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Application Data Sheet

No.75

GC-MS

Gas Chromatograph Mass Spectrometer

Analysis of Psychotropic Drugs in Whole Blood Utilizing Simultaneous Scan/MRM Measurements (2)

In GC-MS/MS MRM mode, the MRM transition and collision energy (CE) must be optimized. In addition, when performing a comprehensive analysis of medicinal toxicants in biological samples, optimization of these parameters for each relevant medicinal toxicant is an extremely laborious process. The GCMS-TQ8030 Triple Quadrupole Gas Chromatograph Mass Spectrometer (GC-MS/MS) achieves simultaneous scan/MRM measurements through high-speed scan and high-speed MRM data sampling techniques. Comprehensive analysis of medicinal toxicants is achieved by combining the MRM mode, used to measure toxicants requiring high sensitivity or that are prone to overlap with foreign materials, with the scan mode, which is used to measure toxicants for which sensitivity is sufficient. The “GC/MS Forensic Toxicological Database” contains the retention indices, characteristic *m/z* (quantitative ions and reference ions) mass chromatograms, and mass spectral data for approximately 500 medicinal toxicant components. As a result, screening can be performed by applying this information to the scan data from simultaneous scan/MRM analysis.

This Application Data Sheet introduces the application of scan data from simultaneous scan/MRM measurement to the Forensic Toxicological Database, leading to comprehensive screening for medicinal toxicants in a whole blood sample. Please refer to this in conjunction with Application Datasheet No. 74, which introduces an example of the mass separation of cholesterol from triazolam and etizolam in whole blood, utilizing MRM data from simultaneous Scan/MRM analysis.

GC/MS Forensic Toxicological Database

The “GC/MS Forensic Toxicological Database” contains the names of medicinal toxicants, as well as their quantitative and reference ions, retention indices, and standard mass spectra. In terms of the retention times for the registered medicinal toxicants, reliable simultaneous estimation of everything from low boiling point to high boiling point components can be performed using the measurement data from n-alkane mixed standard samples (C9-C33, provided by Restek Corporation, P/N: 560295) via the GCMSsolution AART function.

The presence or absence of medicinal toxicant content can be determined easily from the registered quantitative and reference ion mass chromatograms .

In addition, relative response factors utilizing internal standards have been registered for medicinal toxicants often involved in cases of abuse, enabling the calculation of approximate concentration values (semi quantitative values).

Experimental

The liquid-liquid extraction method via EXtrelut® NT3 was used for pretreatment of the whole blood sample. For details on the pretreatment procedure, refer to Application Data Sheet No. 74. Simultaneous scan/MRM measurements were performed on the extracted sample. The MRM measurement targeted triazolam and etizolam, and the scan data was used for simultaneous screening for medicinal toxicants utilizing the “GC/MS Forensic Toxicological Database”. The analytical conditions are shown in Table 1.

In order to calculate semi-quantitative values, the custom internal standard (provided by Restek Corporation P/N: 560294), which contains 8 PAH-d isomers, was adjusted to a concentration of 1 µg/mL. The extracted sample and the adjusted internal standard sample were injected simultaneously into the GC-MS/MS system using the AOC-20i+s solvent flush mode.

Table 1 Analytical Conditions

GC-MS	:GCMS-TQ8030
Column	:Rxi®-5Sil MS (length: 30 m; 0.25 mm I.D., df=0.25 µm)
Glass Liner	:Splitless insert with glass wool (P/N: 221-48876-03)

[GC]		[MS]	
Injection Temp.	:260 °C	Interface Temp.	:280 °C
Column Oven Temp.	:60 °C (2 min) → (10 °C /min) → 320 °C (10 min)	Ion Source Temp.	:200 °C
Injection Mode	:Splitless	Acquisition Mode	:Scan//MRM
Flow Control Mode	:Linear velocity (45.6 cm/sec)	Scan Event Time	:0.15 sec
Injection Volume	:1 µL	Scan Mass Range	:*m/z* 45 – 700
		Scan Speed	:5,000 u/sec

MRM Monitoring *m/z*

Compound Name	Retention Time	Quantitative Transition		Qualitative Transition 1		Qualitative Transition 2	
		Precursor>Product	CE (V)	Precursor>Product	CE (V)	Precursor>Product	CE (V)
Etizolam	27.149	342.00>272.00	24	342.00>245.00	33	342.00>266.00	20
Triazolam	27.171	313.00>277.00	25	313.00>278.00	18	313.00>242.00	35

Results

Fig. 1 shows the scan chromatogram for the extracted whole blood sample, measured in the simultaneous scan/MRM analysis. By applying the Forensic Toxicological Database to the scan data, it was possible to identify the benzodiazepine psychotropic drugs (diazepam and desmethyldiazepam). To date, the method utilized for compound identification has involved a library search of the peak mass spectrum detected with the total ion current chromatogram (TIC). However, the method utilizing the Forensic Toxicological Database uses the estimated retention times and characteristic *m/z* mass chromatogram (MC) for detection as shown in Fig. 2. Accordingly, it enables quick and easy determination of the presence or absence of low-concentration medicinal toxicants that cannot be confirmed with the conventional method. In addition, the mass spectra of the detected medicinal toxicants can be compared to registered standard mass spectra, thereby improving the reliability of the data analysis. By performing simultaneous scan/MRM analysis, it becomes possible to simultaneously screen for components not targeted by MRM measurements.

