SHIMADZU

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Screening of organic acids for nutritional and metabolic profiling from dried urine spots using Gas Chromatograph Mass Spectrometer

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1. Introduction

Organic acids are products of bacterial metabolism on dietary polyphenols, unassimilated amino acids or carbohydrates and are required as intermediates in major metabolic pathways.
Urinary organic acids find clinical relevance as nutritional markers and are tested to gain insights into body functionality with regards to nutrient levels, hormone function and even the state of microbiome. Screening of abnormally elevated levels of organic acids is an important tool to assess specific and deeper medical issues which cause nutrient deficiencies and digestive system imbalances that conventional medicine often overlooks [1].
This study provides a proof of concept for comprehensive screening of organic acids from dried urine spots as potential markers for nutritional and metabolic profiling using Shimadzu Gas Chromatograph Mass Spectrometer. (Figure.1)

Figure.1 : Shimadzu GCMS-QP2020 NX

2. Methods

In this study, an easy and reliable sample preparation process coupled with a rapid analytical method was developed to screen organic acid metabolites in urine matrix using GC-MS system.
The urinary filter paper samples were prepared by first determining required sample volume for pre-treatment based on creatinine measurement. This was followed by adding internal standards, extraction and trimethylsilyl derivatization. Post derivatization, the metabolites were analysed in Scan mode. Shimadzu® capillary column coated with 5% phenyl and 95% dimethylpolysiloxane (SH-Rxi-5Sil MS; 30m, 0.25mm ID, 0.25µm df film thickness) was used for the analysis. The instrument method parameters and detailed sample preparation protocol have been provided in Table. 1 and Figure.2 respectively.

Table. 1 : Instrument method parameters

System configuration	
Instrument	Shimadzu GCMS-QP2020 NX
Auto injector	AOC-20i+s
Column	SH-Rxi-5 Sil MS (30 m,0.25 mm,0.25 um)
Liner	Restek Topaz™ Liner (With wool)
GC	
Injector Temp.	250 °C
Column Oven Temp	60 °C (2 min), 15 °C/min to 310 °C (5 min)
Run Time	23.67 min
Injection mode	Split
Injection Volume	1.0 µl
Linear velocity	38.9 cm/sec (Constant Linearity mode)
MS	
Interface Temp.	280 °C
Ion source Temp.	200 °C
Ionization mode	EI
Event Time	0.3 sec

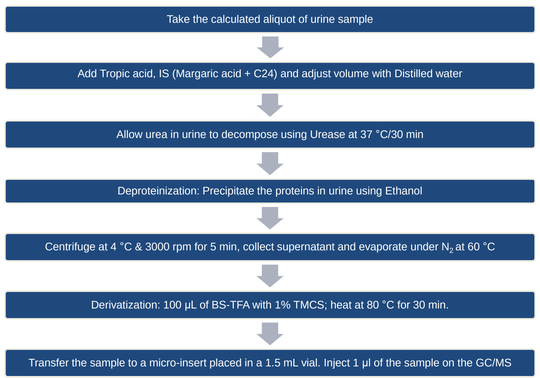

Figure. 2 : Flow chart of sample preparation

3. Results

Qualitative screening -
An abnormal level of organic acids in urine was determined using the Smart metabolite database Ver. 2 containing about 700 metabolites. The Automatic Adjustment of Retention Time (AART) function of GCMS Solution enabled to identify these organic acids independent of the analytical parameters. The AART function makes use of a custom retention index standard (n-alkane) to predict the retention times of the analytes of interest based on their retention indices. This facilitated in considerably reducing the time for qualitative screening of the organic acids. Thereafter, matching their mass spectra with library spectra increases the likelihood of precise identification.
A total of 65 organic acids (Table. 2) analysed by the method provide a metabolic snapshot while seeking additional information related to underlying gut derived chronic health problems.

