The Identification and Quantitation of Ethanol in Blood and Urine from a Fatal Aircraft Accident

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16. Abstract

Triamterene, a diuretic drug used in combination with other drugs for the treatment of hypertension, was found in the blood and urine of a fatal aircraft accident victim. The extraction and identification of triamterene is difficult. It exhibits poor extraction efficiency using some standard base extraction procedures and the parent drug is unsuitable for analysis using gas chromatography. In this case a thin layer chromatography solvent system and high performance liquid chromatography were used to identify and quantitate triamterene in blood and urine. Triamterene is a strong absorber in the ultraviolet region and has an unusual UV spectrum, which simplifies the identification and quantitation of this substance by High Performance Liquid Chromatography.

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INTRODUCTION

The mission of the Federal Aviation Administration (FAA) Civil Aeromedical Institute (CAMI) is to:

- analyze specimens from fatal aircraft accident victims for the presence of therapeutic and subtherapeutic levels of drugs and
- assist the FAA and the National Transportation Safety Board (NTSB) in determining if the drugs (or the associated diseases for which the drugs are given) may be a contributing cause in the accident.

In a recent case, triamterene (Figure 1), a diuretic drug, was identified in urine. The identification and quantitation of triamterene in biological specimens is complicated because the parent drug is unsuitable for analysis by gas chromatography and its presence could be missed in the routine analysis of specimens. An analytic sequence utilizing Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) is presented, which can be used to identify and quantitate triamterene in urine or blood.

In a recent consecutive review of 87 pilots certified to fly with antihypertensives, 35 (40%) had a diuretic in their single or multiple treatment regimens. Of those 35 using diuretics, at least 12 (34%) had been prescribed triamterene. Thus, the ability to search for this specific chemical in the assessment of approved or surreptitiously-initiated medication regimens for hypertension must be maintained in FAA post-accident evaluations.

In this study, an accident victim, a pilot, had previously been prescribed Inderal (propranolol), Vasotec (enalapril maleate), Hytrin (terazosin HCL), and Maxzide (triamterene / hydrochlorothiazide). The pilot's history indicated that Maxzide had been discontinued, and we anticipated that Maxzide, or its components would not be detected in this case.

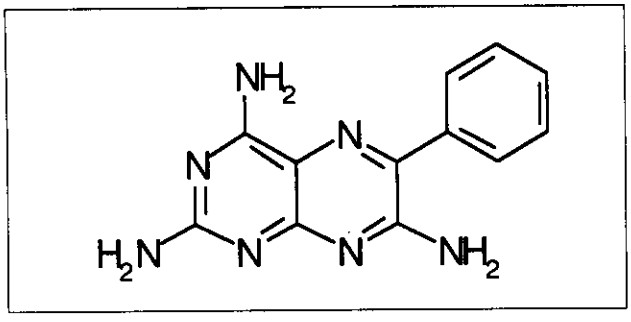

FIGURE 1. The molecular structure of triamterene.

METHOD

Specimens were collected by pathologists near the accident and placed in evidence containers provided by CAMI. These samples were refrigerated and shipped by overnight air. Upon receipt, the specimens were inventoried and accessioned for the analysis of drugs, alcohol, carbon monoxide, and cyanide.

The analysts are not permitted in the accessioning area. All batches submitted for analysis contain at least two blind controls, one negative and one positive. All positive results are confirmed with a second batch run using a different methodology.

Enzyme Multiplied Immunoassay Technique (EMIT), Fluorescence Polarization Immunoassay (FPIA), Gas Chromatography (GC), and Thin Layer Chromatography (TLC) were used to screen the urine for drugs. The urine received for analysis in this case was initially screened using EMIT on a Syva ETS Plus System. In addition, TLC was run using the TOXI-LAB base procedure. Urines screened positive on the EMIT were run by FPIA on the ABBOTT ADx to obtain a semi-quantitative answer and to confirm the initial screen.

An HP 5890 Gas Chromatograph with a 30 meter SPB1 Supelco 0.53mm i.d. column and a nitrogen detector are used to screen for base drugs. The GC has an injection temperature of 250°C detector temperature of 300°C, and the oven is programmed from 110°C to 290°C at 7°C/min.

An internal standard of 2.5 mL of 1.0 ug/mL quinine in water was added to 4 mL of blood or urine prior to the extraction of triamterene, with the standard TOXI-LAB base extraction procedure. Prior to extraction, the blood was precipitated with an equal volume of acetonitrile, centrifuged at 2500 rpm, and the supernatant was transferred into a beaker and the acetonitrile evaporated off, leaving the water component. This remaining water solution containing the drugs of interest was extracted using the standard urine TOXI-LAB base extraction.

A Hewlett Packard 1090 II HPLC with diode array detector was used in the confirmation and quantitation process of triamterene. HPLC was run using the procedure reported by Logan (1). The HPLC peaks were scanned from 190.0nm to 400.0nm using an HP diode array detector. The HPLC oven temperature was set for 35°C. A 25uL sample was injected using an HP autoinjector.

