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Application Data Sheet

No.88

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Metabolites in Serum Using GC-MS/MS

Single quadrupole GC-MS provides excellent chromatographic resolution and enables stable measurements, and is therefore widely utilized for metabolome analyses involving the comprehensive analysis of in vivo metabolites. However, biological samples contain many metabolites and various matrices, so separation with single quadrupole GC-MS can be difficult. With triple quadrupole GC-MS/MS MRM, MS separation is performed twice, with Q1 and Q3. This helps remove the impact of overlapping peaks due to interfering components in comparison with scan mode, in which MS separation is performed with a single quadrupole, and thus enables the acquisition of accurate quantitative results with high-sensitivity detection.

This application data sheet presents an analysis of metabolites in standard human serum using the scan and MRM methods included in the GC/MS Metabolite Database Ver. 2, as well as a comparison of the results.

Analysis Conditions

In the pretreatment process, 2-isopropylmalic acid was added as an internal standard to 50 μL of standard human serum, after which metabolites were extracted with a methanol/water/chloroform (2.5:1:1) solution. Methoxime and trimethylsilyl derivatives were then formed to obtain the samples[1]. The respective samples were measured six times each in scan and MRM modes using methods included in the GC/MS Metabolite Database Ver. 2. Table 1 shows the analysis conditions.

Table 1: Analysis Conditions

GC-MS:	GCMS-TQ8030
Column:	DB-5 (Length 30 m; 0.25 mm I.D.; df = 1.00 μm)
Glass insert:	Splitless insert with wool (P/N: 221-48876-03)

[GC]	
Sample injection unit temp.:	280 °C
Column oven temp.:	100 °C (4 min) → (4 °C/min) → 320 °C (8 min)
Injection mode:	Splitless
Carrier gas control:	Linear velocity (39.0 cm/sec)
Injection volume:	1 μL

[MS]	
Interface temp.:	280 °C
Ion source temp.:	200 °C
Measurement mode:	Scan
Mass range:	*m/z* 45-600
Event time:	0.3 sec
Measurement mode:	MRM
Loop time:	0.3 sec

MRM monitoring *m/z* (MRM measurement of four metabolites and an internal standard (I.S.) compared with scan measurement)

		Quantitative Transition		Qualitative Transition	
Compound name	RT (min)	Precursor>Product	CE (V)	Precursor>Product	CE (V)
3-Hydroxyisovaleric acid-2TMS	15.480	131.10> 73.00	12	247.10> 73.00	18
Homocysteine-3TMS	30.360	234.10> 73.00	27	234.10>128.10	9
Aconitic acid-3TMS	32.490	285.10>147.10	15	375.10>147.10	15
Kynurenine-3TMS	43.890	307.10>218.10	9	307.10>192.10	18
2-Isopropylmalic acid-3TMS (I.S.)	27.930	349.10>259.10	6	349.10>147.10	24

[1] S. Nishiumi, M. Shinohara, A. Ikeda, T. Yoshie, N. Hatano, S. Kakuyama, S. Mizuno, T. Sanuki, H. Kutsumi, E. Fukusaki, T. Azuma, T. Takenawa, M. Yoshida, Metabolomics 6 (2010) 518-528

Analysis Results

Fig. 1 shows mass chromatograms for serum metabolites obtained in scan and MRM modes. In scan mode, some of the four components shown were not detected due to interfering components and insufficient sensitivity, resulting in a repeatability of 14 % or more. In contrast, favorable results were obtained with MRM, which eliminated the impact of interfering components, enabling high-sensitivity measurements with a repeatability of 6.5 % or less (see Table 2).

