Case Report

Benztropine Identification and Quantitation in a Suicidal Overdose*

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Abstract

For a human fatality involving suspected overdose with the anticholinergic agent benztropine, GC–MS analysis was utilized for indentification, quantitation, and investigation of metabolism. Organic extracts of blood and urine were analyzed by a capillary-column gas chromatograph interfaced with an ion-trap mass spectrometer, which was programmed for wide-spectrum data acquisition. Electron impact and chemical ionization were used for benztropine detection. The chemical structures of the ion fragments are proposed. Benztropine-d_3 was synthesized and used as an internal standard. Quantitative determinations of benztropine revealed 0.183 mg/L in blood and 7.12 mg/L in urine from the decedent. Drug concentrations were interpreted relative to the case findings, published data, and a limited evaluation of the therapeutic concentrations in psychiatric patients. In addition, the possible metabolic conversion to norbenztropine was investigated by the synthesis of the norbenztropine derivative. Chromatographic evaluation of samples from the case study did not reveal significant bioconversion via the *N*-desmethylation pathway.

Introduction

Benztropine is an anticholinergic agent that has been used for more than 40 years in the treatment of Parkinson's disease (1) and is currently dispensed as the myselate salt under the trade name of Cogentin (Merck & Co., West Point, PA). The drug has anticholinergic activity similar to a number of other medications as well as to several natural herbs and plants (2). The structure (Figure 1) of benztropine, however, combines the benzhydral portion of diphenhydramine and the tropine portion of atropine, thus imparting antihistaminic properties in addition to its more clinically important anticholinergic effects. Benztropine has been used widely in the control of extrapyramidal reactions associated with neuroleptic therapy for the treatment of psychotic and drug-abuse disorders.

Anticholinergic intoxication is a well-documented syndrome that has been reported primarily in anticholinergic abuse for stimulatory and euphorian drug effects (3–5). In reports of benztropine abuse, the anticholinergic intoxication syndrome has been documented in a number of nonfatal cases (6–11). Diagnosis of benztropine intoxication in the clinical setting has been established primarily by history, clinical presentation, and response to physostigmine treatment (9–11), whereas toxicological support of this diagnosis has been reported in only one case (11). Clinical manifestations of toxicity in these cases include peripheral signs of mydriasis, dry mucosa and skin, urinary retention, and tachycardia, as well as a central anticholinergic syndrome including reports of psychosis (6) and coma (7). Fatal cases of benztropine overdose have not been reported, except as a statistical mention (12) or as a personal communication with limited documentation (13). In the postmortem setting, gross and microscopic autopsy findings may be of limited value in identifying a potential benztropine-associated fatality, and antemortem drug findings along with postmortem toxicology may be required for the determination of the cause of death.

We report a fatality associated with benztropine overdose and describe the use of gas chromatography–mass spectrometry (GC–MS) in the detection and quantitation of the drug. This is the first reported case in which benztropine measurements in blood and urine have been utilized in a postmortem investigation. Because of the limited information on benztropine metabolism, an evaluation of its bioconversion via the *N*-desmethylation pathway was also conducted.

Case History

A 41-year-old white male with a history of paranoid schizophrenia and cocaine abuse was found dead in a motel where he was residing. The decedent had been missing for several days before the discovery of his death, and investigation of the scene showed no forcible entry or signs of a struggle. Postmortem examination revealed an obese male with only mild hypertrophy of

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the left myometrium and severe hepatic steatosis. Postmortem blood and urine samples were submitted for toxicological testing along with a recent medication history that included prescriptions for benztropine and haloperidol. Medications found near the decedent in the motel room included three bottles of benztropine with 201 tablets (402 mg) missing.