RESULTS

The Syva ETS Plus EMIT gave a weak positive reading using the monoclonal Amphetamine/Methamphetamine assay, and a negative reading using the Amphetamine class assay. The Abbott ADx instrument results were negative for the Amphetamine/Methamphetamine II assay. The base TOXI-LAB had a spot with an Rf value of 0.38. This spot was pale yellow in the first stage, gave no reaction in the second stage, showed a blue fluorescence in the third stage, and was brown in the fourth stage. The retention times of an unknown HPLC peak and the triamterene standard were 6.7 minutes (Figure 2).

The ultraviolet (UV) spectrum (Figure 3) of the unknown peak had absorption maxima of 358.5 nm, 250.5 nm, 214.5 nm, 202.5 nm and a shoulder at 282.0 nm, which is the same as those for the triamterene standard. The procedure had a lower limit of quantitation of 25 ng/mL with an R2 of 0.985 and a lower limit of detection of 10 ng/mL. A triamterene standard could not be chromatographed by GC. Attempts to derivatize the specimen for GC analysis failed. The blood was found to contain a therapeutic drug level of 173 ng/mL of triamterene. The therapeutic range of triamterene is 30 ng/mL to 180 ng/mL in blood. Triamterene was also identified in urine, but not quantitated.

DISCUSSION

Initially, the presence of triamterene was not considered a strong possibility in this case, based on information received from investigators indicating that the drug had been discontinued; the TLC did not match the reference library values for triamterene. It was only after finding an unidentified peak with an unusual UV spectrum (Figure 3) in the HPLC analysis that we considered

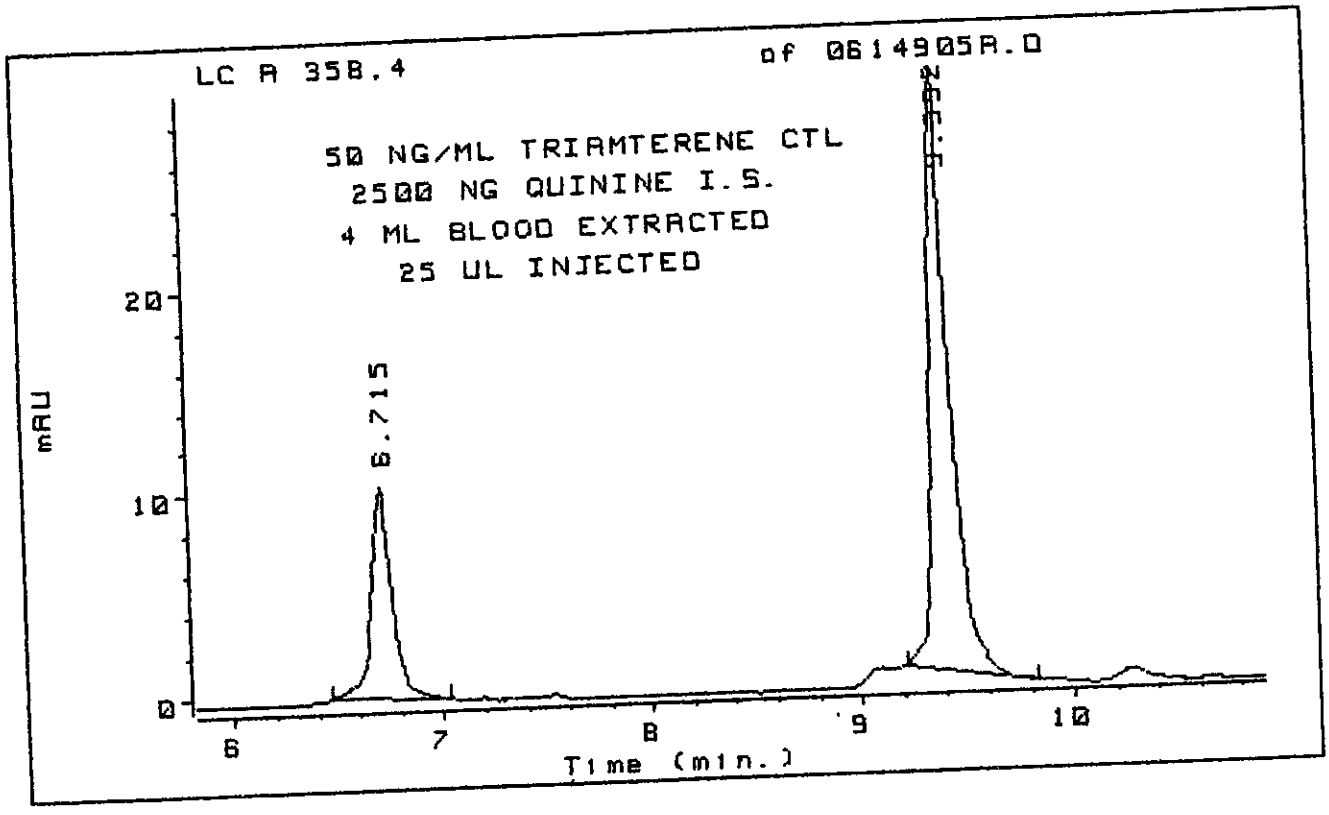

FIGURE 2. The HPLC chromatograph peaks of triamterene at 6.7 mins and quinine (the internal standard) at 9.4 mins.

