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Measurement of azacyclonol in urine and serum of humans following terfenadine (Seldane) administration using gas chromatography–mass spectrometry☆

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ABSTRACT

A gas chromatographic–mass spectrometric (GC–MS) method is presented for the analysis of azacyclonol (AZA), a metabolite of terfenadine in serum and urine specimens. Following an alkaline extraction, AZA and an internal standard were derivatized using heptafluorobutyric anhydride. Fourier transform infrared spectrometry suggested that two sites on the AZA molecule were derivatized. GC–MS of the extracts had a limit of quantitation (LOQ) of 1 ng/ml and linear range of 1–1000 ng/ml in urine. Four volunteers were administered a therapeutic regimen of terfenadine followed by urine and serum specimen collection(s) during the next seven days. The results indicated that following a 60-mg dose of terfenadine each 12 h for five days, (1) AZA appears in urine within 2 h, (2) urine AZA concentrations were above the LOQ 72 h following the last dose, (3) peak urine concentrations were as high as 19 000 ng/ml, and (4) mean serum concentration following the ninth dose was 59 ng/ml.

INTRODUCTION

Terfenadine (Seldane, T) is an antihistaminic drug which is a selective histamine H1-receptor blocker that is essentially devoid of central nervous system depressant activity [1]. Its lack of sedative properties makes it a popular antihistamine prescribed to aviators. The challenge for a toxicology laboratory is detecting its presence without prior knowledge of administration.

T undergoes rapid and extensive (99.5%) biotransformation in man into two main metabolites: a carboxylic acid metabolite with some antihistaminic activity and a piperidine-carbinol metabolite devoid of antihistaminic activity, azacyclonol (α,α-diphenyl-4-piperdinemethanol, AZA) [1]. The structures of T and AZA are provided in Fig. 1.

In most routine gas chromatographic (GC) basic drug screening procedures performed by toxicology laboratories, neither T nor its carboxylic acid metabolite would be detected. Previous studies have detected T and the carboxylic acid metabolite by radioimmunoassay and high-performance liquid chromatography [1–6]. Another study has reported using computerized gas chromatography–mass spectrometry (GC–MS) as a screening method for T and two metabolites following a hydrolysis step [7]. Most of these procedures are generally not applicable to routine basic drug screening.

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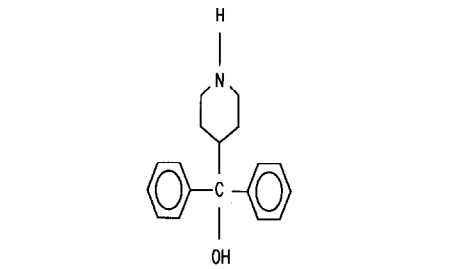
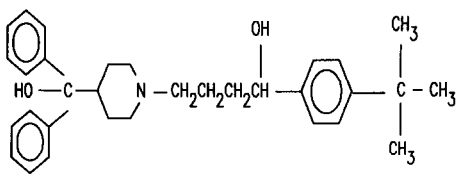


Azacyclonol



Terfenadine

Fig. 1. Structures of terfenadine and azacyclonol.

Our laboratory provides toxicological support in the investigation of aviation mishaps and accidents. We use the detection of the AZA metabolite by nitrogen–phosphorus GC as an indication of T use. This study consisted of two parts: (1) the development of a sensitive GC–MS assay for the quantitation of AZA and (2) measurement of AZA concentrations in urine and serum specimens from individuals receiving therapeutic dosages of T.

EXPERIMENTAL

Materials

AZA was obtained from Merrill Dow Pharmaceuticals (Cincinnati, OH, USA) and a 100 mg/l methanolic solution was prepared. The internal standard (I.S.), 2,2-dimethyl-3-(1,2-diphenylethylamino)-1-propanol, was purchased from Aldrich (Milwaukee, WI, USA) and a 100 mg/l methanolic solution was prepared. Toxitube A extraction tubes were obtained from Marion Analytical (Kansas City, MO, USA). The derivatizing agent, heptafluorobutyric anhydride (HFBA), was acquired from Sigma (St. Louis, MO, USA). Solvents were pesticide grade.