Materials and Methods

Reagents. Fluorescence polarization immunoassays (FPIAs) were performed with reagents obtained from Abbott Laboratories (North Chicago, IL). Benztropine was obtained from Merck & Co. Benztropine-d_3 and norbenztropine were synthesized in Dr. Jindal's laboratory by treatment of benztropine-d_0 with 2,2,2-trichloroethyl chloroformate (14,15). The completeness of the reaction was monitored by GC analysis, and the crude carbamate was washed with equal volumes of 0.1N aqueous HCl and 0.1N base and then purified by preparative thin-layer chromatography. For synthesis of norbenztropine, the resultant carbamate was treated with zinc dust and acetic acid, as described previously (16). For synthesis of benztropine-d_3, the carbamate was reduced with lithium aluminum deuteride (17) to generate benztropine-d_3 with a 70% yield. The material was then crystalized as benztropine-d_3-HCl from a 1:1 mixture of acetone–hexane.

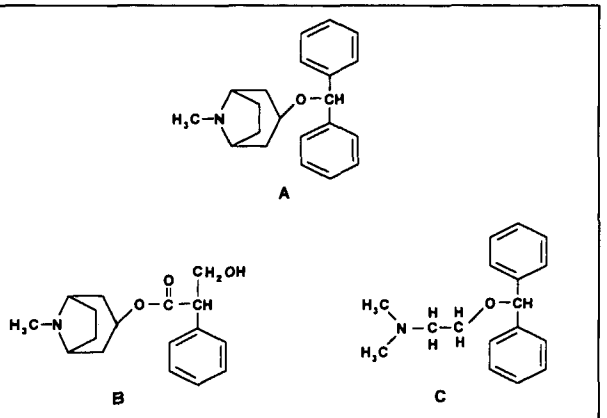

Figure 1. Structures of (A) benztropine, (B) atropine, and (C) diphenhydramine.

Screening procedures. FPIA screening for amphetamine, barbiturates, benzodiazepines, cannabinoids, benzoylecgonine, opiates, phencyclidine, and tricyclic antidepressants was performed on hemolyzed blood using Abbott Laboratories' procedures after an initial methanolic extraction (18). Urine was screened by FPIA using the manufacturer's procedure without modification.

GC–MS extraction procedure. Extraction of benztropine was performed by a previously reported method (16). Briefly, 5 mL of appropriately diluted postmortem urine or 1 mL of patient serum, serum calibrators, or postmortem blood diluted to 5 mL with distilled water was adjusted to pH 9.2 and extracted twice with 10 mL hexane. The organic phases were combined and back extracted with 1 mL of 1N HCl. The aqueous phase was collected and adjusted to pH 9.2 and extracted twice with

