

# 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.

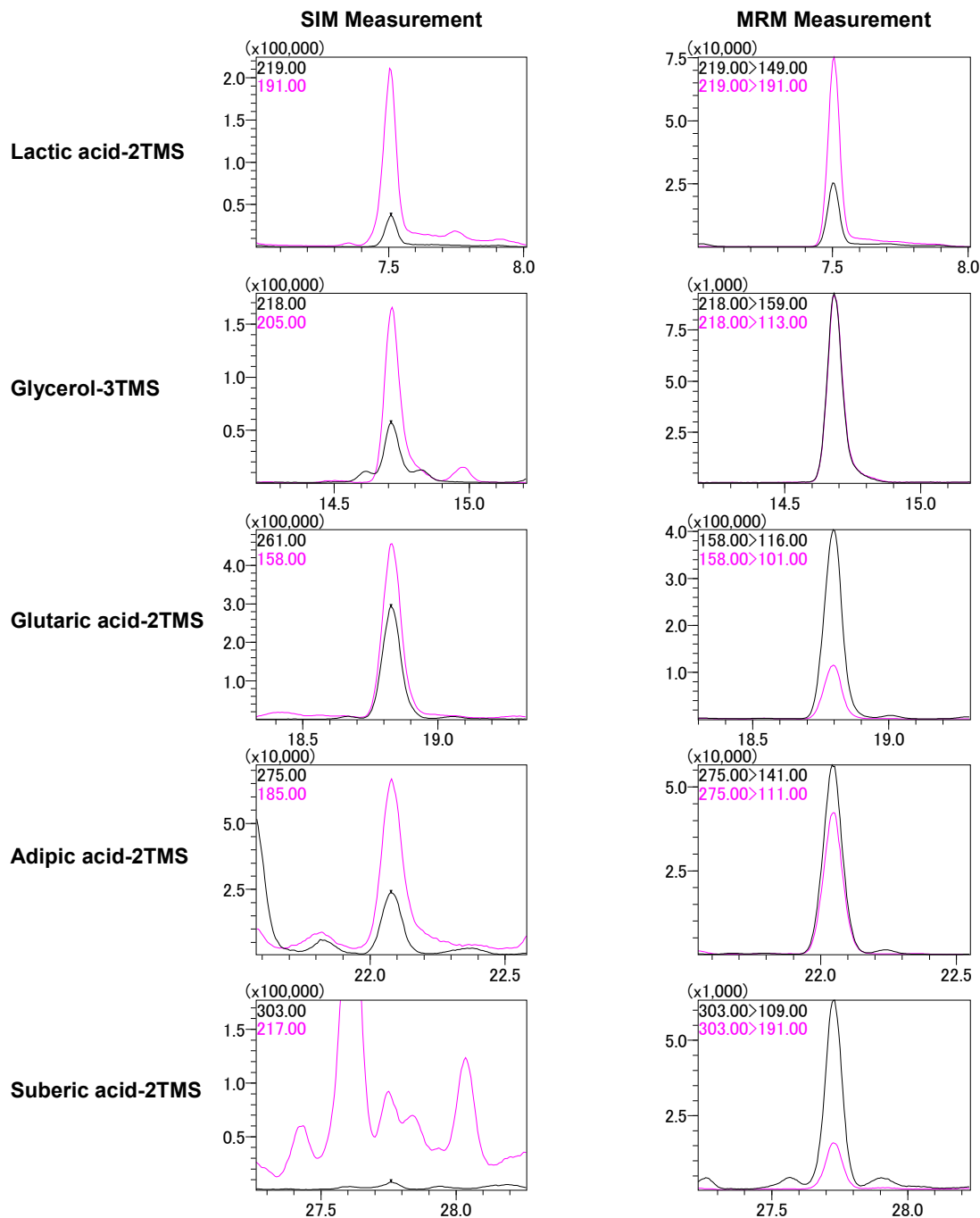


Fig. 1 Comparison of SIM (Left) and MRM (Right) Mass Chromatograms for Metabolites in Rat Urine