine	2.08	27.48	N.D.	3.23	1.74	2.71	1.88	4.50	2.87	1.96	3.63	4.07	3.68
Threonine	22.64	103.44	N.D.	13.47	14.13	14.46	6.17	15.58	19.37	3.48	7.88	24.28	25.61
Cysteine	6.86	62.98	N.D.	4.99	5.49	10.83	3.59	11.68	7.90	5.77	12.12	11.32	8.97
Isoleucine	0.79	7.63	N.D.	0.81	0.59	0.83	0.54	1.42	1.35	0.73	1.42	1.51	1.38
Leucine	1.88	27.10	N.D.	1.95	1.36	1.71	1.52	3.00	2.25	1.47	2.92	3.43	2.40
Asparagine	12.00	122.52	N.D.	10.02	10.35	13.79	5.74	17.53	18.31	8.69	7.43	22.43	22.16
Aspartic acid	0.28	14.50	N.D.	0.51	0.59	1.33	0.52	1.24	0.34	0.33	3.10	0.97	1.46
Glutamine	103.42	1076.34	N.D.	125.24	108.99	146.08	47.41	169.92	116.31	51.82	114.25	209.47	294.76
Lysine	8.60	78.24	N.D.	4.31	5.56	5.83	2.82	14.26	5.81	2.60	4.78	28.98	6.69
Glutamic acid	0.92	21.37	N.D.	0.50	0.64	1.63	0.30	2.79	0.99	0.82	3.45	1.48	1.62
Methionine	0.73	<LLOQ	N.D.	0.82	0.37	<LLOQ	0.28	<LLOQ	0.71	0.44	<LLOQ	0.93	0.88
Histidine	83.98	481.30	N.D.	53.78	39.36	67.46	28.83	84.61	65.25	21.99	27.79	114.42	68.83
Phenylalanine	3.61	33.97	N.D.	5.30	2.22	4.38	2.10	5.21	3.85	2.02	3.27	5.63	4.27
Arginine	9.49	70.99	N.D.	4.88	3.73	6.00	7.65	11.90	8.21	8.99	12.92	8.01	14.58
Tyrosine	1.98	48.47	N.D.	7.29	3.51	7.92	3.03	6.05	4.67	3.12	4.96	4.65	9.43
Tryptophan	5.75	23.28	N.D.	6.79	2.94	5.21	2.41	4.68	4.62	2.22	2.39	7.50	4.93

N.D. – not determined (the concentration of creatinine was under the lowest level of the analytical measurement range, i.e. < 250 μM). The amino acid concentrations are expressed as μM/mM of the creatinine.

Table 4. Mass spectrometric analysis conditions for UHPLC-MS determination of derivatized amino acids.

Analyte	MW	*m/z* [M +H]$^+$	Cone Voltage (V)
Glycine*	75.07	246.1	30
Alanine*	89.09	260.1	30
Serine*	105.09	276.1	30
Proline*	115.13	286.1	30
Valine*	117.15	288.1	30
Threonine*	119.12	290.1	30
Cysteine*	121.16	581.0	34
Isoleucine*	131.18	302.1	30
Leucine*	131.18	302.1	30
Asparagine*	132.12	303.1	30
Aspartic acid*	133.10	304.1	30
Glutamine*	146.15	317.1	30
Glutamic acid*	147.13	318.1	30
Lysine*	146.19	487.1	18
Methionine*	149.21	320.1	30
Histidine*	155.16	326.1	20
Phenylalanine*	165.19	336.1	30
Arginine*	147.20	345.1	30
Tyrosine*	181.19	352.1	30
Tryptophan*	204.23	375.1	30

*Amino acids were analyzed as AccQTag (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) derivatives.

Table 5. Patients' characteristics.

Patient No.	Sex	Age	Azathioprine dose per day
1	Female	39	125 mg
2	Female	33	50 mg
3	Female	72	50 mg
4	Female	30	100 mg
5	Male	30	100 mg
6	Male	41	50 mg
7	Male	35	100 mg
8	Female	42	50 mg
9	Female	23	100 mg
10	Male	31	100 mg
11	Female	68	50 mg
12	Female	35	100 mg
13	Female	46	100 mg

Table 6. Determined creatinine concentrations in the human urine samples.

