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Analysis of Plasma Metabolites Using Gas-Chromatography Tandem Mass Spectrometry System with Automated TMS Derivatization

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Introduction

Gas-Chromatography Tandem Mass Spectrometry (GC-MS/MS) is one of highly suitable techniques for metabolome analysis because of the high separation, reproducible retention times and sensitive selective mass detection. However, metabolites are commonly derivatized before GC-MS/MS analysis. In case of TMS derivatization, the samples should be analyzed within 24 hours after derivatization, because the TMS derivatives will deteriorate after 24 hours. Moreover, metabolome analysis requires the measurement of more than 100 samples. Therefore, an effective analysis procedure and a reduction of exposure of operators to toxic reagents are required. In order to improve analysis accuracy, efficiency and safety, an Automated TMS Derivatization GC-MS/MS System was developed. In this study, the system is evaluated for analysis of metabolites in human plasma.

Methods and Materials

Sample Preparation

Reference; Nishiumi S et. al., Metabolomics, 2010 Nov;6(4):518-528

50 µL of human plasma

↓ Add 10 µL of Internal Standard (I.S., 0.5 mg/mL 2-Isopropylmalic acid)

Extraction of metabolites

↓ Add 250 µL of Methanol / Water / Chloroform (2.5 : 1 : 1) mixture, voltex, then centrifuge
Take 225 µL of supernatant after the 30 min incubation at 37°C with shake
Add 200 µL of Water to the supernatant, voltex, then centrifuge
Take 250 µL of supernatant and dry the sample by SpeedVac

Methoxyamination

↓ Add 40 µL of 20 mg / mL methoxyamine - Pyridine mixture
Incubate at 30°C for 90 min with shake

Trimethylsilylation using AOC -6000

↓ Add 20 µL MSTFA and Incubate at 37°C for 30 min with shake

Measure the metabolites by GCMS-TQ8040

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Analytical Conditions

GC	
Inj. Temp.	: 250°C
Column oven Temp.	: 60°C (2.00 min) → (15°C/min) → 330°C (3.00 min) : Total 23 min
Linear velocity	: 39.0 cm/sec
Split Ratio	: 30
Injection volume	: 1 µL
Column	
BPX-5 (30m x 0.25mm I.D. df=0.25µm, SGE)	
MS	
Interface Temp.	: 280°C
Ion source Temp.	: 200°C
Data acquisition	: MRM
	475 compounds 950 transitions Avg. dwell time : 3.5ms Min. dwell time : 1.0ms
Database	
Smart Metaboites Database (Shimadzu)	

