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Application News

Gas Chromatograph Mass Spectrometer GCMS-QP 2020 NX

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

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## User Benefits

- Rapid screening of organic acids for nutritional and metabolic profiling
- Easy, quick and reliable sample preparation from urine matrix
- Use of Smart Metabolites Database™ Ver.2 and Automatic Adjustment of Retention Time (AART) function for quick identification

## ■ Introduction

Humans live in symbiosis with different bacteria. While some bacteria can contribute to a plethora of health benefits like protecting the host from the colonization of pathogenic bacteria, regulating the development of the gut or producing vitamins and hormones for the host, while some bacteria are also known to cause diseases. One of the important metabolites that can be produced by microbial processes are organic acids. Organic acids are compounds that are characterized by weak acidic properties. It is well established that almost all organisms produce organic acids of low molecular mass. Several organic acids are known to be specific products of bacterial metabolic action on dietary polyphenols or unassimilated amino acids or carbohydrates and are required as intermediates in major microbial metabolite pathways [1].

### Role of organic acids in human health

Organic acids are the intermediary products in Kreb's cycle and act as an energy source preventing the tissue breakdown. The main activity of organic acids is associated with a lowering in gastric pH converting the inactive pepsinogen to active pepsin. Excretion of supplemental minerals and nitrogen is minimized with organic acids as these form complexes with minerals and aid in their bioavailability. Also, organic acids have antimicrobial activity and can be both bacteriostatic and bactericidal [2]. Due to this, they can inhibit pathogenic bacterial proliferation, improve the disturbed intestinal microflora balance and thus enhance total gastrointestinal tract digestibility.

### Clinical relevance of urinary organic acids as nutritional markers

Organic acids may be tested to gain insights into how the body is functioning – nutrient levels, hormone function, and even the state of the microbiome which are immensely important for understanding our overall health. Screening of abnormally elevated levels of organic acids is a useful tool for individuals having specific medical issues known to cause nutrient deficiencies, such as gut disorders that impair nutrient absorption. Analysis of organic acids is an important tool to assess deeper layers of human biochemistry and digestive system imbalances from opportunistic organisms that conventional medicine often overlooks.

In this application note, we have provided a proof of concept for rapid 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

## ■ Experimental

### Sample preparation

The urine sample or urine filter paper sample were prepared by first determining required sample volume for pre-treatment based on creatinine measurement, followed by adding internal standards, extraction and trimethylsilyl derivatisation. 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<br>310 °C (5 min) |
| Run Time | 23.67 min |
| Injection mode | Split |
| Injection Volume | 1.0 µl |
| Linear velocity | 38.9 cm/sec (Constant mode) |
| MS | |
| Interface Temp. | 280 °C |
| Ion source Temp. | 200 °C |
| Ionization mode | EI |
| Loop Time | 0.3 sec |

