SHIMADZU
Excellence in Science

Application Data Sheet

No.100

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Toxicological Substances in Whole Blood Using Smart Forensic Database (1)

By providing mass separation in two stages, GC-MS/MS is capable of separating out interferences in biological samples and toxicological substances. Therefore, it is simple to determine whether toxicological substances are present, significantly reducing the time required for data analysis. In order to analyze toxicological substances in MRM mode, however, MRM transitions and collision energies (CE) must be optimized, which is very labor intensive.

Smart Forensic Database is an MRM database containing retention indices, MRM transitions, collision energies, and quantitation/confirmation ion ratios for 201 toxicological substances often involved in poisonings. The retention times for the registered toxicological substances are accurately estimated simultaneously from low-boiling point components to high-boiling point components, using measurement data from a standard n-alkane mixture via the GCMSsolution AART function. Smart MRM, which is provided with the GCMS-TQ8040, can then create MRM analysis methods automatically using the database.

This article introduces an example of applying Smart Forensic Database to the analysis of toxicological substances in a whole-blood sample.

Experimental

Liquid-liquid extraction via EXtrelut® NT3 was used to pretreat the whole-blood sample. The collected whole-blood sample was measured into 1 mL portions for acidic fractionation and basic fractionation, and each portion was diluted with 1 mL of Milli-Q water. The acidic fraction was adjusted to a pH 5 using 10 % hydrochloric acid, and the basic fraction was adjusted to a pH 9 using 10 % ammonia water. The respective solutions were added to the EXtrelut® NT3 columns and left to stand for 30 minutes, after which each was eluted with a 10 mL chloroform:isopropanol (3:1) mixture. The extracted solutions of acidic fraction and basic fraction were then mixed, and after dessication with silica gel and drying in a nitrogen airflow, the sample solution was re-dissolved in a 200 μL chloroform:isopropanol (3:1) mixture. To check the MRM sensitivity, the sample obtained was spiked with promethazine, phenobarbital, chlorpromazine, and triazolam so that the concentration of each compound becomes 50 ng/mL in whole blood.

Analytical Conditions

The conditions registered in Smart Forensic Database were used as the GC-MS/MS analysis conditions. For the compounds subject to MRM measurement, a simultaneous Scan/MRM analysis method was created, in which the 201 components registered in the database were set.

Table 1: Analytical Conditions

GC-MS:	GCMS-TQ8040
Column:	Rxi® -5SilMS (Length 30 m, 0.25 mm I.D., df=0.25 μm)
Glass liner:	Splitless insert with wool (PN: 221-48876-03)

[GC]	
Injection temp.:	260 °C
Column oven temp.:	60 °C (2 min) → (10 °C /min) → 320 °C (15 min)
Injection mode:	Splitless
Flow control mode:	Linear velocity (45.6 cm/sec)
Injection volume:	1 μL

[MS]	
Interface temp.:	280 °C
Ion source temp.:	200 °C
Acquisition mode:	Scan/MRM
Scan event time:	0.1 sec
Scan mass range:	*m/z* 43 – 600
Scan speed:	10,000 u/sec
MRM event time:	0.3 sec
Total loop time:	0.4 sec

Results

The extracted whole-blood sample was spiked with four toxicological substances so that the concentration of each substance becomes 50 ng/mL in whole blood, and then measured using Scan/MRM mode. Fig. 1 shows the mass chromatograms obtained, and Fig. 2 shows the repeatability obtained by repeating analyses five times. With the Scan mode analysis, confirmation ions were not detected, there was an overlap with cholesterol, and the peak for triazolam could not be confirmed. With the MRM mode, however, each component was clearly detected, and favorable repeatability results of 4.29 % max. were obtained.