Automated TMS Derivatization GC-MS/MS system

Figure 1 Automated TMS Derivatization GC-MS/MS system

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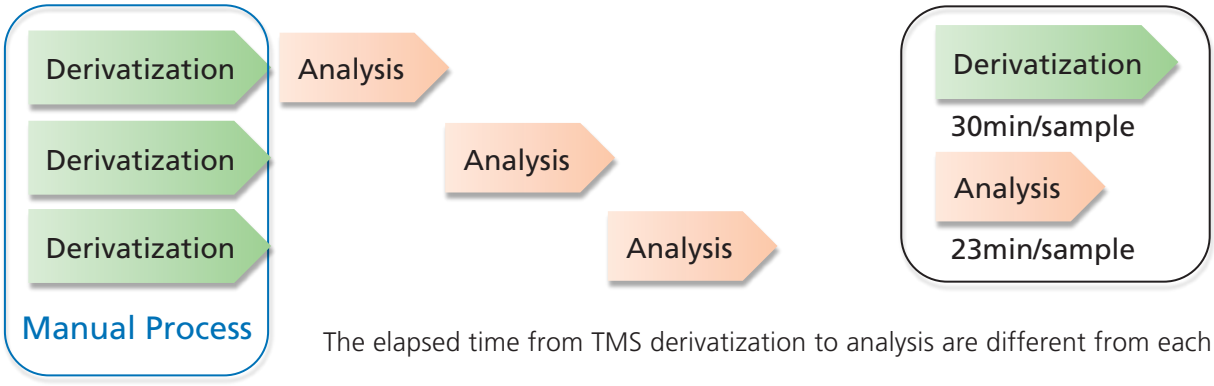
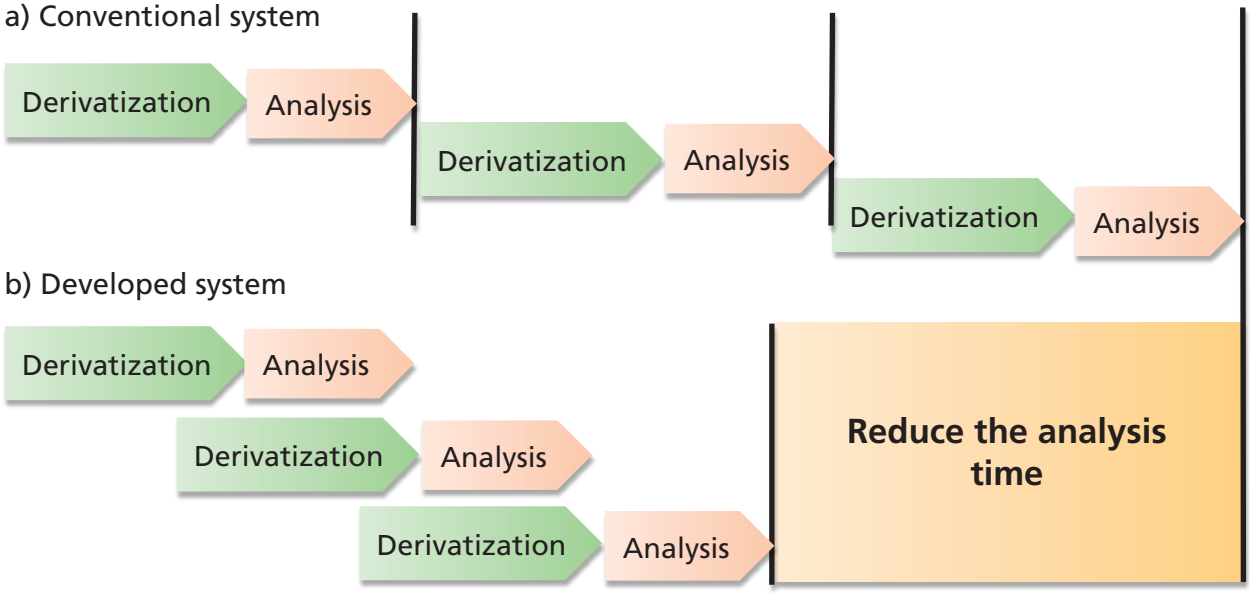
Analysis Workflow

1) Conventional Manual Derivatization Method



The elapsed time from TMS derivatization to analysis are different from each sample.

2) Automated TMS Derivatization GC-MS/MS System



The developed system can derivatize plural samples simultaneously and analyze the samples immediately after TMS derivatization.

Result

Analysis of Plasma metabolites using Automated TMS derivatization GC-MS/MS System

The 179 metabolites were detected in pooled human plasma by the Automated TMS Derivatization GC-MS/MS System. To validate the system, 7 replicate samples were analyzed and the relative standard deviation (RSD) value in the 179 metabolites was calculated. For 2-Isopropylmalic acid as an internal standard, RSD% of peak area was 8.47%.