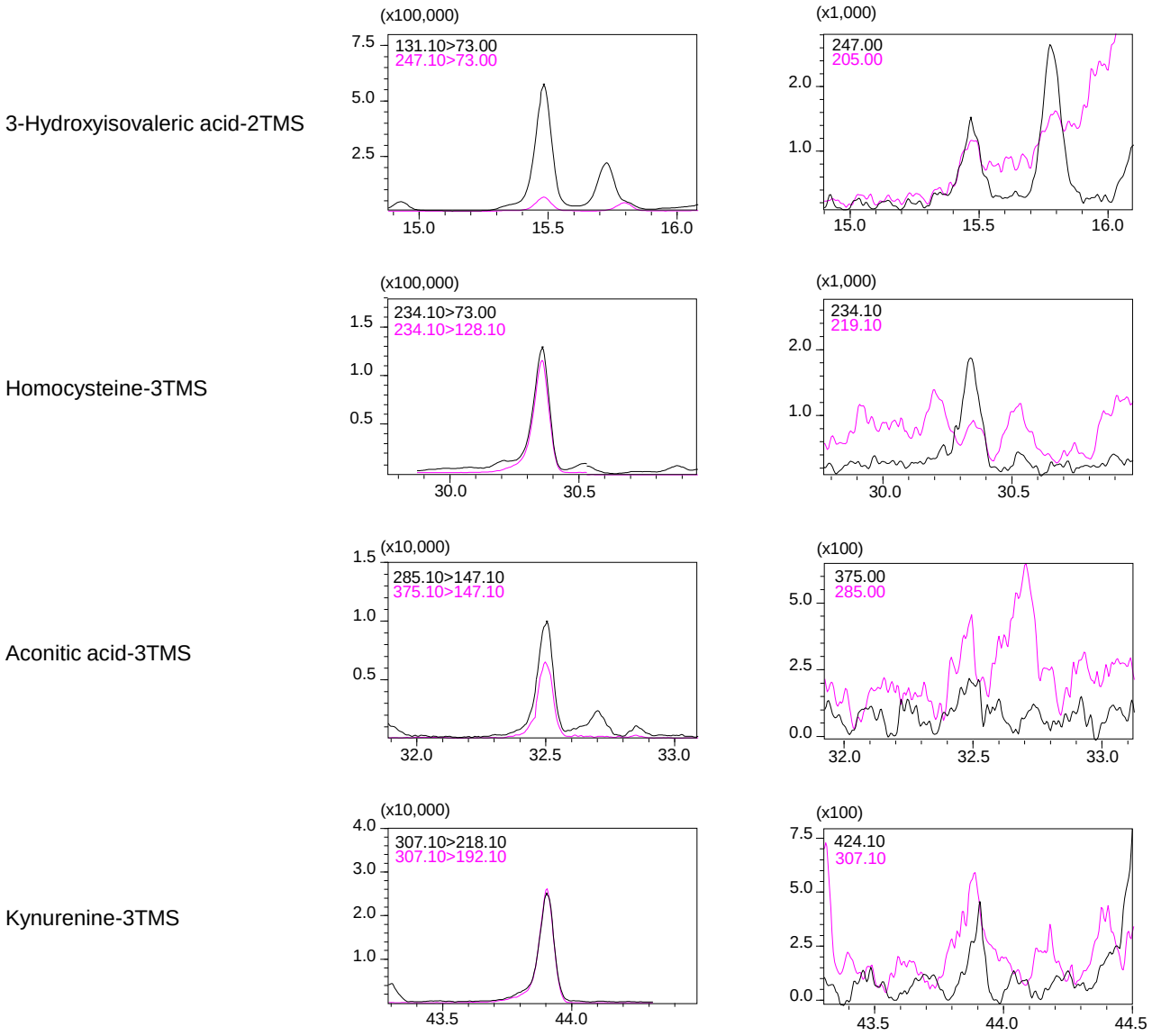

Fig. 1: Comparison of MRM (Left) and Scan (Right) Mass Chromatograms for Metabolites in Standard Serum

Table 2: Repeatability for Scan and MRM Modes (corrected with internal standard)

	%RSD (n=6)	
Compound name	MRM	Scan
3-Hydroxyisovaleric acid-2TMS	3.99	14.0
Homocysteine-3TMS	5.04	23.4
Aconitic acid-3TMS	5.98	N/A
Kynurenine-3TMS	6.48	24.5

First Edition: August, 2013

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