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Determination of Triazolam by AccuTOF™ GC/Time-of-Flight Mass Spectrometry

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Introduction

Triazolam is a benzodiazepine with a very short elimination half-life. The range is reported to be 1.5 to 5.5 hours[1]. Due to its frequent use as a sedative and its potential to affect human activities such as driving, an unambiguous and sensitive analysis method is needed for its identification and quantitation. Generally, for determination of triazolam, screen tests are performed in biological samples followed by confirmation and quantitation with GC/MS [2]. Here, we demonstrate the feasibility by using the JEOL AccuTOF™ GC, time-of-flight mass spectrometer with accurate mass measurement and negative ion chemical ionization (NCI) capabilities for triazolam determination. D_4-triazolam was used as internal standard. The mass accuracy without internal reference is smaller than 2 mmu. The limit of detection is 5 ng/mL. The quantitation standard curve can be linear from 5 ng/mL to 1000 ng/mL with R^2 of 0.9992. To the best of our knowledge, this is the first time that triazolam has been detected by GC/time-of-flight mass spectrometry with accurate mass measurement.

Experimental

1. Solvents and standards

All solvents used were of HPLC grade. Triazolam and d_4-triazolam standard solutions were purchased from Cerilliant (Round Rock, TX). A series of triazolam standard solutions, with concentrations from 5 ng/mL to 1000 ng/mL, was prepared in methanol. A stock solution of d_4-triazolam with 100 ng/mL was also prepared in methanol.

2. Sample preparation

An aliquot of 100 µL of sample was transferred into a small silanized glass tube, and then 100 µL of the internal standard was added. The solvent was evaporated under a gentle stream of nitrogen gas. Fifty microliters of ethyl acetate followed by 50 µL BSA/TMCS (5/1) were added to the tube. All tubes were heated at 80 °C for 30 min. The liquid was transferred to an autosampler vial for injection.

3. GC/MS analysis

The system included a JEOL AccuTOF™ GC time-of-flight mass spectrometry system set at NCI mode and an Agilent 6890 N GC. The system was controlled by a JEOL MassCenter™ workstation. The GC column was a DB5-MS capillary column (30 m, 0.25 mm i.d., 0.25µm). The initial oven temperature of 60 °C was held for 1

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min, and then increased to 325 °C at the rate of 25 °C/min and held at the final temperature for 3 min. The carrier gas was helium with a constant flow rate of 1 mL/min. The temperatures for injection port, transfer line and ion source were 275 °C, 250 °C, and 200 °C, respectively. The reagent gas was methane with a flow rate of 0.83 mL/min. The ionizing voltage and current were 200 V and 300 µA, respectively. The MCP detector was set at 2,500 V. One microliter sample was injected onto the column with splitless mode.

Results and Discussion

Triazolam has very short elimination half-time. It is metabolized via hepatic microsomal oxidation. The hydroxylated metabolites, which are inactive, are excreted primarily in the urine as conjugated glucuronides. The level of parent drug in the biological fluid is usually very low after a few hours administration. It is generally known that negative-ion chemical ionization (NCI) provides very high sensitivity for analyzing compounds containing halogen atoms. Triazolam contains 2 chlorine atoms, making it possible to obtain very high sensitivity under NCI detection. Treatment with BSA/TMCS (5/1) improved the peak shapes of triazolam. Theoretically, only its hydroxylated metabolites – not the drug itself - should form TMS derivatives. However, TMS may enhance the chromatography for triazolam by either associating with the drug or by deactivating the GC column [3]. We first tested the detection limit under the current experimental conditions. An average signal-to-noise ratio of 70 was achieved when 5 ng/mL triazolam was injected. The error for mass accuracy is less than 2 mmu. Bigger than 3 mmu mass accuracy error was obtained if lower than 5 ng/mL samples were injected. Figure1 shows the high-resolution mass chromatogram (Δm = 0.01) and mass spectrum for m/z 306, $[M\text{-}Cl\text{-}H]^-$, in the standard solution with 5 ng/mL triazolam and 100 ng/mL internal standard.