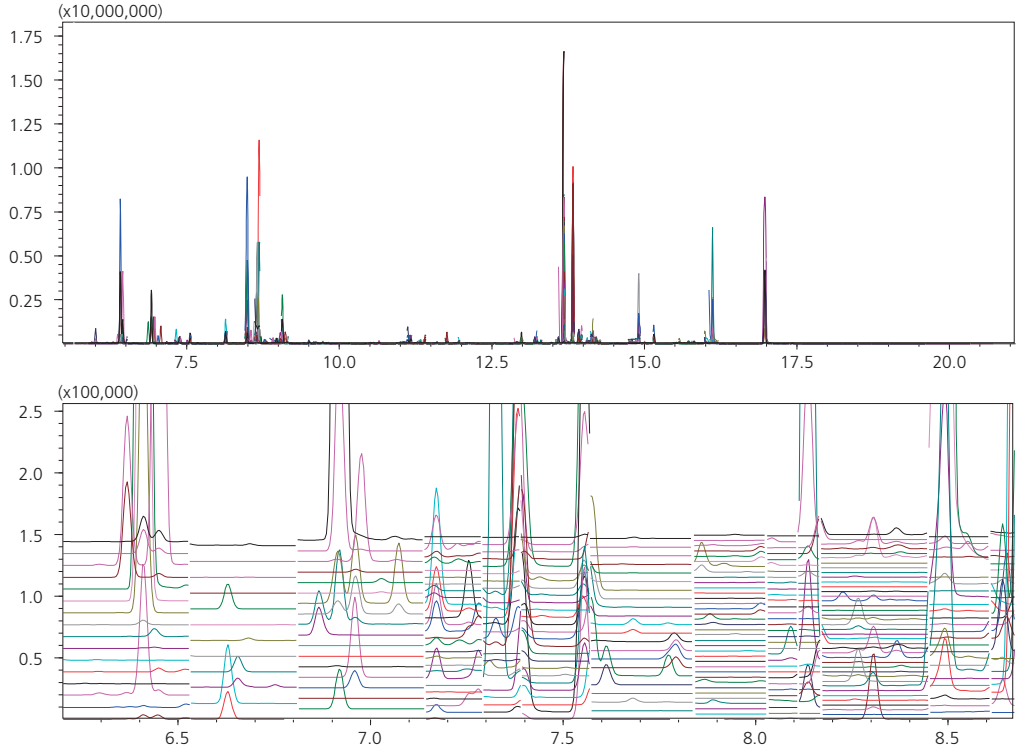


Figure 2 MRM Chromatograms of metabolites in pooled human plasma

For the detected metabolites, the peak area of each ion was calculated and normalized to the peak area of 2-isopropylmalic acid as an internal standard. The RSD% of 134 metabolites were less than 20%. The result was almost the same as the conventional manual derivatization method.

Table 1 Reproducibility of relative peak area in pooled human plasma

%RSD (n=7)	Number of Compounds
- 5.00%	43
5.01 - 10.00%	42
10.01 - 15.00%	27
15.01 - 20.00%	22
≤ 20.00%	134
> 20.00%	45
Total	179

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Signal intensities with lapse of time

We have measured the samples which passed from 0 to 24 hours after TMS derivatization. The signal intensities of some metabolites like Lysine, Tyrosine, Kynurenine and Tryptophan were decreased gradually during 24 hours. As time passed after TMS derivatiztion, the TMS derivatives ratio in some compounds were changed by the reaction progress. On the other hand, Automated TMS Derivatization system can analyze samples immediately after TMS derivatization. The samples which passed from 0 to 24 hours after the methoximation were analyzed. As the result, the signal intensities in these compounds were not changed even with the lapse of time.