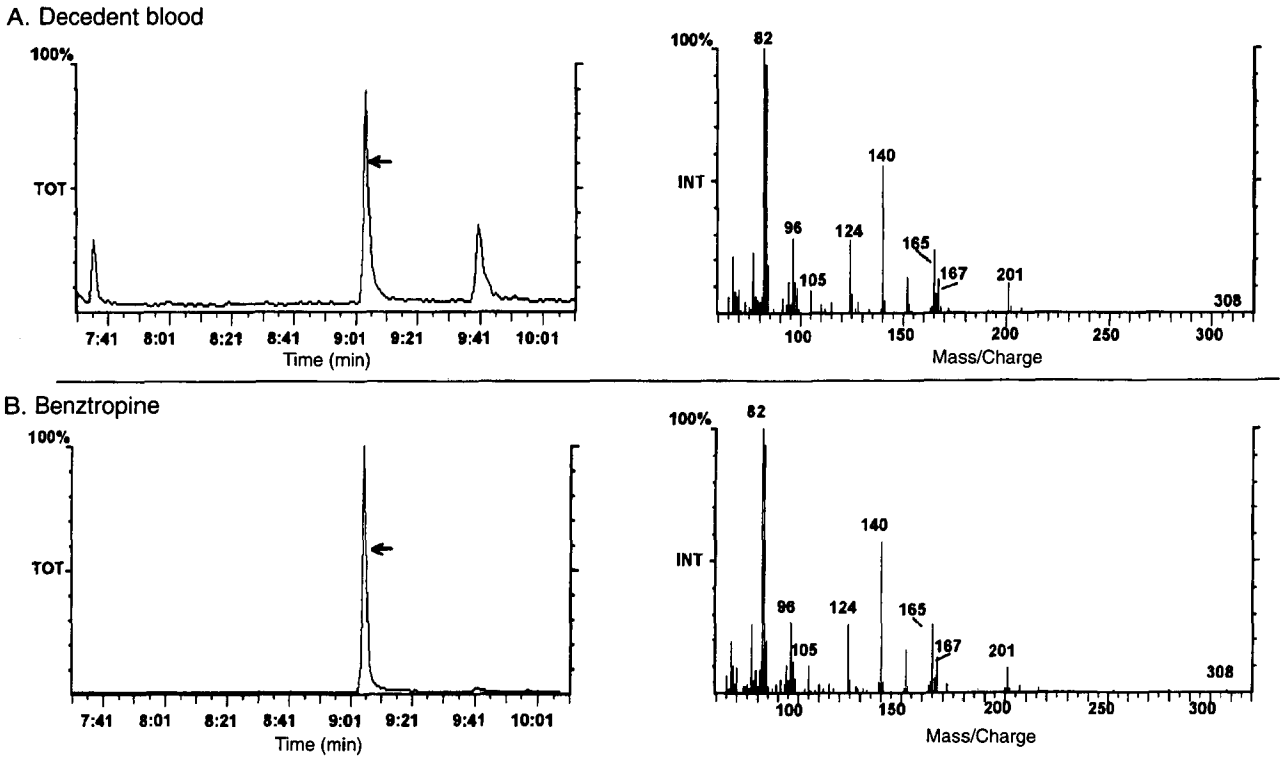

Figure 2. Total ion gas chromatogram and partial mass spectra of benztropine from (A) extract of postmortem blood of decedent and (B) drug-free serum supplemented with benztropine.

5 mL hexane. The organic fractions were combined, evaporated to dryness, and reconstituted with 50 µL ethyl acetate, and 1 µL was injected onto the GC–MS column for chromatographic separation and mass spectral analysis. Recovery of benztropine averaged 79.1% (range, 73–87%), which is in agreement with previously published data (16). For quantitation, 100 µL of benztropine-d_3 (0.10 mg/L) was added to an appropriate dilution of sample prior to the extraction. Calibrators with benztropine concentrations up to 3.00 mg/L were analyzed along with patient and postmortem specimens. Benztropine was quantitated by measuring the ion intensity at *m/z* 140 and 143. Calibration curves based on relative ion intensities were linear up to a concentration of 3.00 mg/L. Negative control urine and postmortem blood showed no significant background ions at *m/z* 140 and 143.

GC–MS instrumentation. Benztropine identification and quantitation were performed with a Perkin Elmer GC–MS system that included a Model 8420 capillary gas chromatograph with an open-split interface to an ion-trap mass spectrometer. A (5% phenyl)-methylpolysiloxane capillary column (DB-5Ms, 15 m × 0.25-mm i.d.) with a film thickness of 0.25 µm (J&W Scientific) was used. Helium was used as the carrier gas with a flow rate of 1 mL/min. A sample injection volume of 1 µL was used in the splitless mode. A column temperature program (125–290°C, 20°C/min) with a final hold of 5 min was used along with an injection temperature of 230°C and an ion source temperature of 275°C. For the electron impact (EI) method of ionization, a mass spectral scan from *m/z* 50 to 400 was employed. Methane was used for chemical ionization (CI) analyses with a scanning range of *m/z* 50–400. Ion-trap control, data acquisition, and analysis were performed with INCOS software (Perkin-Elmer).

Postmortem and therapeutic samples. Postmortem urine and heart blood were obtained at autopsy for the case study. Postmortem blood, as well as urine, was stored at –10°C until analysis. To evaluate therapeutic concentrations, blood was collected from patients receiving daily 4- to 8-mg doses of benztropine for at least a 2-week period. Samples were obtained just prior to the morning dosage, and serums were stored at –10°C until analysis.

