ANALYSIS OF URINARY ORGANIC ACIDS

Human urine is a complex biofluid of diverse small polar metabolites such as organic acids. Organic acidurias are inborn errors of metabolism given rise to by defections of inherited enzymes which lead to accumulation excess amounts of normal organic acids in human urine. For this reason qualitative and quantitative analyses of urinary organic acids are prominent diagnostic tools for the interpretation of metabolite profile that reflects inherited metabolic disorders. Conventionally gas chromatography coupled to mass sepectrometry (GC-MS) is used in spite of disadvantages as limititaton to volatile and thermally stable analytes thereby obligating of time consuming derivatization process.

At this point, “Jasem-Organic Acids” method overcomes abovementioned drawbacks by using liquid chromatography tandem mass spectrometry (LC-MS/MS). Jasem method ensures quantification of 54 organic acids by absence of derivatization simplified sample preperation to diluting. Jasem offers two panels achieving sufficient chromatographic separation for crucial isomers by LC-MS/MS.

ANALYSIS OF URINARY ORGANIC ACIDS BY LC-MS/MS

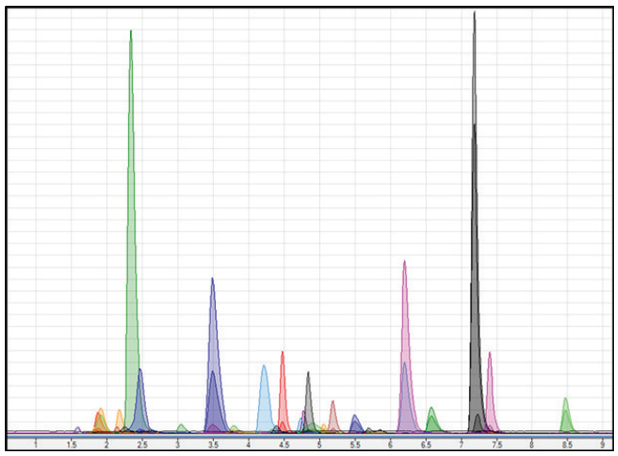
MATRIX: Urine

FEATURES

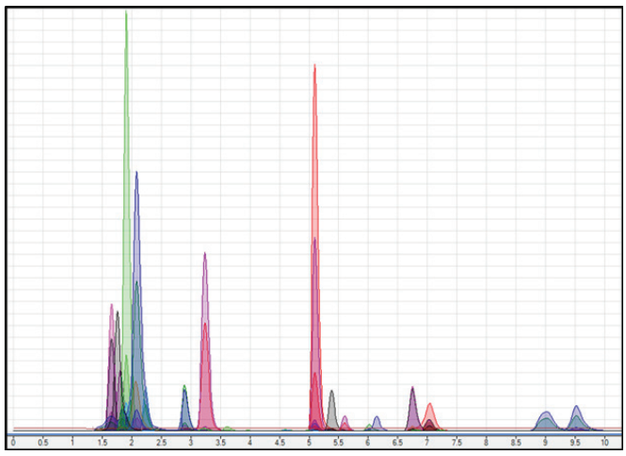
Analysis of 54 organic acids without derivatization.
Quick and easy sample preparation; just dilution!
12 minutes run for each panel (Total analysis time 24 minutes-Panel 1&2).
Successful chromatographic separation of isomers.

List of organic acids analysed in Panel-1 and Panel-2:

	PANEL-1		PANEL-2
1	2-Methylcitrate	29	2-OH-Glutaric acid
2	2-OH-Phenylacetic acid	30	2-OH-3-Methylpentanoic acid
3	2-OH-Butyric acid	31	2-OH-Isocaproic acid
4	2-Oxoadipic acid	32	2-OH-Isovaleric acid
5	3-Methylglutaconic acid	33	3-OH-Glutaric acid
6	3-OH-2-Methylbutanoic acid	34	3-OH-Propanoic acid
7	3-OH-3-Methylglutaric acid	35	3-Methylcrotonyl glycine
8	3-OH-Butyric acid	36	3-Methylglutaric acid
9	3-OH-Isobutyrate	37	3-Methyl-2-oxovaleric acid
10	3-Phenyllactic acid	38	3-OH-Isovaleric acid
11	4-OH-Phenylacetic acid	39	3-OH-Pentanoic acid
12	Fumaric acid	40	4-Methyl-2-oxovaleric acid
13	Glycolic acid	41	4-OH-Phenylpyruvic acid
14	Hexanoylglycine	42	Adipic acid
15	Homogentisic acid	43	2-ketoglutaric acid
16	Malic acid	44	Citric acid
17	Malonic acid	45	Ethylmalonic acid
18	N-(3-Phenylpropionyl) glycine	46	Glutaconic acid
19	N-Acetylaspartic acid	47	Glutaric acid
20	N-Acetyltyrosine	48	Lactic acid
21	N-Isovalerylglycine	49	Methylmalonic acid
22	Oxoproline	50	Orotic acid
23	4-OH-Phenyllactic acid	51	Phenylpyruvic acid
24	Propionylglycine	52	Pyruvic acid
25	Sebacic acid	53	Succinic acid
26	Suberic acid	54	Tiglylglycine
27	Suberylglycine		
28	Succinylacetone		