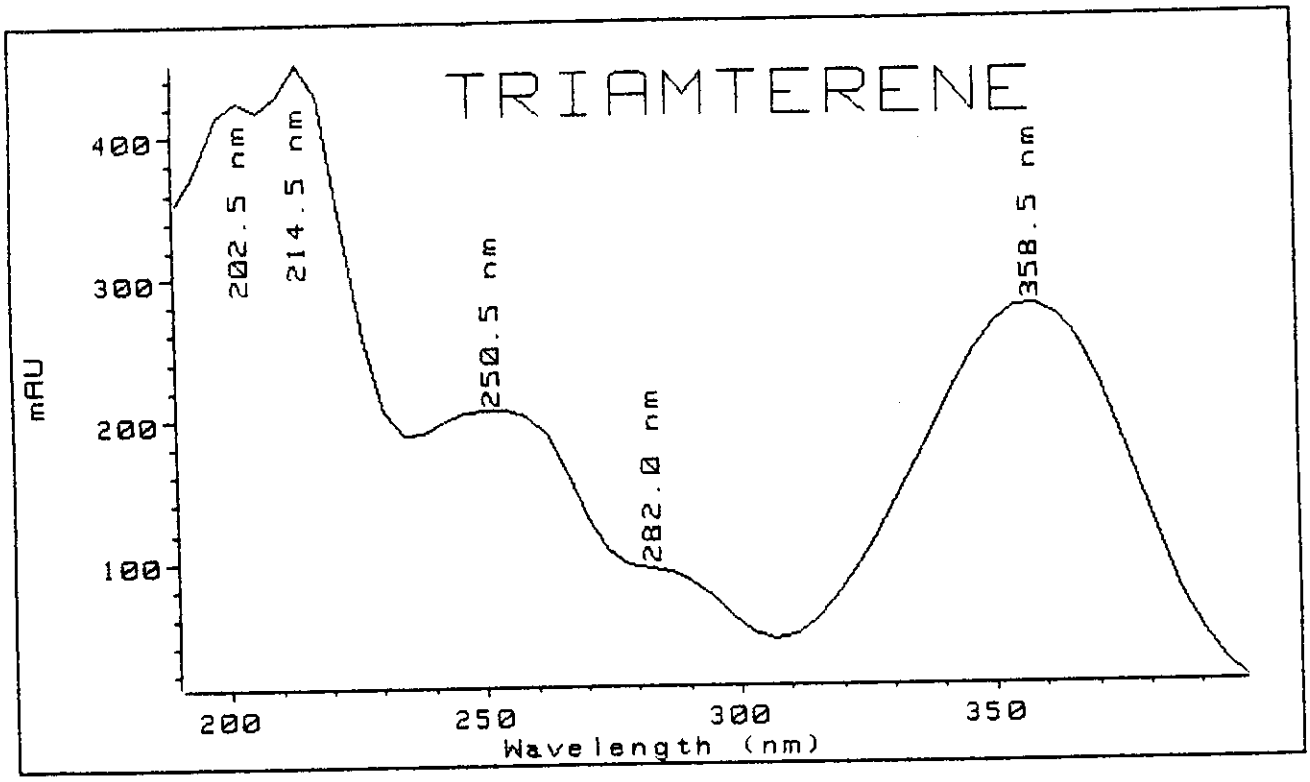

FIGURE 3. The UV spectrum for triamterene run on HPLC using a diode array detector.

triamterene as a possible drug in the specimen. Triamterene was suspected after finding a match between the reference spectrum in *Clark's Isolation and Identification of Drugs* (2) and the unknown UV spectrum found by HPLC. A triamterene standard was obtained from a local pharmacy for comparison. The retention times and complete UV spectra from the triamterene standard and the unknown were identical.

CONCLUSION

Analysis for triamterene can easily be unsuccessful because of unanticipated problems in extraction, detection, and confirmation. It is advantageous to know from prior experiments that the TOXI-LAB base extraction procedure successfully extracts triamterene from urine or blood, whereas several other base extraction procedures have failed to extract this drug. In the present case, the unknown Rf value and visual patterns on the TOXI-LAB sheet were inconsistent with the triamterene values in the TOXI-LAB reference library, which lists the Rf value for triamterene at 0.31 with an additional yellow spot in the second stage. Subsequent tests using a low concentration triamterene standard with TOXI-LAB have shown that triamterene matches the patterns found in our current case. The inability of gas chromatography to be used in the identification of triamterene can be bypassed by using a combination of TLC and HPLC to identify and quantitate triamterene in urine or blood.

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