K1 Urinary Excretion of α-Hydroxytriazolam Following a Single Oral Dose of Halcion®

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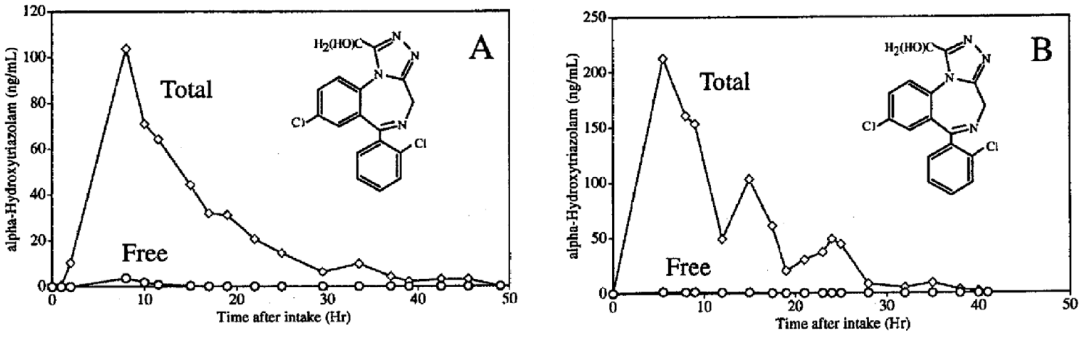
The goals of this presentation are to establish an effective procedure for analysis of a-hydroxytriazolam and to characterize human urinary excretion following of this compound following a single oral dose of triazolam.

Triazolam is a very short-acting triazolobenzodiazepine with sedative hypnotic properties. Urinary excretion following an oral dose of this drug includes approximately 2% parent compound and 70% α-hydroxytriazolam glucuronide [1]. Approved for medicinal use in Taiwan, it is also controlled at the same level (Level III) as Flunitrazepam. Alleged misuses *of* this substance have been associated with case specimens submitted to this laboratory.

In this study, urine specimens were screened by TDx® followed by sample preparation (without and with enzymatic hydrolysis) and GC-MS protocols for quantitative determination *of* free and total α-hydroxytriazolam. Enzymatic hydrolysis was carried out by mixing the specimen with *Helix pomatia* β-glucuronidase for 2 hr at 56°C. The mixture was then adjusted to pH 9.5, extracted with ethyl acetate, dried, and derivatized using MSTFA. Deuterated α−hydroxytriazolam was used as the internal standard. Confirmation test was carried out using a HP 5973N GC-MSD equipped with a 30-m HP 5MS fused silica capillary column under the following conditions: Injector and interface temperature 260°C and 280°C, respectively; column oven temperature initiated at 150°C for 1 min, then programmed to 300°C at 20°C/min, and held at the final temperature for 6.50 min. Data acquisition included full-scan 50-500 amu) and selected-ion-monitoring *of* the following ions: *m/z* 415, 417, and 430 for α-hydroxyttiazolam; *m/z* 419, 421, and 434 for a-hydroxytriazolam-d_4-. Standard criteria were used to confirm the presence *of* the analyte prior to its quantitation.

The overall protocol achieved the following results when applied to the analysis of 2-mL drug-free urine specimens fortified with 10-200 ng/mL a-hydroxytriazolam: Recovery, 95%; interday and intraday precision ranges, 1.50-3.52% and 0.93-4.71%, respectively; linearity, $r^2 > 0.99$; limits of detection and quantitation, 0.05 and 0.1 ng/mL, respectively.

This protocol was applied to the analysis *of* urine samples collected from two volunteers (A and 13) taking one oral dose of Halcion® (0.25 mg triazolam). Excretion profiles *of* free and total α-hydroxytriazolam are shown in Figure 1. Free α-hydroxytriazolam is detectable, but at very low levels (<5 ng/mL). Peak excretion of total α-hydroxytriazolam occurs at approximately 5-10 hr following the drug intake. Total α-hydroxytriazolam is excreted at detectable levels approximately 2-35 hr following an oral dose *of* 0.25 mg triazolam. Total free and conjugated α-hydroxytriazolam excreted by A and B are 0.61% and 31.6%; and 0.36% and 57.2% *of* the dose, respectively.



Urinary excretion profiles of two volunteers taking one oral dose of Halcion® (0.25 mg triazolam).

Fraser AD, Bryan W, Isner AF: Urinary screening for a-OH triazolam by FPIA and EIA with confirmation by GOMS; *J Anal Toxicol* 16:347-350! 1992.

Halcion®, α-Hydroxytriazolam, Drug Excretion

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