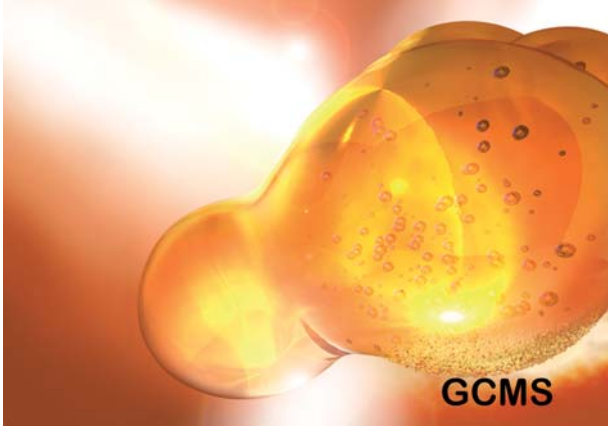
Application Note



FAST GCMS DETERMINATION OF URINARY ORGANIC ACIDS FOR DIAGNOSIS OF METABOLISM INBORN ERRORS

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Introduction

During normal metabolism activities, the nutrients obtained from food are metabolized in the body, which means that they are either stored or used as energy. In case of inherited metabolic disease, the patient lacks certain enzymes, which lead to an accumulation of unmetabolized compounds. These accumulated compounds cause a severe toxicity acting on the nervous system, then they restrict and delay development and growth resulting in premature death. For a quick screening Liquid Chromatography with Tandem Mass Spectrometry is sometimes employed, however, for a close inspection and a high risk screening of the organic acids, the best analytical technique is Gas Chromatography coupled to Mass Spectrometry (GCMS). Here, we present a Fast GCMS method that increase the daily throughput of analysis.

Sample preparation

The urine or urine filter paper are prepared for analysis by adding internal standards, extraction and TMS derivatisation (Please refer to Shimadzu Method Package). Then, this solution is directly injected in the GCMS.

Analytical method

Instrument:
GCMS-QP2010
AOC-20i+s

Injection:
Split, ratio 1/100
Temperature 280 °C

Column:
Supelco SLB 5 ms
(J&W DB-5 recommended)
15m x 0.1mm x 0.1 µm
Linear velocity 35 cm/s
T°C Prog. 100 °C (1.6 min)-
14 °C/min-280 °C (3 min)

MS:
I/F 280 °C
Ion source 200 °C
EI mode
Scan m/z 50 to 500
Event time 0.2 s
(scan speed 2500)

The Figure 1 compares the conventional GCMS and Fast GCMS analysis of the same standard solution.

The analysis time of Fast GCMS is 3 to 4 times shorter. The zoom on the 3-hydroxy-3-methylglutaric acid and pimelic acid peaks shows that thanks to the small diameter column there is no loss of resolution.

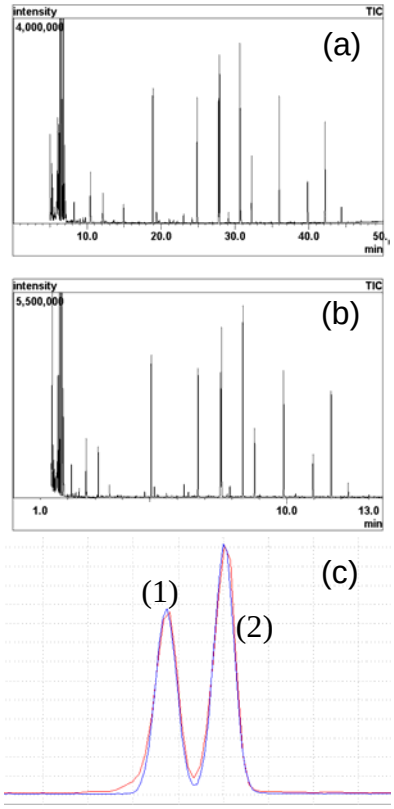

Fig. 1: Chromatograms of organic acids standard (500 µmol.L^{-1}) in conventional (a) and Fast GCMS (b). Comparison of the 3-hydroxy-3-methylglutaric acid (1) and pimelic acid (2) separation in conventional (blue line) and Fast GCMS (pink line).

Quantitative analysis

Among the hundreds of organic acids screened, around 20 of them are available on the market. Those can be used for internal calibration together with, for example, tridecanoic acid trimethylester and margaric acid as internal standards, to quantify them precisely and consequently get a more precise diagnosis for the concerned disorders. The calibration ranges from 50 to 1000 µmol.L^{-1}, depending on the compound. The chromatogram of the 500 µmol.L^{-1} standard, for nine organic acids and two internal standards is presented in Figure 1. The Figure 2 shows the calibration curve for the glutaric acid.

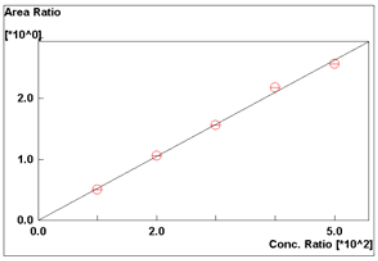

Fig. 2: Calibration curve of glutaric acid, $R^2 = 0.9962$.

Screening with the LRI

To observe an abnormally high or low level of all the other organic acids, we need to identify them in order to compare their peak intensities to the internal standard peak intensities. Two solutions are available. The first one is to use the Shimadzu Method package containing a table compound with 135 organic acids. The AART function of GCMSsolution enables to identify these organic acids whatever the analytical parameters are. Furthermore, an excel macro produce an automatic diagnosis from the identification.

The second solution is to screen the organic acids in one to five public or private libraries. GCMSsolution makes the screening technique very reliable thanks to the use of Linear Retention Indices (LRI). The Figure 3 compares the results obtained from library search with and without LRI similarity search. The LRI search reduces the hits from ten to only one.

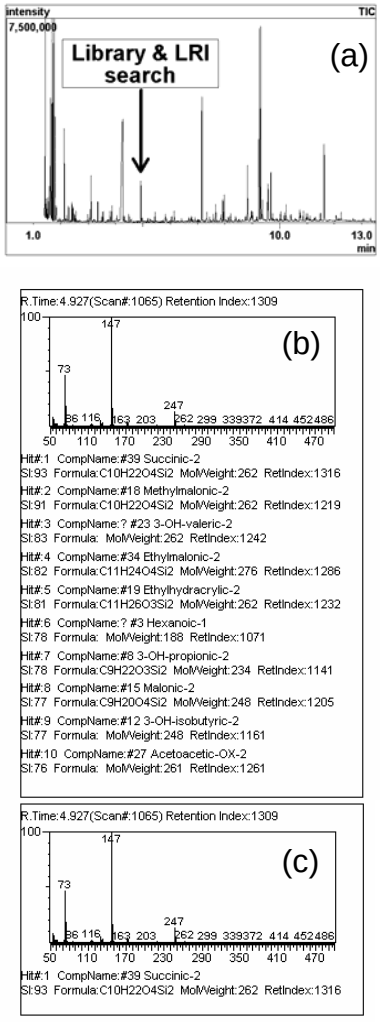

Fig. 3: Chromatogram of urinary sample (a), hits without (b) and with the use of the LRI search (c).

Conclusions

The Fast GCMS abilities of the Shimadzu GCMS-QP2010 series, offered as standard, enable to increase the daily throughput by a factor of 3 to 4.

The new and unique features of GCMSsolution, AART and LRI search functions, using Linear Retention Indices, transform the difficult analysis of organic acids in a simple and reliable analysis.

Acknowledgements

The standard solutions and the urinary sample were prepared by the Biochemistry department of the CHU Rouen, France.