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Structural analysis of quazepam metabolites in bile by ion trap time-of-flight mass spectrometry

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ABSTRACT

Quazepam (QZP) is a long-acting benzodiazepine-type hypnotic. We searched for novel QZP metabolites in bile and determined their structures by liquid chromatography-ion trap time-of-flight mass spectrometry (LC-IT-TOF MS). The metabolites were extracted with ethyl acetate after β-glucuronidase treatment. First, a single MS spectrum was acquired. Second, MS^n spectra were acquired for peaks that consisted of ions with the isotope pattern corresponding to molecules bearing one chlorine atom. The novel QZP metabolites found in this study were hydroxyquazepam, hydroxy-methoxyquazepam, hydroxy-oxoquazepam, and hydroxy-methoxy-oxoquazepam, which have the hydroxy and methoxy groups on the fluorophenyl group, and dihydroxy-oxoquazepam and dihydroxy-methoxy-oxoquazepam, which have one hydroxy group at the 3-position of the seven-membered ring and the other hydroxy group and the methoxy group on the fluorophenyl group. We demonstrated that LC-IT-TOF MS was a useful tool for determining the structure of the metabolites. However, the exact locations of the hydroxy and methoxy groups on the fluorophenyl group could not be identified.

1. Introduction

In drug analysis, the target analyte is usually the parent drug or one of its major metabolites. The concentrations of these substances in biological matrices, such as blood, urine, and bile, are usually higher than other metabolites. Moreover, a metabolite that has a long elimination time can be a valuable analyte in forensic toxicology, even if it is derived from only a small fraction of the administered drug, because it can offer a long detection window that makes it possible to prove the usage of a certain drug long after the parent drug has been eliminated. Ethyl glucuronide and ethyl sulfate have attracted much attention recently because they offer longer detection windows than ethanol [1–3]. However, these kinds of valuable metabolites have not been discovered for most other drugs. Therefore, metabolites such as these need to be identified and studied to create new possibilities for drug analysis.

Quazepam (QZP, Fig. 1) is a long-acting, trifluorinated benzodiazepine, marketed under the brand name Doral in Japan. QZP is first metabolized by substitution of oxygen for sulfur to give oxoquazepam (OQ), which then transforms along two main pathways (Fig. 1): (a) hydroxylation to give 3-hydroxy-oxoquazepam (HOQ); and (b) *N*-dealkylation to form *N*-desalkyl-oxoquazepam (DOQ), which is further hydroxylated to 3-hydroxy-*N*-desalkyl-2-oxoquazepam (HDOQ) [4]. We previously investigated the concentrations of QZP and its four metabolites in blood, urine, and bile obtained from a man who had been prescribed QZP approximately 3 weeks before his death [5]. We found that HOQ selectively accumulated in bile (56.2 μg/mL). This result indicates that HOQ can remain in bile longer than QZP or any of its other metabolites and offers a long detection window. This result inspired us to investigate other QZP metabolites in the bile sample.

Liquid chromatography-ion trap time-of-flight mass spectrometry (LC-IT-TOF MS) is thought to be one of the best methods for elucidating structures of unknown metabolites of drugs in biological matrices. This method can identify the formula of a metabolite from single MS. Furthermore, the fragments obtained from MS^2 can provide information about the partial structure of the metabolite. These fragments can be further fragmented into smaller fragments by MS^n ($n \geq 3$), which in turn can offer more information about the structures. Assuming that the structures of unknown metabolites are only partially different from that of the parent drug, they can be determined by comparison of MS^n. We conducted LC-IT-TOF MS on the bile samples to search for novel QZP metabolites.

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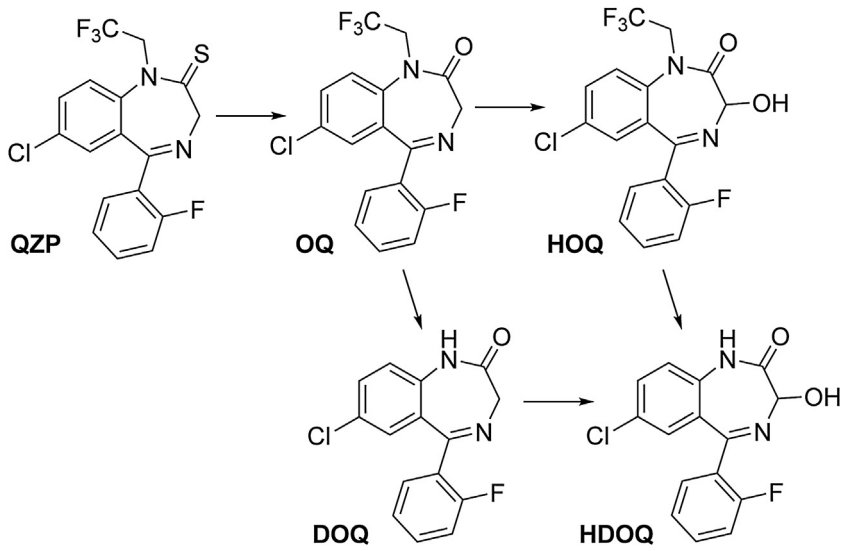

