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Application Data Sheet

No.74

GC-MS

Gas Chromatograph Mass Spectrometer

Analysis of Psychotropic Drugs in Whole Blood Utilizing Simultaneous Scan/MRM Measurements (1)

When analyzing medicinal toxicants using GC-MS, the presence of fatty acids and cholesterol, which exist in large quantities in whole blood, can interfere with detection. In fact, profiles for triazolam and etizolam, benzodiazepine psychotropic drugs, overlap with the cholesterol chromatogram, making data analysis difficult with a single GC-MS system. Furthermore, the retention indices for triazolam and etizolam are adjacent, and both have characteristic *m/z* ratios of 313 and 342, respectively (Fig. 1), making it even more difficult to distinguish these compounds.
Consequently, there are high expectations for utilizing GC-MS/MS. This Application Data Sheet introduces an example of simultaneous scan/MRM measurements for the mass separation of cholesterol from triazolam and etizolam in whole blood. Refer to this in conjunction with Application Datasheet No. 75, which introduces an example of simultaneous screening for other medicinal toxicants by applying scan data from simultaneous scan/MRM measurements to the “GC/MS Forensic Toxicological Database”.

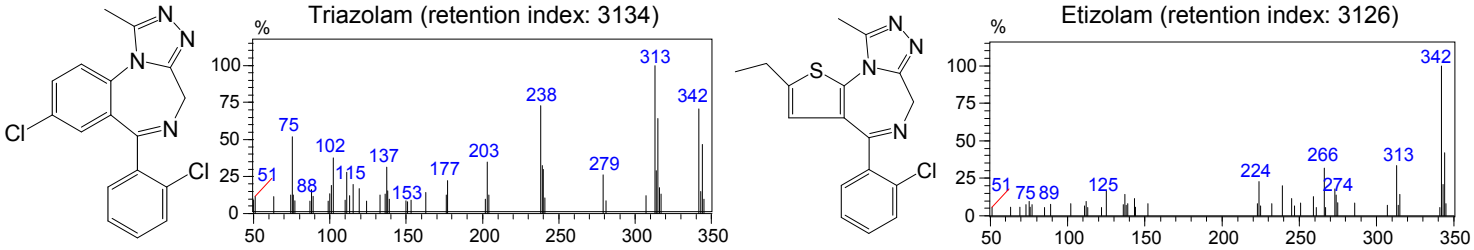


Fig. 1: Scan Mass Spectra and Retention Indices for Triazolam and Etizolam

Sample Pretreatment

The liquid-liquid extraction method via EXtrelut® NT3 was used for pretreatment of the whole blood sample. For both the acidic fraction and basic fraction, 1 mL of the collected whole blood sample was measured, and each was then diluted with 1 mL of Milli-Q water. The acidic fraction was adjusted to a pH of 5 using 10 % perchloric acid, and the basic fraction was adjusted to a pH of 9 with 10% ammonia water. Each solution was poured into EXtrelut® NT3, and after leaving to stand 30 minutes, eluted with a 10 mL mixed solution of chloroform and isopropanol (3 :1). Afterwards, the extracted acidic fraction and basic fraction liquids were mixed, and following desiccation with silica gel, dried and hardened under a nitrogen gas flow. The resulting sample was then re-dissolved with a 200 µL mixed solution of chloroform and isopropanol (3 :1).

In order to calculate semi-quantitative values utilizing the “GC/MS Forensic Toxicological Database”, the custom internal standard (P/N: 560294, from Shimadzu GLC), which contains 8 PAH-d isomers, was adjusted to a concentration of 1 µg/mL for use as an internal standard sample. The adjusted extracted sample and the internal standard sample were injected simultaneously into the GC-MS/MS system using the AOC-20i+s solvent flush mode.