Extraction

AZA was extracted and identified by a previously published method [8]. The relative retention time of AZA to the SKF-525 (Smith Kline & French, Philadelphia, PA, USA) internal standard was measured at 0.961 (±0.003) using a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA, USA) equipped with a nitrogen–phosphorus detector and a J&W (Folsom, CA, USA) DB-5 column (15 m × 0.25 mm I.D., 0.25 μm film thickness). To quantify AZA, 5 ml of standard or clinical specimen and 15 μl of I.S. were added to a Toxitube A and mechanically rotated for 20 min. After centrifugation, the organic layer was evaporated to dryness. The residue was reconstituted in 100 μl of HFBA, covered and heated at 70°C for 20 min. Excess derivatizing agent was removed by evaporating the mixture to dryness, and the residue was reconstituted in 40 μl 1-chlorobutane and chromatographed.

Instrumentation

A Hewlett-Packard 5890 gas chromatograph and 5970 mass-selective detector were used for AZA analysis. Helium was the carrier gas flowing at 1.5 ml/min. The injector temperature was 260°C and the transfer line temperature was 280°C. A J&W DB-5 (15 m × 0.25 mm I.D., 0.25 μm film thickness) column provided analytical separation. The oven temperature began at 200°C, ramping at 15°C/min to 260°C. The mass spectrometer was operated in the selected-ion mode with the following ions being monitored: *m/z* 205, 232, and 405 for AZA and *m/z* 388 for the I.S. The quantitating ratio was 405/388; 205/405 and 232/405 ratios served as qualifiers for identification.

Infrared spectra of AZA and derivatized AZA were obtained using a Bio-Rad (Cambridge, MA, USA) 60A Tracer gas chromatograph–Fourier transform infrared (FT-IR) interferometer. The samples were introduced into the GC–FT-IR system via a Hewlett-Packard 5890 gas chromatograph equipped with a J&W DB-5 column (15 m × 0.25 mm I.D., 0.25 μm film thickness). The temperature program was 200°C to 260°C at 15°C/min. Helium was used as the carrier gas and

the split ratio was 30:1. Delivery from the gas chromatograph to the interferometer was through a transfer line made of fused silica and maintained at a temperature of 250°C. The samples were cryogenicly deposited from the transfer line onto a ZnSe plate held at liquid nitrogen temperatures. The plate was translated as the GC effluents were deposited, and the spectra were obtained on-line as the ZnSe plate translated into the infrared beam. Schwartzchild microscope optics were used for detector collection.

Human drug administration

Four healthy male volunteers (ages 30–43 years) participated in this study. The investigation protocol was approved by the Armed Forces Institute of Pathology Human Subjects Committee, and each participant gave written informed consent before taking part. Prior to the beginning of the study, a urine specimen from each volunteer was collected. Each subject was then given Seldane, 60 mg every 12 h up to a total of nine doses. Urine specimens were collected at each voiding for three days and then, first morning voidings until day 8. Blood (10 ml) was drawn from each volunteer 2 h after the ninth dose. Urine and serum specimens were refrigerated after collection.

RESULTS AND DISCUSSION

Although AZA can be detected and quantitated using a routine alkaline drug extraction and GC without derivatization, the sensitivity and chromatography were unacceptable for quantitating low-dose studies. Therefore, a method was developed using HFBA as the derivatizing reagent. This compound reacts with alcohols and primary and secondary amines to form amides and esters with improved chromatographic characteristics. AZA has a secondary amine and a tertiary alcohol, thus providing two potential sites of derivatization. The mass spectrum of underivatized and derivatized AZA are shown in Fig. 2 (mass range scanned to 800 a.m.u.). The mass spectrum of derivatized AZA was inconclusive in determining whether both functional groups were derivatized. FT-IR spectra of underivatized and derivatized AZA were obtained (Fig. 3). The IR spectra indicate that the derivatization occurs at both the NH and OH site. The spectrum of the underivatized sample has strong bands indicative of the OH and NH ring moieties in the high-frequency end of the spectrum. The fingerprint region of the spectrum is also consistent with the AZA structure. The derivatized spectrum has very little features in the high-frequency end of the spectrum indicating removal of the OH and NH ring functional groups. The remainder of the spectrum is also consistent with an RCOOR and R_2NCOR moiety. Those bands which do occur in the high-frequency end of the spectrum of the derivatized sample can be attributed to other functionalities in the molecule.