Extracted LC-MS/MS chromatogram of Panel-1



Extracted LC-MS/MS chromatogram of Panel-2

Number	Organic Acids	LOQ (ppm)	(%) RSD
1	Glycolic acid	0.72	6.64
2	Malic acid	0.06	4.54
3	Malonic acid	0.07	6.91
4	Fumaric acid	0.06	9.67
5	N-Acetylaspartic acid	0.10	4.67
6	Oxoproline	0.05	3.53
7	2-Oxoadipic acid	0.06	6.50
8	2-Methylcitrate	0.72	29.64
9	Propionylglycine	0.18	3.36
10	3-OH-3-Methylglutaric acid	0.18	1.74
11	2-OH Butyric acid	0.06	5.86
12	3-OH Butyric acid	0.26	9.17
13	3-OH Isobutyrate	0.55	0.71
14	Homogentisic acid	0.04	2.26
15	4-OH-Phenyllactic acid	0.08	10.32
16	N-Acetyltyrosine	0.13	4.53
17	3-Methylglutaconic acid	0.05	2.92
18	Succinylacetone	0.05	5.19
19	N-Isovalerylglycine	0.07	3.26
20	Suberylglycine	0.13	2.26
21	2-OH-Phenylacetic acid	0.07	1.80
22	4-OH Phenyl acetic acid	1.40	1.58
23	3-Phenyllactic acid	0.06	6.14
24	N-(3-Phenylpropionyl) glycine	0.05	1.97
25	Hexanoylglycine	0.09	3.07
26	Suberic acid	0.08	1.44
27	3-OH-2-Methylbutanoic acid	0.09	12.17
28	Sebacic acid	0.07	2.93
29	Pyruvic acid	0.22	12.45
30	2-Ketoglutaric acid	0.61	1.35
31	Citric acid	0.39	3.68
32	Orotic acid	0.04	2.98
33	Lactic acid	3.54	10.83
34	2-OH-Glutaric acid	0.37	0.73
35	3-OH-Propanoic acid	1.49	3.66
36	Methylmalonic acid	0.34	2.84
37	3-OH-Glutaric acid	0.05	1.17
38	Succinic acid	0.10	1.92
39	Ethylmalonic acid	0.05	2.07
40	Glutaconic acid	0.20	6.07
41	4-OH-Phenylpyruvic acid	0.39	4.88
42	3-OH-Isovaleric acid	0.11	2.64

43	Glutaric acid	0.23	1.73
44	3-Methyl-2-Oxovaleric acid	0.13	6.15
45	2-OH-Isovaleric acid	0.07	2.46
46	3-OH-Pentanoic acid	0.16	4.06
47	4-Methyl-2-oxovaleric acid	0.15	4.44
48	Tiglylglycine	0.05	3.17
49	3-Methylcrotonyl glycine	0.10	2.47
50	3-Methylglutaric acid	0.08	4.07
51	Phenylpyruvic acid	0.10	3.12
52	Adipic acid	0.08	4.00
53	2-OH-3-Methylpentanoic	0.08	1.46
54	2-OH-Isocaproic acid	0.07	4.92

Sample Preperation

STEP 1

- Dilute urine sample 10 times using Reagent-1

STEP 2

- Transfer 100 µl of diluted urine sample to a vial

STEP 3

- Add 50 µl of IS mix and 850 µl of Reagent-1 into the sample and inject to LC-MS/MS system

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