Patient No.	Creatinine concentration (mM)	Volunteer No.	Creatinine concentration (mM)
1	20.80	1	9.41
2	0.262	2	7.19
3	< 0.250	3	42.60
4	13.60	4	24.70
5	5.45	5	6.06
6	2.40	6	2.84
7	23.40	7	20.50
8	3.80	8	6.99
9	22.90	9	32.20
10	13.50	10	1.96
11	1.13		
12	4.32		
13	4.87		

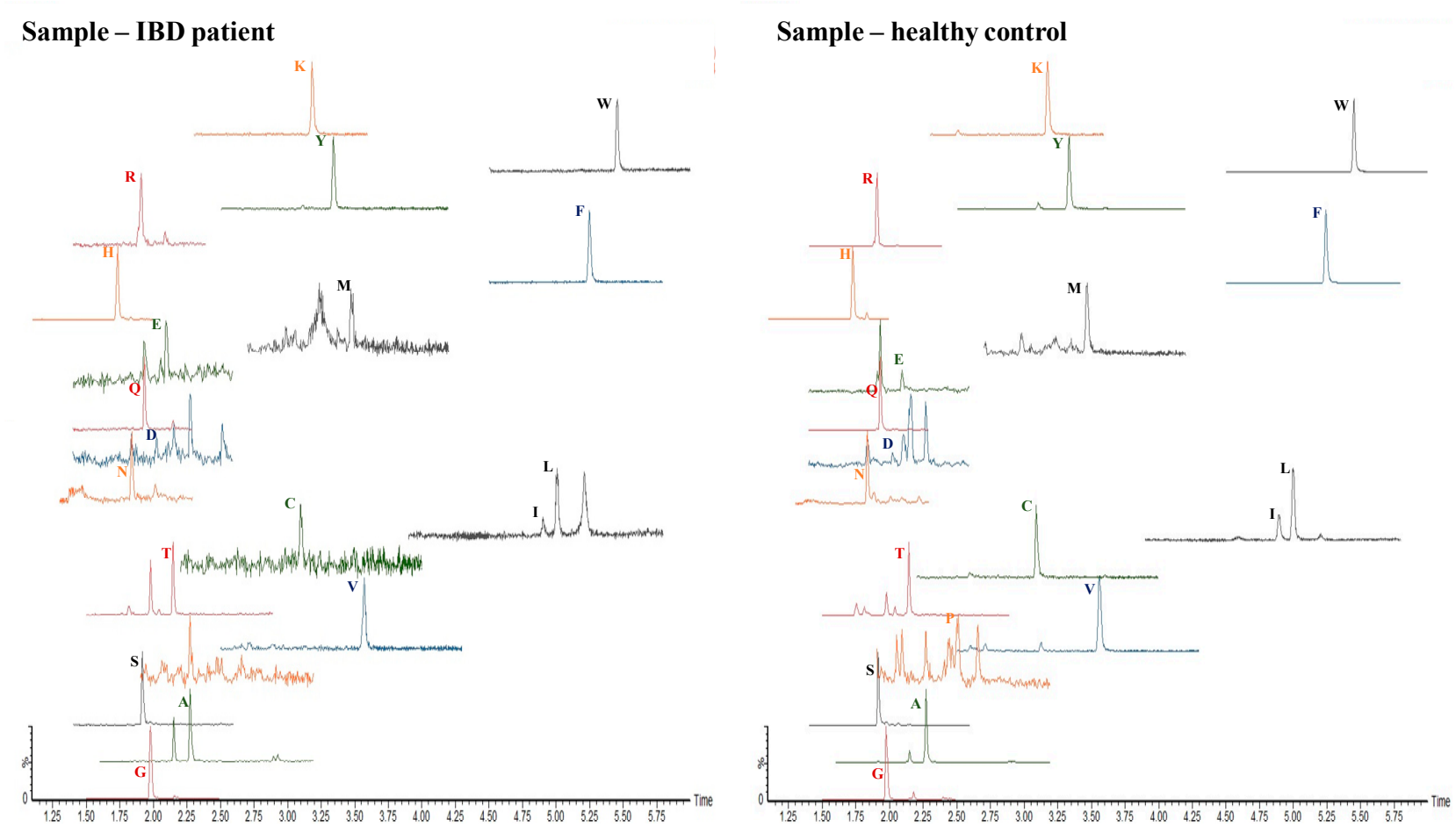

Figure 1. Selected ion chromatograms of derivatized amino acids provided by the UHPLC-MS analysis of clinical human urine samples obtained from a patient suffering from inflammatory bowel disease (left panel) and from a healthy control (right panel). For the sample preparation and other working conditions, see the section 3.