The assay limit of quantitation (LOQ) was determined by analyzing urine standards of AZA at decreasing concentrations until one or more qualifying ion ratios failed. The LOQ was 1 ng/ml. Similarly the linear range was established by analyzing standards at increasing concentrations until either qualifying ion ratio failed or the correlation coefficient of the linear regression line was less than 0.98. The assay was linear from 1 to 1000 ng/ml. Concentrations greater than 1000 ng/ml were measured by making appropriate dilutions and reanalyzing.

Assay specificity was assessed by adding a large number of alkaline extractable drugs and carrying them through the procedure. The following drugs did not interfere with the assay: alprazolam, amitriptyline, amoxapine, amphetamine, brompheniramine, caffeine, chloroquine, chlorpheniramine, chlorpromazine, codeine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, flurazepam, hydroxyzine, imipramine, lidocaine, meperidine, methamphetamine, nicotine, nordiazepam, nortriptyline, oxycodone, phencyclidine, phenylpropanolamine, pseudoephedrine, quinidine, thioridazine and verapamil.

The identification of AZA as a metabolite of T occurred by accident. The Division of Forensic Toxicology provides toxicological support in the investigation of aviation mishaps and accidents.

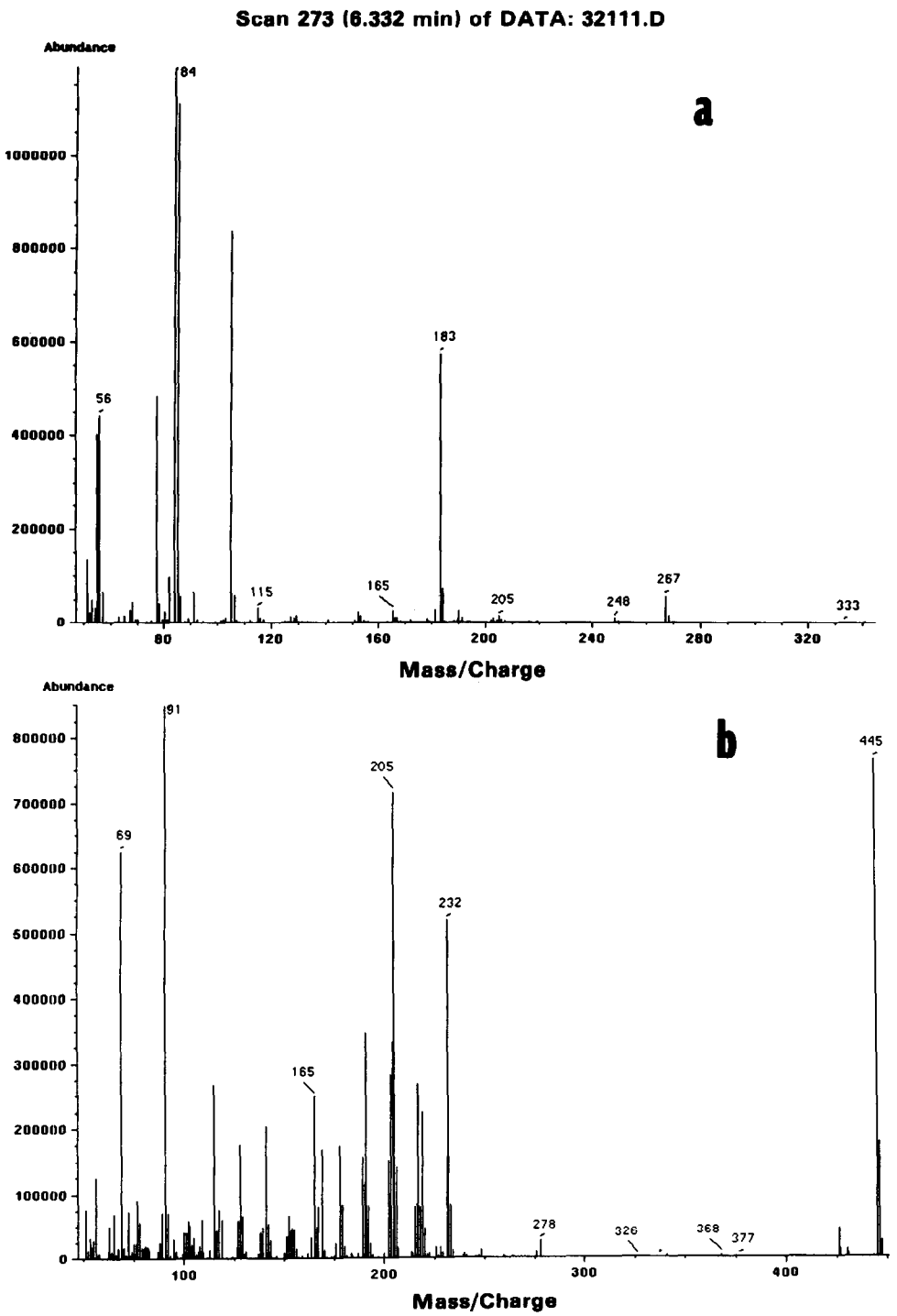


Fig. 2. Mass spectra of (a) underivatized and (b) derivatized azacyclonol.

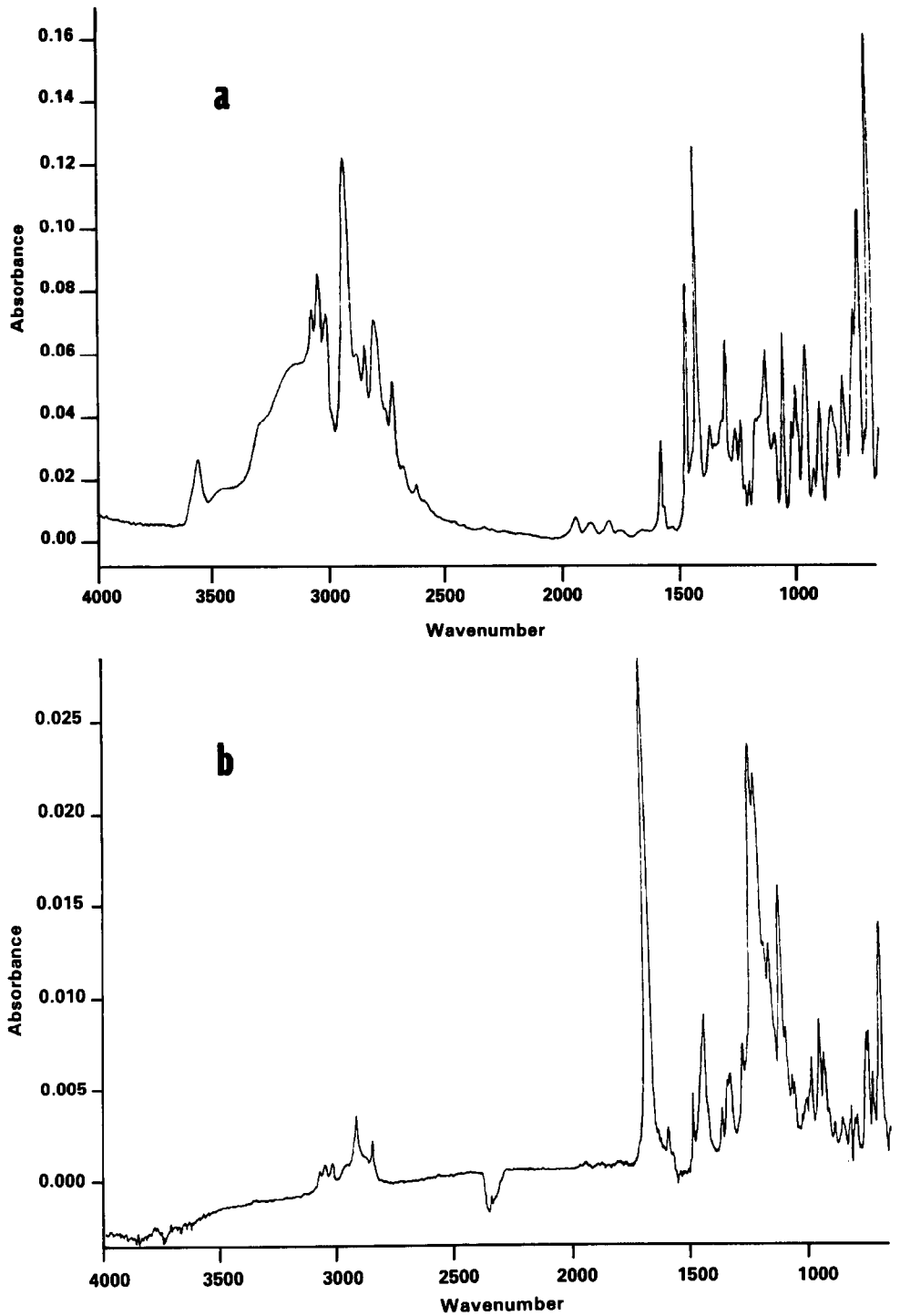


Fig. 3. FT-IR spectra of (a) underivatized and (b) derivatized azacyclonol.