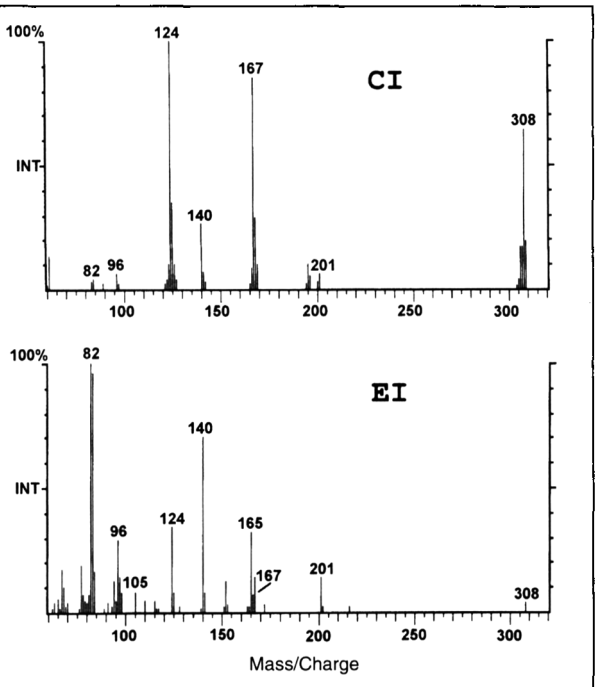

Figure 3. Comparison of chemical ionization (CI) and electron impact (EI) mass spectral analysis of benztropine.

Results and Discussion

The screening of the decedent's postmortem blood by FPIA methods for amphetamines, barbiturates, benzodiazepines, cannabinoids, benzoylecgonine, opiates, phencyclidine, and tricyclic antidepressants was negative. Identification of benztropine in postmortem blood was by GC–MS analysis. Figure 2 shows the total ion chromatogram and partial EI spectra obtained from (A) analysis of an extract from the decedent's postmortem blood as compared with (B) a similar analysis of an extract from drug-free serum that was supplemented with benztropine. On the left are shown the total ion chromatograms, which reveal a major peak (indicated by an arrow) with a retention time of 9.2 min in both analyses. A similar analysis utilizing drug-free serum as a negative control showed no detectable ions in this region (chromatogram not shown). Other chromatographic peaks found in the analysis of the decedent's blood were also found in the negative serum control. On the right side of each panel in Figure 2 is presented the mass spectra for the 9.2-min peak. Both spectra show ion intensities at *m/z* 82 (base peak), 96, 105, 124, 140, 165, 167, 201, and 308. A computer library fit of the mass fragments for the decedent and reference compounds showed greater than 95% concordance. Further confirmation of benztropine presence in the decedent's blood was obtained by CI mass spectral analysis. A comparison of the spectra obtained by EI and CI analysis of benztropine is shown in Figure 3. The CI mass spectrum shows a protonated molecular ion with ion intensity at *m/z* 308 and additional ion intensities at *m/z* 124, 140, and 167. The molecular ion is consistent with benztropine's molecular weight of 307 daltons, further confirming the presence of benztropine in the decedent's blood at the time of death.

Based on the EI and, to a limited extent, the CI mass spectra for benztropine, the fragmentation scheme previously reported by Jindal et al. (16) was expanded, as structurally illustrated in Figure 4. We hypothesize that with a loss of an electron from the nitrogen atom, the formed molecular ion undergoes a β-cleavage process, losing a stable diphenylalkoxy radical to give an even electron ion at *m/z* 124 (19–21). The fragment ion at *m/z* 124 is the base peak under CI conditions but, with EI analysis, undergoes an additional series of one electron shifts with the loss of C_2H_4 and C_3H_6 groups to give conjugated, thermodynamically stable ions at *m/z* 96 and 82, respectively (22). Under both EI and CI conditions, the molecular ions with charge on the oxygen atom may undergo a homolytic cleavage to give an even electron oxonium ion at *m/z* 140 or a heterolytic cleavage to give a highly delocalized diphenylmethyl cation at *m/z* 167. Under EI conditions, subsequent cy-

clization of some diphenylmethyl cations results in a tricyclic ion fragment at *m/z* 165. In an alternate pathway, the molecular ion with charge on the oxygen atom undergoes migration of a phenyl moiety to give odd electron ions at *m/z* 201 and 106, with an ion at *m/z* 105 resulting from the loss of a single proton. The mass spectra of benztropine by EI and CI analysis for the postmortem samples were consistent with the proposed scheme, adding further support to the identification of benztropine in this case.