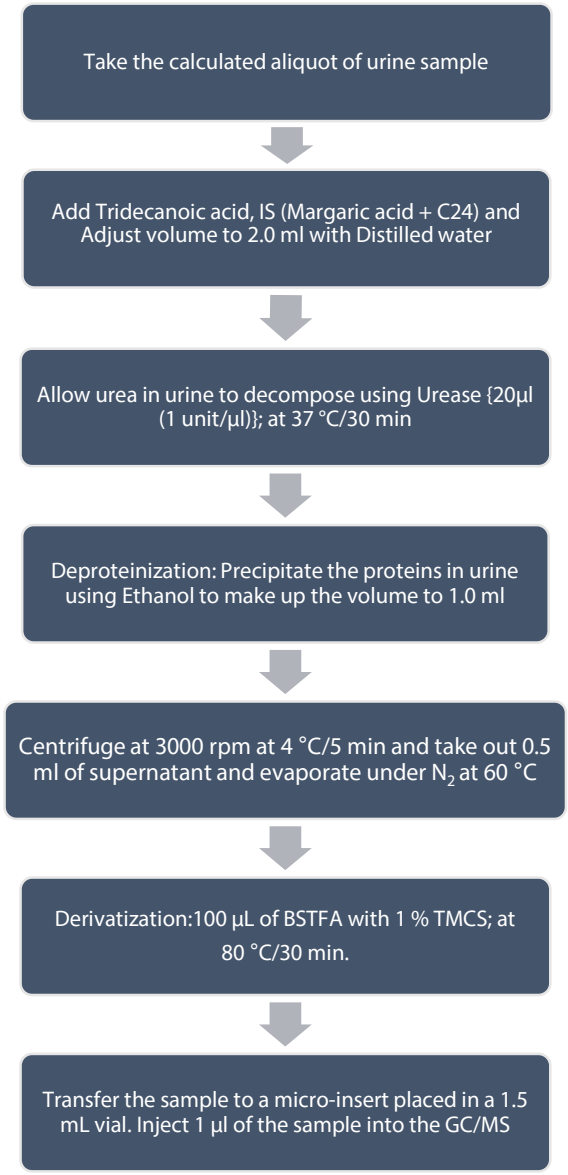



Figure 2. Flow chart of sample preparation

## ■ Results

Qualitative analysis:

To determine an abnormally high or low level of organic acids in urine or any biological matrix, the primary step required was to identify them first and to compare their peak intensities to the internal standard peak intensity. For this purpose, two approaches can be used.

1. The first solution is to screen the organic acids in up to 10 available public or private mass spectral libraries. Although it is the simplest approach, its not the most reliable way to accurately identify organic acids during scan analysis, owing to their lower response in sample matrix.
2. The second one is to use the Smart metabolite database containing about 700 metabolites. The AART function of GCMS Solution enables to identify these organic acids independent of the analytical parameters. The AART function makes use of a custom retention index standard (n-alkane) in order to predict the retention times of the analytes of interest based on their retention indices.

This helped to considerably reduce the time for qualitative screening of the organic acids. Thereafter, matching their mass spectra with those present in the library increases the likelihood of precise identification.

The total ion chromatogram of 65 organic acids in a urine sample and representative library search results of one such organic acid have been displayed in Figure 3 and Figure 4, respectively.

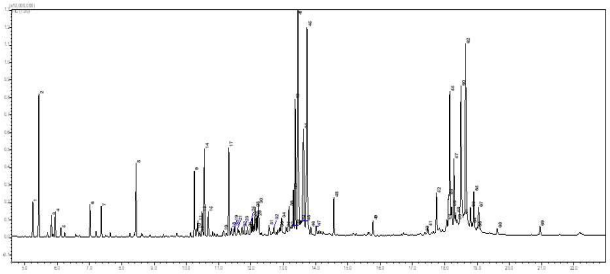
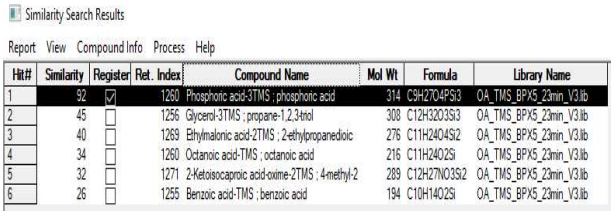


Figure 3. Total ion chromatogram of organic acids in a representative urine sample