The non-sedative properties of Seldane make it a popular antihistamine used by aviators. After identification of AZA in urines of aviators and consultation with flight surgeons, Seldane was found to be the common characteristic in several cases. This precipitated a literature search of T to provide an explanation for the presence of AZA. It was found that T is hydrolyzed in man, with AZA being one of the metabolic products. This in turn generated the question as to how long and at what concentrations would AZA appear in the urine following a therapeutic regimen of T. To answer this question, a study using human volunteers was undertaken. Urine specimens were collected and quantitated for AZA. Figs. 4 and 5 summarize the data collected following the initial dose and the entire dosing regimen, respectively. From the data, the following conclusions were drawn: (1) AZA appears in the urine within 2 h after the initial dose of terfenadine; (2) AZA was detected in the urine of all individuals 72 h after the final dose, when specimen collection ceased; and (3) concentrations as high as 19 000 ng/ml in urine were measured during the dosing regimen. The serum specimens were quantitated using the same procedure as the urine specimens. The four concentrations found are presented in Table I.

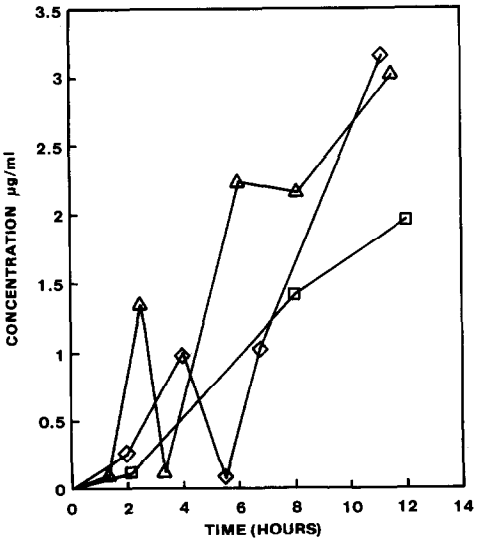


Fig. 4. Urine azacyclonol concentrations in three volunteers following the initial dose. One of the four subjects failed to produce a urine specimen in the first 12 h after dosing.

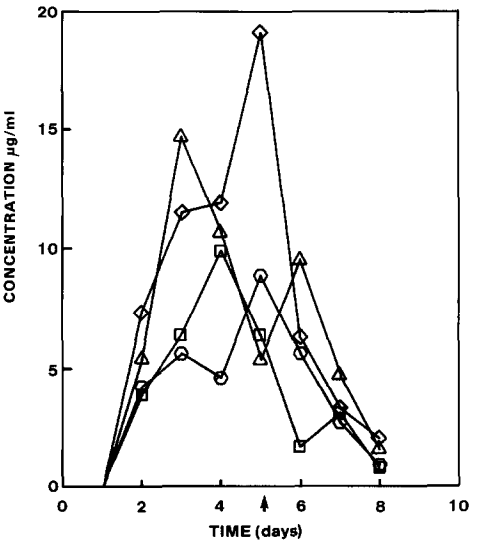


Fig. 5. Urine azacyclonol concentrations in four volunteers throughout the dosing regimen.

TABLE I

SERUM CONCENTRATIONS OF AZACYCLONOL IN FOUR VOLUNTEERS AFTER THE LAST SELDANE DOSE

Subject	Concentration (ng/ml)
A	45
B	67
C	70
D	54
Mean	59 (± 11.6)

The mean serum concentration of 59 ng/ml represents the serum concentration of AZA found at steady state after a therapeutic regimen of T.

In summary, this study indicates that T usage can be easily identified by detection of its metabolite, AZA, in urine and that AZA can be quantitated using a routine GC–MS procedure. It also provides information about the concentrations of AZA that may be expected in urine and serum specimens.

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