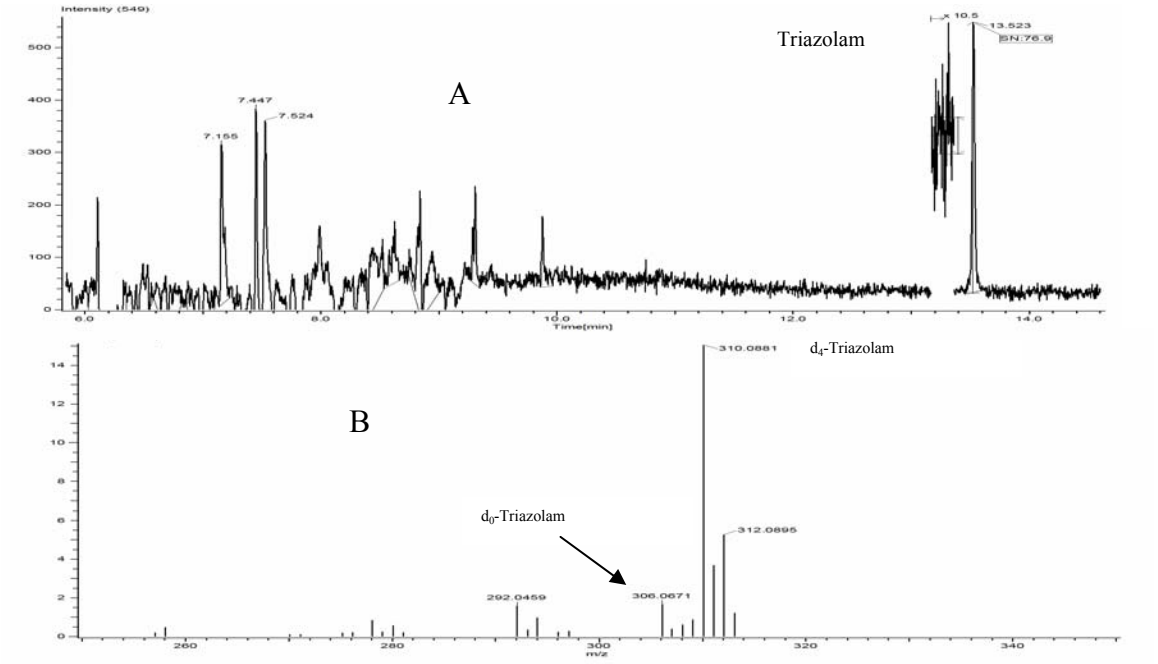

Fig.1 (A) Mass chromatogram of triazolam (m +/- 0.01) with concentration of 5 ng/mL. (B) Mass spectrum of traizolam with concentration of 5 ng/mL

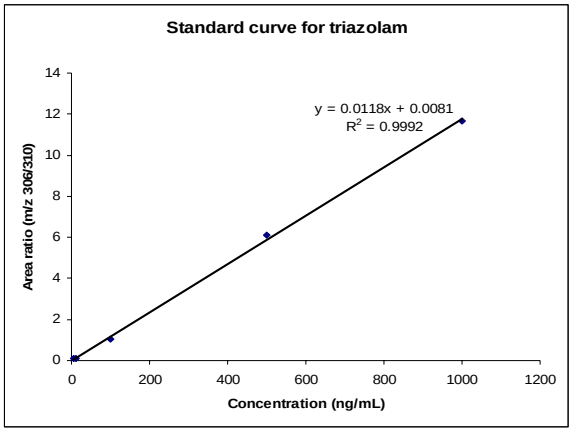

Fig. 2 Standard curve for triazolam from 5 ng/mL to 1000 ng/mL with d4-triazolam as the internal standard.

Concentration (ng/mL)	Precision % CV	Mass Accuracy (mmu)
5	16.2	1.44
10	3.2	1.12

Table 1. Precision and mass accuracy for two different concentrations of triazolam (n = 5)

Peak-area ratios (d_0/d_4) from high-resolution mass chromatograms were calculated for each standard and plotted against the known concentrations of the standard. Correlation coefficient (R^2) is 0.9992. The standard curve is shown in Figure 2.

The method precision was determined by analyzing two different concentrations of standard solution. The samples were analyzed five times in duplicate. The percent coefficient of variation (CV) is 3.2% for the 10 ng/mL sample and 16.2% for the 5 ng/mL sample. Higher than 20% CV was obtained if samples lower than 5 ng/mL were injected. Therefore, the quantitation limit for the assay is 5 ng/mL. The results are listed in Table 1.

Conclusion

To the best of our knowledge, this is the first time the feasibility of using GC/time-of-flight mass spectrometry to determine triazolam has been evaluated. The accurate mass measurement capability of a high-resolution time-of-flight mass spectrometer makes the determination unambiguous even in very low concentrations. The method was sensitive, precise and simple. In order to apply this method for biological samples, additional method development including sample extraction and validation may be required.

References

1. http://www.mentalhealth.com/drug/p30-h01.html
2. Hold, KM, Forensic Science International, 84(1997), 201-209
3. Hold, KM, J. Mass Spectrometry, 31(1996), 1033-1038