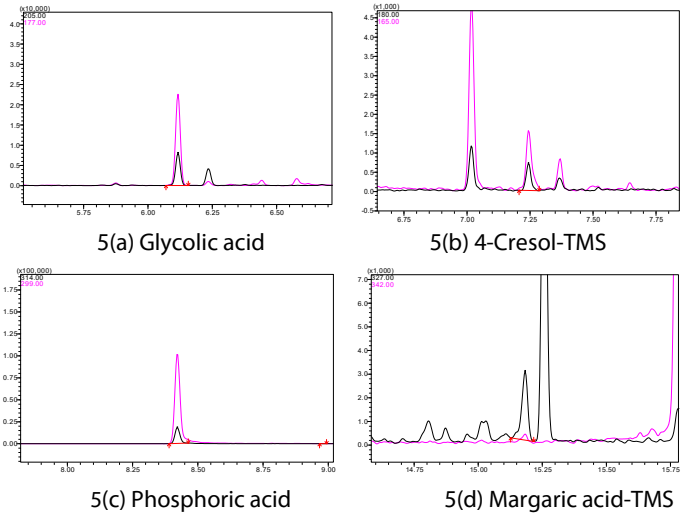
Similarity Search Results

Report View Compound Info Process Help

| Hit# | Similarity | Register | Ret. Index | Compound Name | Mol Wt | Formula | Library Name |
|---|---|---|---|---|---|---|---|
| 1 | 92 | ☑ | 1260 | Phosphoric acid-3TMS ; phosphoric acid | 314 | C9H27O4PSi3 | OA_TMS_BPX5_23min_V3.lib |
| 2 | 45 | ☐ | 1256 | Glycerol-3TMS ; propane-1,2,3-triol | 308 | C12H32O3Si3 | OA_TMS_BPX5_23min_V3.lib |
| 3 | 40 | ☐ | 1269 | Ethylmalonic acid-2TMS ; 2-ethylpropanedioic | 276 | C11H24O4Si2 | OA_TMS_BPX5_23min_V3.lib |
| 4 | 34 | ☐ | 1260 | Octanoic acid-TMS ; octanoic acid | 216 | C11H24O2Si | OA_TMS_BPX5_23min_V3.lib |
| 5 | 32 | ☐ | 1271 | 2-Ketoisocaproic acid-oxime-2TMS ; 4-methyl-2 | 289 | C12H27NO3Si2 | OA_TMS_BPX5_23min_V3.lib |
| 6 | 26 | ☐ | 1255 | Benzoic acid-TMS ; benzoic acid | 194 | C10H14O2Si | OA_TMS_BPX5_23min_V3.lib |

Figure 4. Representative Library search of Peak No.8 from Figure 3 denoting Phosphoric acid TMS derivative

Along with library search results and spectral match, the reference ions intensity ratio of analytes was also evaluated as per defined criteria. This further helped in the accurate identification of analytes obtained after using Linear Retention Index (LRI) and AART function. Figure 5(a), 5(b), 5(c), 5(d) are representative target and reference ion chromatograms of some typical organic acids analysed in one of the urine sample matrices. A total of 65 organic acids analysed by the method (Table 2), provide a metabolic snapshot while seeking additional information related to underlying gut derived complicated and chronic health problems.



5(a) Glycolic acid

5(b) 4-Cresol-TMS

5(c) Phosphoric acid

5(d) Margaric acid-TMS

Figure 5. Chromatograms of organic acids in urine

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 |

Elevated levels of certain organic acids have found strong correlation in autoimmune disorders like Systemic Lupus Erythematosus (SLE). Also, the data reveals phosphoric acid levels play an important role in distinguishing between SLE patients and control samples [3]. Such studies furthermore validate the significance of elevated organic acid levels in urine or fecal matter 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, anxiety, cardiovascular disease, diabetes, cancer, anorexia and many more.

Quantitative analysis:

Among the hundreds of organic acids screened, respective organic acid standards which may be present in the sample or those which are commercially available in the market can be procured. Those can be used for calibration together with tridecanoic acid trimethylester and margaric acid as internal standards, to quantify them precisely and consequently get a more accurate diagnosis for the concerned disorders. The calibration ranges can then be decided depending on the targeted analytes of interest. Below is an example of calibration plot for glutaric acid with clinical relevance as a dysbiosis marker (Figure 6)

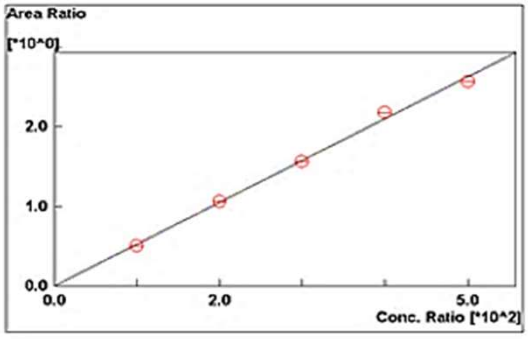



Figure 6. Calibration curve of glutaric acid

## ■ Conclusion

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

## ■ References

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3. Yan, R., Jiang, H., Gu, S., Feng, N., Zhang, N., Lv, L., & Liu, F. (2020). Fecal metabolites were altered, identified as biomarkers and correlated with disease activity in patients with systemic lupus erythematosus in a GC-MS-based metabolomics study. Frontiers in immunology, 2138.

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