

# Application Data Sheet

## No. 59

### GC-MS

Gas Chromatograph Mass Spectrometer

## Analysis of Metabolites in Rat Urine Using MRM via GC-MS/MS

GC-MS provides excellent chromatographic separation, and is capable of highly sensitive and stable measurements. Consequently, GC-MS is widely utilized when measuring low-molecular-weight metabolites in living organisms and performing metabolomic analysis to assess their fluctuations. When analyzing biological samples featuring a large number of matrix components, however, separation from the matrix can be difficult using GC-MS. An alternative is GC-MS/MS, which provides excellent separation performance via MS. In this Application Datasheet, an analysis of metabolites in rat urine is performed using GC-MS/MS. Results comparing this data with GC-MS data are also introduced.

### Experimental

Rat urine was treated using the urease-treated direct drying method [1]. The samples were then subjected to trimethylsilylation prior to measurement.

### Analysis Conditions

SIM was used as the GC-MS measurement mode and MRM was used for the GC-MS/MS measurements.

Table 1: Analysis Conditions

GC-MS	:GCMS-TQ8030		
Column	:DB-5 (Length 30 m, 0.25 mm I.D., df=1.0 μm)		
[GC]		[MS]	
Injection Temp.	:280 °C	Interface Temp.	:280 °C
Column Oven Temp.	:100 °C (4 min) → (4 °C /min) → 320 °C (0 min)	Ion Source Temp.	:200 °C
Injection Mode	:Splitless	Tuning Mode	:Standard
Sampling Time	:1 min		
Flow Control Mode	:Linear velocity (39.0 cm/sec)	GC-MS Mode	
Injection Volume	:1 μL	Acquisition Mode	:SIM
		SIM Event Time	:0.3 sec
		GC-MS/MS Mode	
		Acquisition Mode	:MRM

#### SIM monitoring *m/z*

Compound name	RT (min)	Quantitative	Qualitative
Lactic acid-2TMS	7.51	219	191
Glycerol-3TMS	14.711	218	205
Glutaric acid-2TMS	18.827	261	158
Adipic acid-2TMS	22.078	275	185
Suberic acid-2TMS	27.76	303	217

#### MRM monitoring *m/z*

Compound name	RT (min)	Quantitative Transition		Qualitative Transition	
		Precursor>Product	CE (V)	Precursor>Product	CE (V)
Lactic acid-2TMS	7.51	219 > 149	8	219 > 191	5
Glycerol-3TMS	14.711	218 > 159	6	218 > 113	14
Glutaric acid-2TMS	18.827	158 > 116	8	158 > 101	15
Adipic acid-2TMS	22.078	275 > 141	8	275 > 111	10
Suberic acid-2TMS	27.76	303 > 109	12	303 > 191	4

## Analysis Results

A comparison of SIM measurements in GC-MS mode and MRM measurements using the GC-MS/MS mode is shown in Fig. 1. Using the GC-MS SIM mode, the identification and quantification of Glycerol-3TMS and Suberic acid-2TMS compounds are difficult due to matrix effects. In contrast, with GC-MS/MS MRM measurements, the mass separation of matrix effects enabled both identification and quantification.

GC-MS/MS thus proved effective for the analysis of biological samples containing large quantities of impurities.

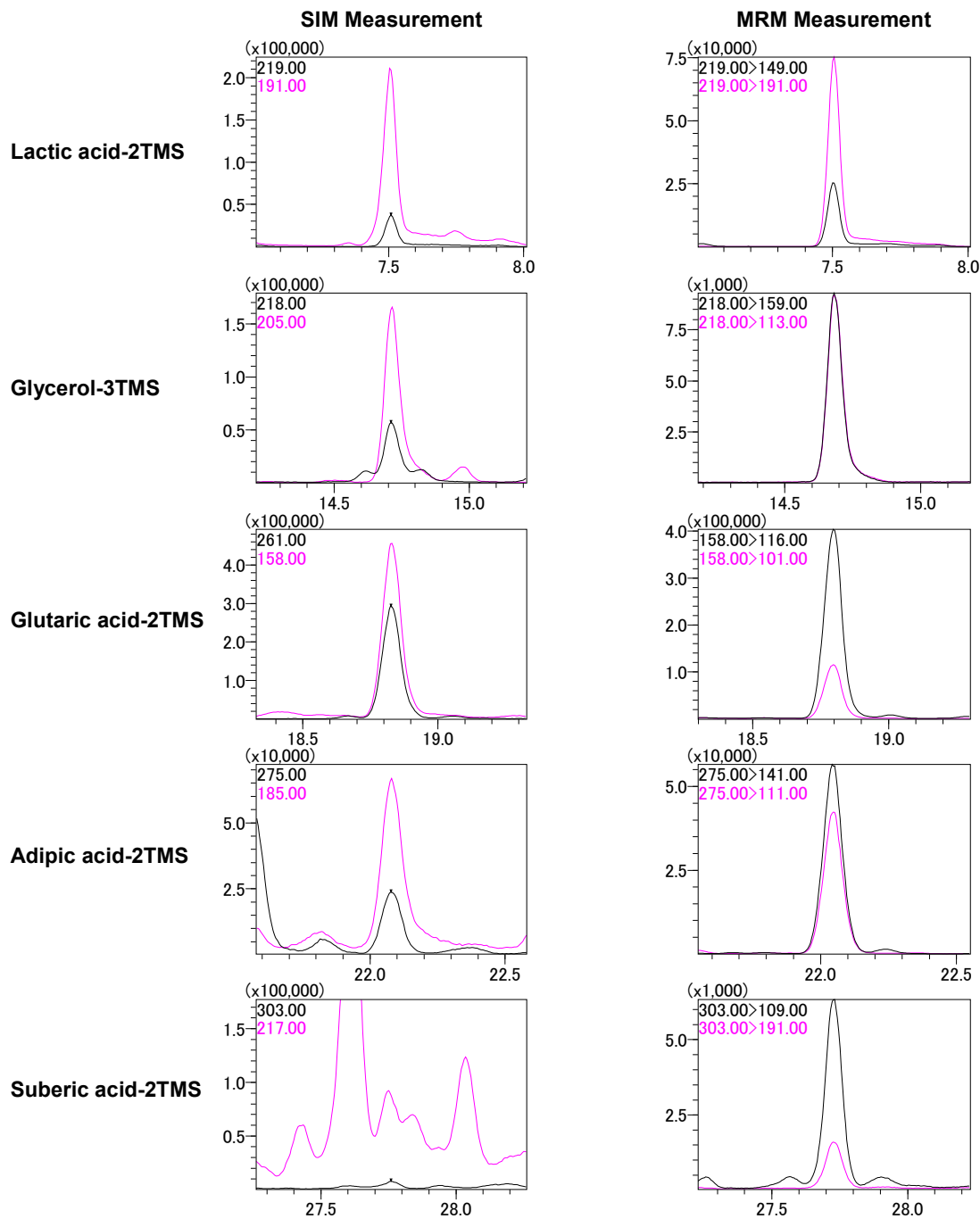


Fig. 1 Comparison of SIM (Left) and MRM (Right) Mass Chromatograms for Metabolites in Rat Urine