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Letter to the Editor

High-performance liquid chromatographic assay of adinazolam and its desmethyl metabolite

Sir,

Adinazolam is a triazolobenzodiazepine derivative currently under investigation as an antidepressant agent [1]. Biotransformation in humans proceeds by N-demethylation, yielding desmethyladinazolam as the major metabolite. This metabolic product has pharmacologic activity [2] and is present in human plasma in concentrations higher than those of the parent drug [3]. Attempts to replicate a previously reported high-performance liquid chromatographic (HPLC) assay for adinazolam and its metabolite [3] were complicated by incomplete chromatographic separation of the internal standard from the compounds to be quantitated. The present report described a modified HPLC assay which overcomes the above problem and also is more rapid and straightforward.

EXPERIMENTAL

Adinazolam mesylate, desmethyladinazolam, and the internal standard (U-31485, I.S.) [4] were kindly provided by the Upjohn Company (Kalamazoo, MI, U.S.A.). Stock solutions of 50–100 μg/ml of each compound (as the free base) were prepared in benzene or toluene, with subsequent dilution to 1 μg/ml. Working solutions of adinazolam and desmethyladinazolam were prepared in methanol, while that of the I.S. was prepared in benzene. To a series of round-bottom culture tubes were added 300 ng of the internal standard. Calibration tubes contained variable amounts of adinazolam and desmethyladinazolam ranging from 25 to 200 ng. Tubes for quantitation of actual biological samples contained only the internal standard. The organic solvents were evaporated to dryness at 40°C under conditions of mild vacuum. Drug-free control plasma (0.5 ml) was added to calibration tubes, together with 0.5 ml of distilled water. A 1-ml aliquot of unknown samples was added to all other tubes. To each tube was added 1.5 ml of benzene–isoamyl alcohol (98.5:1.5), and the tubes were agitated in the upright position on a vortex mixer for 30–60 s. After centrifugation, the organic phase was separated into clean conical centrifuge tubes and evaporated to dryness at 40°C under mild vacuum. The evaporated residue was reconstituted with 200 μl

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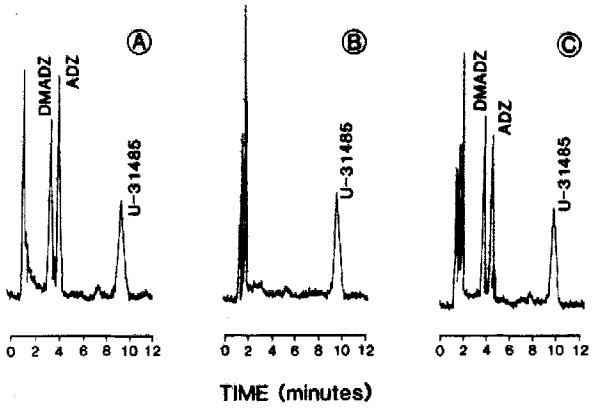

Fig. 1. (A) Extract of a calibration standard containing 200 ng/ml each of adinazolam (ADZ) and desmethyladinazolam (DMADZ), and 300 ng/ml of the internal standard (U-31485). (B) Blank patient sample (internal standard added). (C) Extract of a plasma sample from a patient following ingestion of adinazolam (internal standard added).

of mobile phase and transferred to autoinject microvials, of which 65 μl were injected onto the chromatograph using an automatic injector.

A 0.06 *M* $NH_4H_2PO_4$ buffer was prepared by addition of 0.2 ml of phosphoric acid to 6.9 g of ammonium dihydrogen phosphate; the volume was made up to 1 l with distilled water. Of this buffer 340 ml were mixed with 130 ml of acetonitrile and 60 ml of methanol to form the HPLC mobile phase (organic and aqueous components were separately filtered and degassed prior to mixing). The HPLC was a Waters Assoc. instrument [5–8] consisting of a solvent delivery system, a variable-wavelength UV detector (229 nm; 0.02 a.u.f.s.), data module, and WISP automatic injector. The column was a μBondapak C_{18} cartridge housed in a Z-Module; the mobile phase flow-rate was 3 ml/min. All analyses were performed at room temperature.

The applicability of the method to human pharmacokinetic studies was demonstrated by measurement of multiple plasma concentrations of adinazolam and desmethyladinazolam following a single 30-mg dose of adinazolam in a healthy volunteer.

RESULTS AND DISCUSSION

Under the described chromatographic conditions the compounds of interest gave three well resolved chromatographic peaks (Fig. 1). The relation of peak-height ratio versus plasma concentration was linear for each drug. Blank plasma samples were consistently free of endogenous contaminants. Coefficients of variation for identical samples ($n=6$ at each concentration) were evaluated at plasma concentrations of 25, 50, 75, 100, 150 and 200 ng/ml. For adinazolam, values were: 2.4, 4.7, 4.2, 4.1, 1.4 and 1.4%. For desmethyladinazolam, values were: 6.6, 3.5, 4.5, 7.2, 3.9 and 3.1%. Extraction of all three compounds from plasma was 80% or greater. Plasma concentrations of adinazolam and its metabolite following a single dose of adinazolam are shown in Fig. 2.

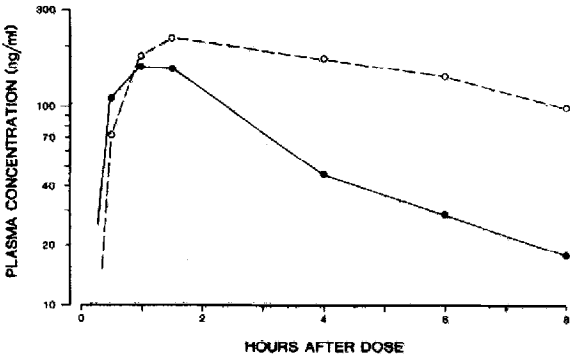

Fig. 2. Plasma concentrations of adinazolam (●) and desmethyladinazolam (○) in a healthy volunteer following ingestion of 30 mg of adinazolam. (Data presented with kind permission of Dr. V. Markku I. Linnoila.)

The present HPLC method modifies a previously described technique [3] by using a different internal standard, as well as a single plasma extraction at physiologic pH using benzene–isoamyl alcohol. This modified method provides consistently high recovery of adinazolam, desmethyladinazolam and the internal standard, and is sensitive enough for single- or multiple-dose pharmacokinetic studies in humans. The straightforward sample preparation procedure, together with the capacity for automated sample injection (which can proceed overnight), allows up to 100 biological samples to be analyzed by one person in a single working day.

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