Analytical Conditions

Simultaneous scan/MRM measurements were performed on the extracted sample. The MRM measurement targeted the triazolam and etizolam, and the scan data was used simultaneously screen for other medicinal toxicants utilizing the “GC/MS Forensic Toxicological Database”. The analytical conditions are shown in Table 1.

Table 1: Analysis 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]	
Injection Temp.	:260 °C
Column Oven Temp.	:60 °C (2 min) → (10 °C /min) → 320 °C (10 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
Data Acquisition Mode	:Scan//MRM
Scan Event Time	:0.15 sec
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

Figs. 2 and 3 show the mass chromatograms obtained from scan/MRM measurements of the whole blood extracted sample (blank), which did not contain etizolam or triazolam, and the sample created by adding etizolam and triazolam to the blank sample in order to reach a concentration of 500 ng/mL. In the scan mass chromatogram, the cholesterol is detected at the same retention time as etizolam and triazolam, making it difficult to determine the presence of these psychotropic drugs. However, 2-stage mass separation via MRM enables separation from the cholesterol, making selective detection of etizolam and triazolam possible. In the scan measurement, similar mass spectra are indicated for etizolam and triazolam, but in the MRM chromatogram, they could be separated and confirmed without mutual influences.

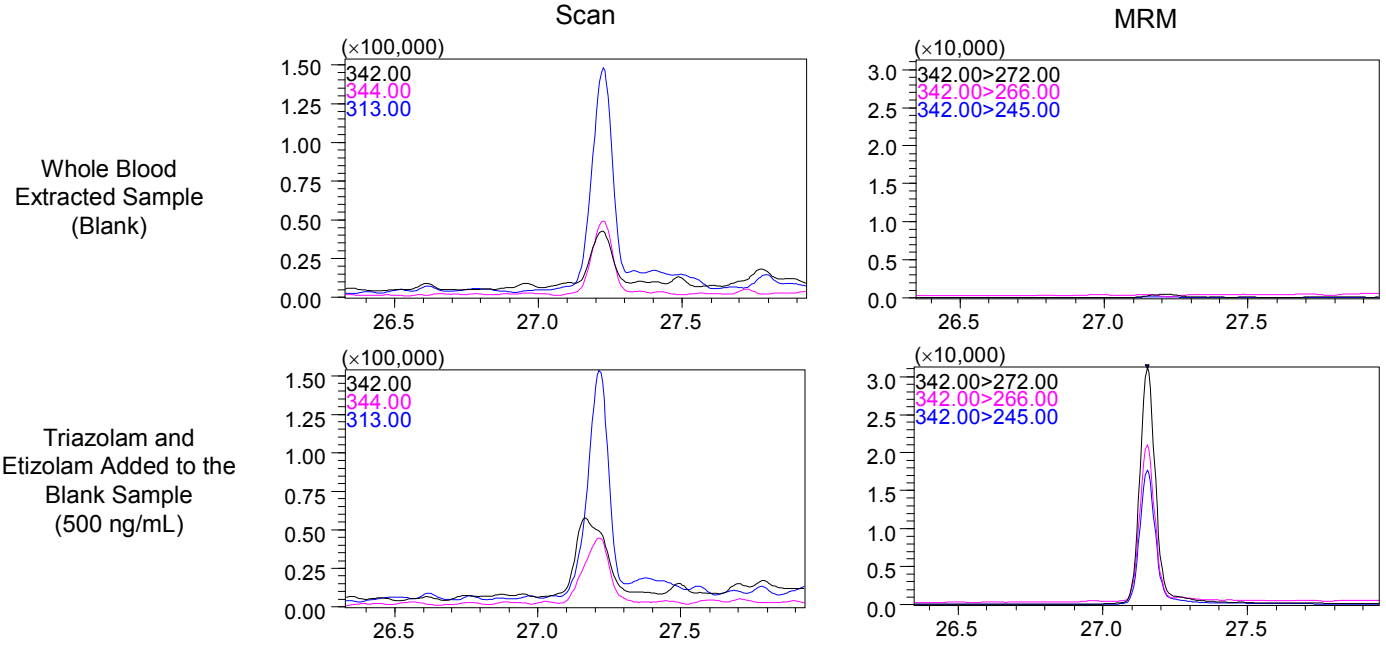


Fig. 2 Scan and MRM Mass Chromatograms of Etizolam in a Whole Blood Sample
(Left: Scan; Right: MRM; Top: Whole blood extracted sample (Blank); Bottom: Sampled created by adding triazolam and etizolam to the blank sample (500 ng/mL))