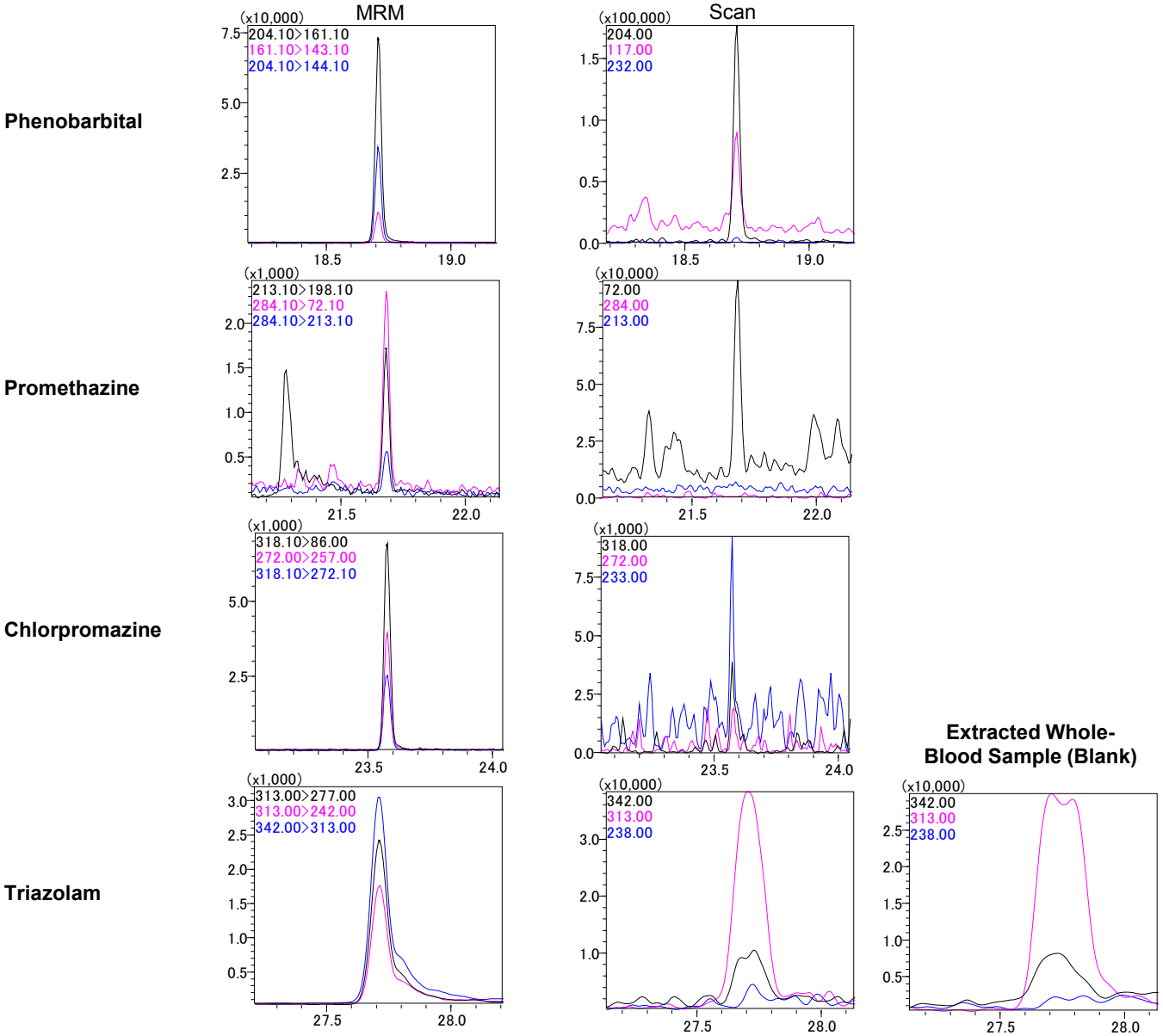

Fig. 1: Scan and MRM Mass Chromatograms for Four Toxicological Substances in Whole-Blood Samples (L: MRM; R: Scan)

Table 1: Area Repeatability at Five Replicates (Concentration in Whole Blood: 50 ng/mL)

	Data 1	Data 2	Data 3	Data 4	Data 5	Average	SD	%RSD
Phenobarbital	131,876	133,119	137,359	136,480	133,656	134,498	2323.7	1.73
Promethazine	2,756	2,873	2,742	2,885	2,829	2,817	65.7	2.33
Chlorpromazine	12,832	12,899	12,657	13,484	14,024	13,179	565.3	4.29
Triazolam	10,909	10,315	10,704	10,838	10,701	10,693	229.5	2.15

The data evaluated in this article was obtained from a sample that was spiked with the substances after extraction. There is no guarantee that a favorable recovery ratio will be obtained with the pretreatment method described above.
This data was provided by Associate Professor Kei Zaitsu in the Department of Legal Medicine & Bioethics, Nagoya University Graduate School of Medicine.

First Edition: September, 2014

Shimadzu Corporation
www.shimadzu.com/an/