学 位 論 文 の 要 旨

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学　位　論　文　名　Improvement of Sample Throughput Using Fast Gas Chromatography Mass-spectrometry for Biochemical Diagnosis of Organic Acid Disorders

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論 文 内 容 の 要 旨

INTRODUCTION

Gas chromatograph mass-spectrometry (GC/MS) and tandem mass spectrometry (MS/MS) are widely used for diagnosis and screening of inborn metabolic disorders (IMD). The GC/MS method, which analyzes organic acids, amino acids and sugars extracted from liquid urine or urine filter paper, is utilized for the diagnosis of a variety of metabolic disorders and diseases. It is claimed that the GC/MS method is more sensitive than MS/MS in detection of organic acid disorders, and also is able to detect a larger case spectrum of these disorders compared with MS/MS. Hence it has been used to confirm the disease detected as positive in the MS/MS screening or even a prenatal diagnosis of high-risk pregnancy. However, this method is time-consuming for the long GC/MS analysis time.

In this study, we developed the fast-GC/MS method using a short and extremely narrow inner diameter capillary column, a high carrier-gas pressure and a fast oven temperature ramp, and studied the usefulness of the method.

MATERIALS AND METHODS

To validate the fast-GC/MS method certain representative compounds were chosen, lactic acid and 2-hydroxyisovaleric acid as hydroxyl fatty acids; glutaric acid, malonic acid, methylmalonic acid, ethylmalonic acid, succinic acid, adipic acid and suberic acid as dicarboxylic acids; 4-hydroxyphenylacetic acid as aromatic acids; urasil as a pyrimidine metabolite; isovalerylglycine as a amino acids. Internal standards are margaric acid (MGA) and tropic acid (TA). To evaluate the diagnostic

utility of inborn error metabolism of the fast-GC/MS method, urine samples from 15 patients with 9 different disorders and 16 healthy controls were analyzed, and then compared with the conventional method. The patients included 3 each with isovaleric acidemia; 2 each with methylmalonic acidemia, propionic acidemia, ornithine transcarbamylase deficiency and multiple carboxylase deficiency); one each with glutaric aciduria type I, glycerolemia, maple syrup urine disease and Lactic aciduria.

In analysis of authentic compounds, 100 µl each of MGA and TA and tetracosane solutions (0.5 mg/ml) were added to one ml of standard solution (each 0.05 mg/ml of compounds). A sample of the mixture was evaporated under a gentle stream of nitrogen gas at room temperature. One ml of BSTFA and TMCS (10:1) were added to the dry residue and heated at 80 °C for 30 min. (Trimethylsilylation; TMS). The standard solution was analyzed five times each by both conventional and fast-GC/MS analysis.

The samples were treated using the solvent extraction method which is popular in GC/MS diagnosis of inborn errors metabolism, and then were analyzed using conventional and fast-GC/MS.

The analyses were performed on a gas chromatograph coupled to a quadrupole mass spectrometer (GCMS-QP2010, Shimadzu, Kyoto, Japan). A DB-5 column, 30 m×0.25 mm i.d.,1.00 µm, J&W (conventional capillary column) was used for the conventional-GC/MS, while a short, and extremely narrow bore DB-5 column, 20 m×0.10 mm i.d., 0.40 µm, J&W (fast-GCMS column) was for the fast-GC/MS method. The temperature program was 100 °C (4 min) – 4 °C/min–280 °C (11.0 min) in the conventional-GC/MS, while was 80 °C (0 min) – 30 °C/min – 325 °C (3.2 min) in the fast GC/MS.

The data were processed using the automated data system, which we developed previously (Shimadzu S. D. Corp and Department of Pediatrics Shimane University). In the system, compounds were automatically identified with retention index and ratios of quantitative ion to confirmation ion for 134 urinary organic acids, and further make an auto-diagnosis with the combination of abnormal compounds detected.

RESULTS AND DISCUSSION

Analytical condition for fast-GC/MS

To realize the 15-min analysis cycle time in the fast-GC/MS, the programmed column temperature was increased from 80 to 325 °C at 30 °C/min using the fast-GCMS column. Since the amount of stationary phase in a fast-GCMS column is less than that in a conventional capillary column used in the conventional-GC/MS. There might be column overload and inversed effects due to accumulated impurities. Although a higher split ratio is desirable for the above-mentioned reasons, a higher split ratio increases the minimum quantitation limit. The split ratio of 1:70 insured that the reproducibility (MGA at 2mg/l) was <10% RSD, which is required by the automated data system.

No less than eight sampling points for each peak are necessary for precise chromatographic peak shapes. The fast-GC/MS set up a data acquisition rate of 0.15 sec (m/z: 50–500, scan speed: 3333 amu/s) to collect above 8 sampling points across the all peaks in the diagnostic compounds.

The retention time of lactic acid-diTMS (first eluted sample) was shortened from 9.606 to 3.330 min (time reduction ratio=0.35). This ratio decreased as the retention time increased, reaching 0.18 (from

47.210 to 8.732min) at C24 (last eluted sample). As those results, the chromatographic separations of fast-GC/MS were almost identical to conventional-GC/MS even though the cycle time shortened from 63-min to 15-min.

Validation of Authentic sample analysis

The standard deviations (SDs) of retention times in both methods were <0.002 min (0.15 s). The SDs of the retention indices were in the range of 0.04 to 0.07 for conventional-GC/MS and in the range of 0.09 to 0.48 for fast-GC/MS. The differences in retention indices between the two methods ranged from −11.31 to +0.82. These results indicated that the allowance of the retention index for fast-GC/MS was ±20 when target compounds were identified with the data determined by conventional-GC/MS.

The relative peak areas by fast-GC/MS tended to be larger than those by conventional-GC/MS (0.91–1.82 times). The reproducibility by fast-GC/MS was <5% RSD.

Analysis of Urine samples from patients.

Urine samples obtained from 15 patients with 9 different disorders and 16 healthy controls were analyzed, and the data were processed using the automated data system. Both methods provided the diagnostic results for all specimens except 1 healthy control, which showed only a slight elevation of 3-hydroxy-isobutyric by fast-GC/MS. The quantitative value of 3-hydroxy-isobutyric acid-diTMS was 0.14 by fast-GC/MS, while the cutoff value was 0.13. It was 0.10 by conventional-GC/MS. The values obtained by fast-GC/MS tend to be higher than those of conventional-GC/MS (1.07–1.82 times). Therefore, the cutoff values for fast-GC/MS should be determined from the results of 16 healthy controls. By using those, diagnosis results of fast-GC/MS method for 15 patients with 9 different disorders are identical to conventional GC/MS.

CONCLUSION

Our study revealed that the fast-GC/MS has good chromatographic separation and reproducibility in chemical diagnosis of organic acid disorder no less than the conventional GC/MS method. The analytical time of the fast-GC/MS method can be extremely shortened, and make analysis of a larger number of samples. The analytical cost can be more saved. Combination of the fast-GC/MS and the automated data system will be more powerful tools in clinical laboratories due to a productivity increase and reduced analysis costs. Furthermore, the fast-GC/MS is expected to contribute to other metabolome analysis