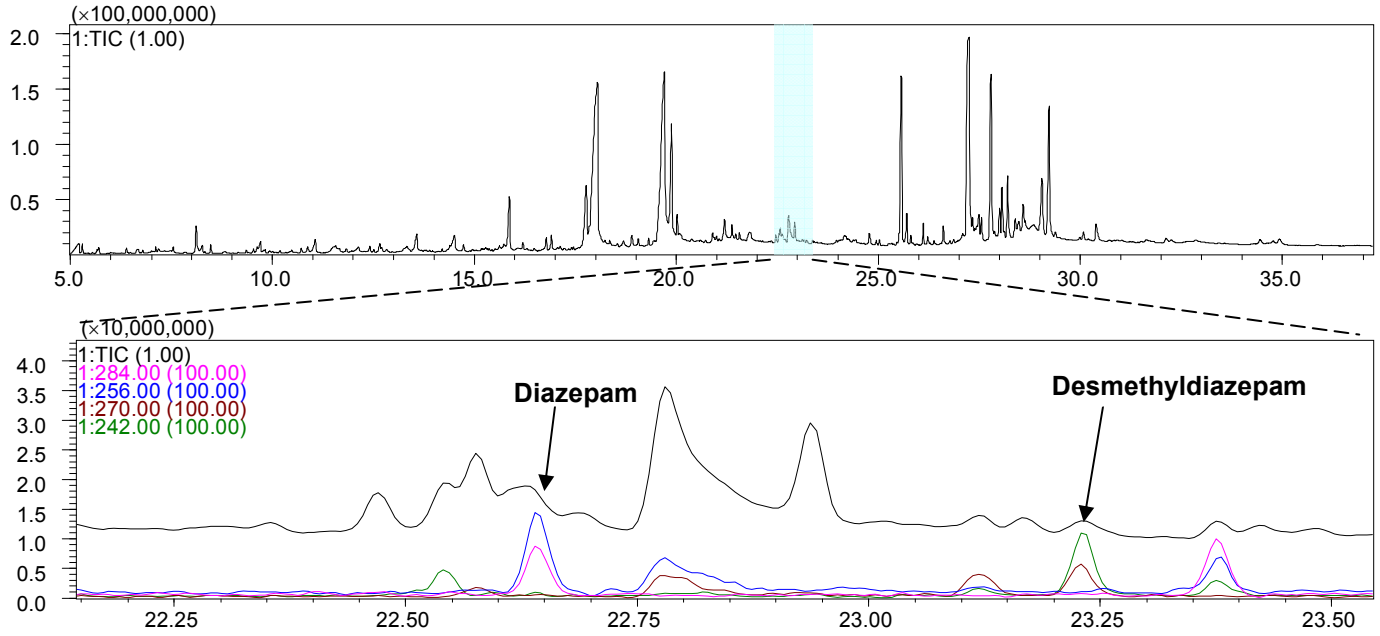

Fig. 1 Scan Chromatogram for Measured Whole Blood Sample
(Top: Scan TIC; Bottom: Enlarged TIC and MC for detected medicinal toxicants)

Comparison of Detected Peak Mass Spectrum and Standard Mass Spectrum

Data Analysis Utilizing Estimated Retention Intervals and Characteristic m/z Mass Chromatograms

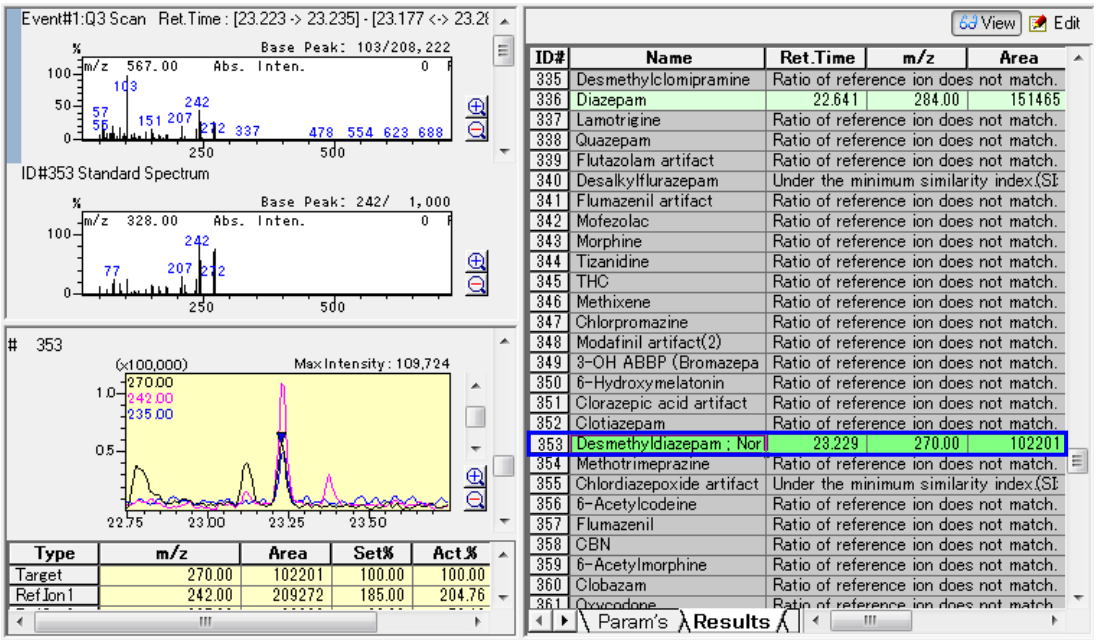

Fig. 2 Scan Data Analysis Window Using the “GC/MS Forensic Toxicological Database”

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