Fig. 1. Metabolic pathway of quazepam (QZP) (OQ, oxoquazepam; HOQ, 3-hydroxy-oxoquazepam; DOQ, *N*-desalkyl-oxoquazepam; HDOQ, 3-hydroxy-*N*-desalkyl-2-oxoquazepam).

2. Case history

The details of the case history are described previously [5]. The deceased man was in his early sixties. Approximately 3 weeks before his death, he was prescribed central nervous system drugs, including QZP at a dose of one 20-mg tablet daily. Blood concentrations of all prescribed drugs were within therapeutic levels. The heart blood concentration of QZP was 19.3 ± 0.8 ng/mL.

3. Materials and methods

3.1. Reagents

Water was purified using a Milli-Q water purification system (Millipore, Billerica, MA). β-Glucuronidase (type HP-2, from Helix pomatia, 152,900 U/mL β-glucuronidase, 714 U/mL sulfatase) was purchased from Sigma-Aldrich (St. Louis, MO). Sep-Pak Vac® PSA (1 mL, 50 mg) cartridges were purchased from Waters (Milford,

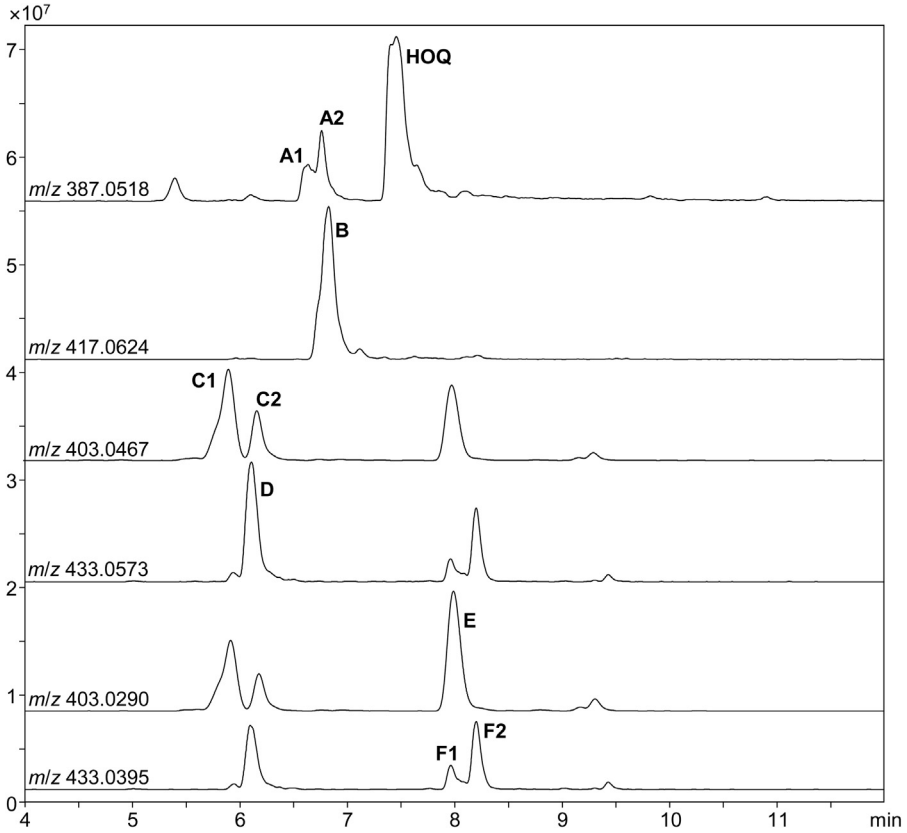

Fig. 2. Mass chromatograms from single MS in positive mode.