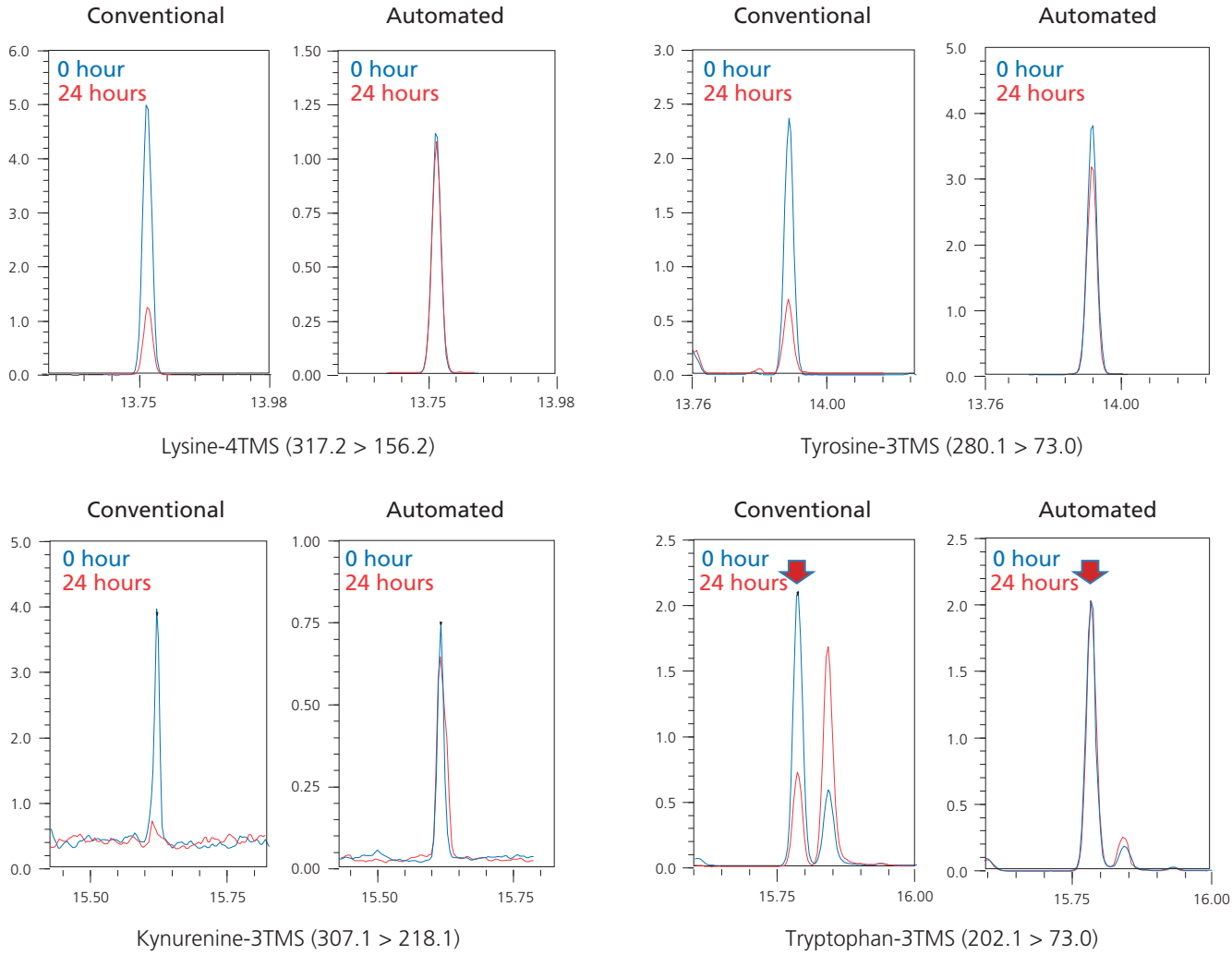

Figure 3 Mass Chromatograms of Lysine-4TMS, Tyrosine-3TMS, Kyunurenine-3TMS and Tryptophan-3TMS. Blue lines are 0 hour and red lines are 24 hours after setting the samples on the auto-sampler.

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Conclusions

The Automated TMS Derivatization GC-MS/MS System was developed and evaluated for analysis of metabolites in human plasma. The novel system improves the accuracy, efficiency and safety for metabolite analysis in human plasma compared to the conventional manual derivatization method. This system contributes to the dissemination of metabolomics studies.

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