Table. 2 : List of organic acids analysed by the method

ID #	Compound Name	ID #	Compound Name	ID #	Compound Name	ID #	Compound Name
1	Lactic acid	17	Phosphoric acid	33	Creatinine	49	Homovanillic acid
2	Glycolic acid	18	Ethylmalonic acid	34	2-Hydroxyphenylacetic acid	50	Citric acid
3	Pyruvic acid	19	Succinic acid	35	3-Hydroxyglutaric acid	51	Hippuric acid
4	2-Keto-isovaleric acid	20	Methylsuccinic acid	36	3-Phenyllactic acid	52	Dopamine
5	2-Hydroxybutyric acid	21	Uracil	37	3-Hydroxy-3-methylglutaric acid	53	Homogentisic acid
6	Oxalic acid	22	Fumaric acid	38	Phenylpyruvic acid	54	Methylcitric acid
7	4-Cresol	23	Thymine	39	2-Ketoglutaric acid	55	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid
8	2-Hydroxyisovaleric acid	24	Glutaric acid	40	Tartaric acid	56	Vanilmandelic acid
9	3-Hydroxybutyric acid	25	3-Methylglutaconic acid	41	4-Hydroxybenzoic acid	57	4-Hydroxyphenyllactic acid
10	3-Methyl-2-oxovaleric acid	26	3-Methylglutaric acid	42	N-Acetylaspartic acid	58	Pyridoxine
11	Malonic acid	27	Mandelic acid	43	Arabinose	59	Sebacic acid
12	Acetoacetic acid	28	Citramalic acid	44	Suberic acid	60	Ascorbic acid
13	Methylmalonic acid	29	Malic acid	45	Quinolinic acid	61	2-Hydroxyhippuric acid
14	2-Ketoisocaproic acid	30	Adipic acid	46	Aconitic acid	62	Pantothenic acid
15	2-Hydroxyisocaproic acid	31	2-Phenyllactic acid	47	Orotic acid	63	Kynurenic acid
16	4-Hydroxybutyric acid	32	5-Hydroxymethyl-2-furoic acid	48	Tricarballylic acid	64	Margaric acid
						65	3-Indoleacetic acid

Along with library search results and spectral match, the reference ions intensity ratio of analytes was also evaluated as per defined criteria. This further aided in the accurate identification of analytes obtained after using Linear Retention Index (LRI) and AART function. Figure.3(a), 3(b), 3(c), 3(d) are representative target and reference ion chromatograms of some typical organic acids analysed in one of the urine sample matrices.

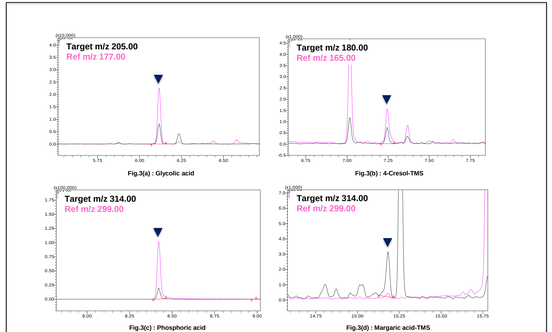

Figure. 3 : Chromatograms of organic acids in urine

Elevated levels of certain organic acids have found strong correlation in autoimmune disorders like Systemic Lupus Erythematosus (SLE) [2]. Such studies furthermore validate the significance of elevated organic acid levels in urine as crucial biomarkers which may lead to various chronic disorders. Rapid and accurate screening of such specific markers of interest can help in early detection of several disorders associated with organic acid levels such as depression, obesity, cardiovascular disease, diabetes, cancer and many more [3].

4. Conclusion

Smart metabolite database with AART and LRI functions coupled with a high-speed scan rate of 20000 u/sec of the GCMS QP2020 NX system, enabled precise and efficient screening of organic acids from biological matrix like dried urine spot. The study also emphasized the role of organic acids from urine as potential markers for nutritional and metabolic profiling.

5. References

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