Quantitation of benztropine in serum, postmortem blood, and urine was performed in the EI mode with benztropine-d_3 as the internal standard. Chromatographic retention and mass spectra for benztropine and benztropine-d_3 in a representative sample are shown in Figure 5. Leading and trailing ends of the peak show predominant mass spectra for benztropine-d_3 and benztropine, respectively. Deuteration of benztropine resulted in a *m/z* shift equivalent to 3 amu for all fragments except for ions at *m/z* 105, 165, and 167. Based on the deuteration of the *N*-methyl group on benztropine, the spectrum shift observed for the deuterated form of benztropine is consistent with the fragmentation scheme illustrated in Figure 4.

Table I shows the benztropine concentration in the decedent's blood in comparison with 12-h trough concentrations in serum from patients treated with benztropine doses ranging

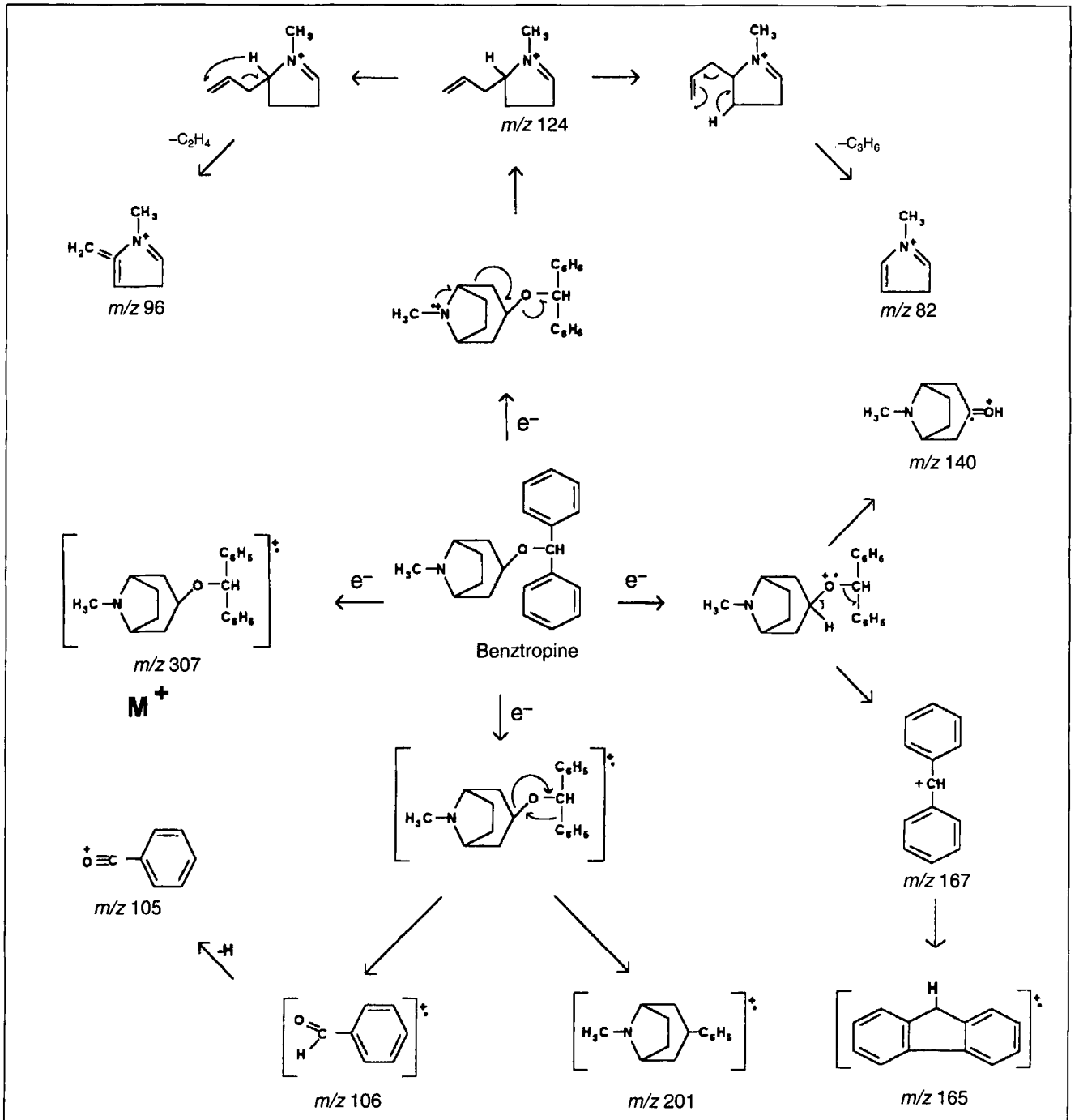

Figure 4. Proposed ion fragmentation scheme for benztropine.

from 4 to 8 mg per day. The postmortem blood concentration in this case was 20-fold higher than therapeutic trough concentrations measured in the five patients. Dose- and time-dependent therapeutic ranges are not well-established for benztropine. Using an HPLC method, a therapeutic plasma concentration of 0.0067 mg/L has been reported for a single patient (23). In a prior publication by Jindal et al. (16), in which information on dosage and time of collection were not available, therapeutic ranges (0.0795–0.126 mg/L) were much higher but still did not reach the concentration determined for the decedent in the present case. In a reported case of benztropine overdose (11) where serial benztropine concentrations were determined, a peak serum concentration of 0.100 mg/L was measured when the patient was experiencing severe central anticholinergic toxicity. The patient survived the overdose through receipt of prompt medical attention and subsequent