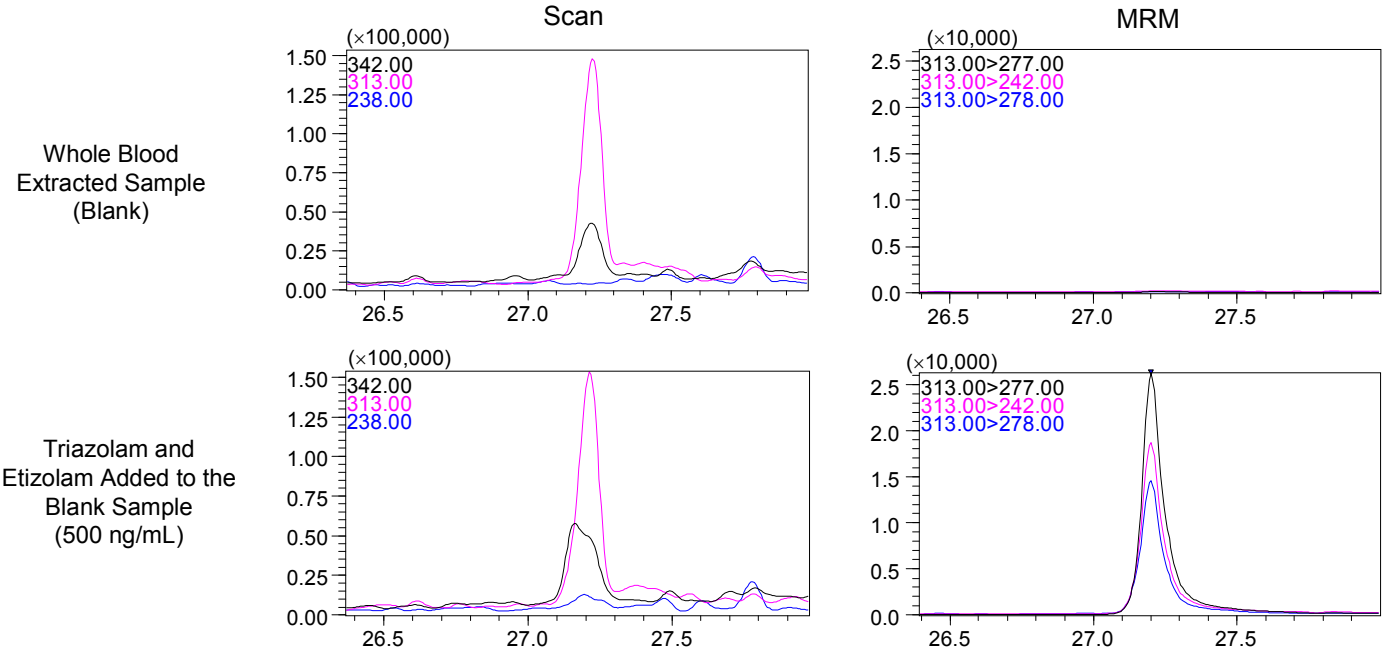


Fig. 3 Scan and MRM Mass Chromatograms of Triazolam in a Whole Blood Sample
(Left: Scan; Right: MRM; Top: Whole blood extracted sample (Blank); Bottom: Sampled created by adding triazolam and etizolam to the blank sample (500 ng/mL)

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