physostigmine treatment but did suffer a prolonged course of intoxication. Evidence of anticholinergic toxicity disappeared only after the benztropine concentration decreased to less than 0.010 µg/L. In our case, in which the individual did not receive medical treatment, blood concentration of benztropine at the time of death exceeded all previously reported therapeutic and toxic concentrations.

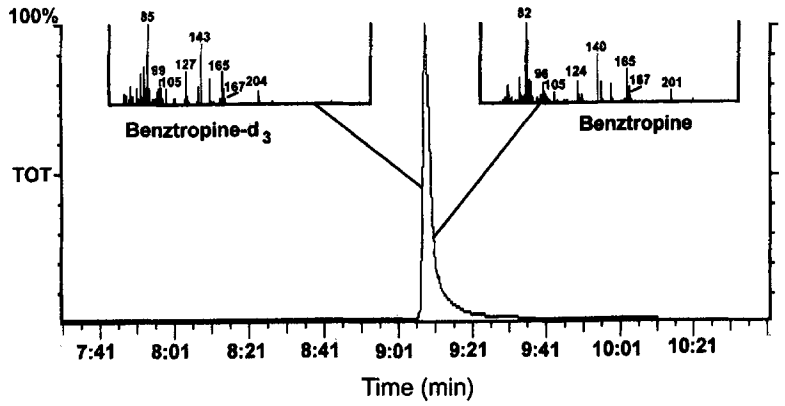

Figure 5. Representative total ion chromatogram and partial mass spectra of benztropine and benztropine-d_3 extracted from postmortem blood.

Benztropine was also detected in the urine of the decedent without evidence of additional metabolic products or other drugs. Figure 6 shows the total ion gas chromatogram and partial mass spectrum for an extract of the decedent's urine. Subsequent quantitation revealed a urinary benztropine concentration of 7.12 mg/L. Elimination and metabolism of benztropine have not been reported except for a positron emission study with *N*-11C-methyl-benztropine (24), which suggested a slow rate of benztropine metabolism in humans as 83% of the drug was unchanged at 30 min post-injection (24). The relatively high concentration of benztropine found in the decedent's urine from the present case indicates significant urinary elimination of the parent drug. In order to make a direct search for possible metabolites in the decedent's serum and urine, we synthesized norbenztropine. Figure 7 illustrates the chromatographic resolution of pure benztropine and norbenztropine and their respective mass spectra. The fragmentation of norbenztropine is consistent with the scheme shown earlier in Figure 4 and has a shift of 14 amu in all ion fragments, with the exception of ions at *m/z* 105, 165, and 167. Analysis of the decedent's urine and blood, however, did not reveal the presence of norbenztropine, indicating no significant bioconversion by the *N*-desmethylation pathway.

Table I. Benztropine Concentration in Serum and Postmortem Blood

Subject	Benztropine (mg/L)	Dose (mg)	Sample (collection)
Patient 1	0.008	2 (BID*)	Serum (trough therapeutic)
Patient 2	0.004	2 (BID)	Serum (trough therapeutic)
Patient 3	0.004	2 (BID)	Serum (trough therapeutic)
Patient 4	0.006	4 (BID)	Serum (trough therapeutic)
Patient 5	0.005	2 (BID)	Serum (trough therapeutic)
Decedent	0.183	240†	Blood (postmortem)

* BID = twice a day.
† Maximum dose based on medication bottles found with decedent.

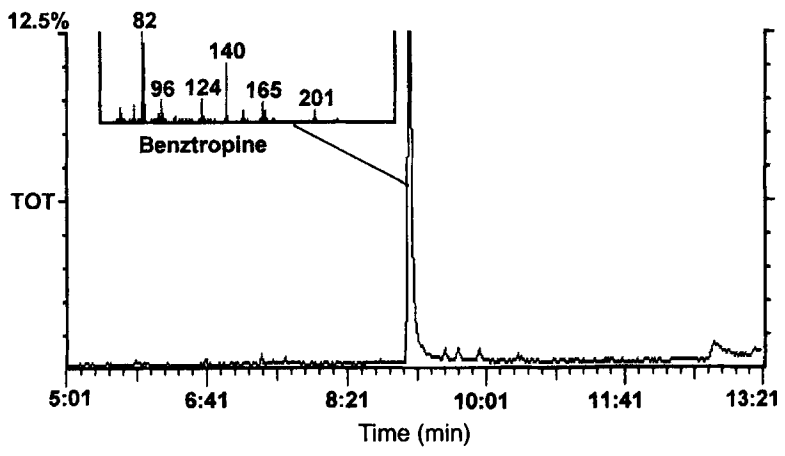

Figure 6. Total ion count chromatogram and partial mass spectrum of extract of urine from decedent.

Conclusions

Benztropine analysis of postmortem blood and urine by GC–MS provided a highly specific method for forensic toxicological evaluation in a potential benztropine overdose fatality. The postmortem blood concentration of benztropine in this case was significantly higher than found for therapeutic use of the drug and also exceeded the peak concentration reported previously

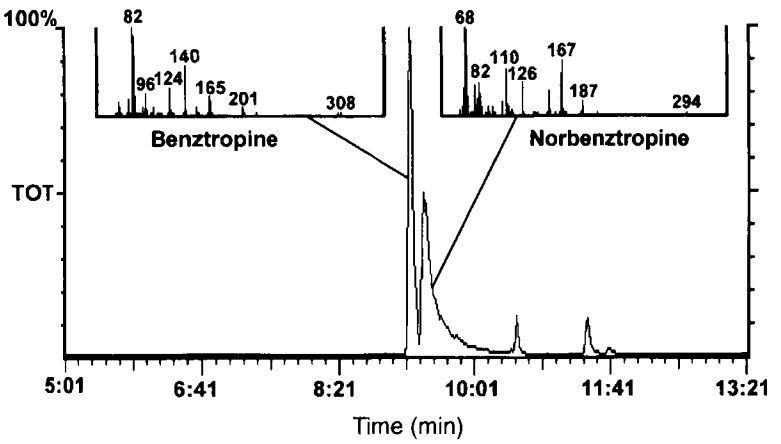

Figure 7. Total ion chromatogram and partial mass spectra of extract from drug-free urine supplemented with benztropine and norbenztropine.

in a severe case of benztropine intoxication. Considering the circumstances in this case and the absence of either gross or microscopic evidence of death from natural or traumatic causes, the death was ruled a suicide resulting from the toxic effects of benztropine overdose, based on the toxicological findings. Additional mass spectra and a fragmentation ion analysis are provided to assist in the forensic toxicological evaluation of this agent.

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