CASE REPORT

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Attempted Murder with Pancuronium

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ABSTRACT: A nurse was accused of attempting to murder her anesthesiologist husband on two occasions by administering to him a neuromuscular blocking agent. In both episodes, urine specimens were obtained from the victim shortly after the suspected assaults. The samples were initially tested fluorometrically using Rose Bengal dye as a pairing agent. Both were presumptively positive for pancuronium. Confirmation of these results was achieved by pairing the drug with potassium iodide, extracting the complex, and submitting the extract to thin-layer chromatography (TLC) cleanup, elution at the appropriate retardation factor (Rf), and, finally, gas chromatography/mass spectrometry (GC/MS) analysis in the selected-ion monitoring mode. The two quaternary amines of pancuronium appear to undergo pyrolytic *N*-demethylation in the injection port to yield an entity amenable to capillary column gas chromatography. The mass spectrum of the compound consists of a base peak of *m/z* 322, with additional fragments of 292, 323, 338, 396, and 397 *m/z*, each of which was monitored. The confirmed positive findings were instrumental in adjudicating the case.

KEYWORDS: toxicology, homicide, pancuronium

Recently, another jurisdiction referred to the authors a case in which it was alleged that a nurse had attempted to murder her ailing anesthesiologist husband by twice administering to him a potent neuromuscular blocking agent. In both episodes, urine specimens from the victim were obtained within 1 to 2 h of the suspected assaults and were submitted to our laboratories for analysis.

Although two drugs, pancuronium bromide and succinylcholine chloride, were readily available to the suspect, the victim's symptoms and prolonged duration of respiratory distress were considered to be more consistent with the effects of pancuronium than with those of succinylcholine.

Pancuronium bromide is a neuromuscular blocking agent that competes for cholinergic receptors at the motor endplate. Unlike succinylcholine, it does not produce depolari-

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zation. Depending upon the dose and the circumstances of administration, demonstrable effects of the drug can persist for over 100 min. It has been reported that pancuronium has an elimination half-life ($t_{1/2}$) ranging from 89 to 161 min. Approximately 40% of an administered dose has been recovered in the urine as parent drug, together with its three known metabolites, 3-hydroxypancuronium, 17-hydroxypancuronium, and 3,17-dihydroxypancuronium (Figs. 1*a*, 1*b*, 1*c*, and 1*d*, respectively), with the 3-hydroxy transformation product predominating [*1*].

Chemically, pancuronium bromide is the dibromide salt of a dipiperidinium derivative of the steroid androstane. Its molecular weight, minus the anions, is 573. Total saturation of the molecule precludes its estimation by liquid chromatography (LC) with an ultraviolet detector. Cursory trials to determine pancuronium by LC and refractive index detection in our laboratory were not successful. Several reports in the literature refer to the use of Rose Bengal dye as an ion-pairing reagent for fluorometric quantification of pancuronium in biologic specimens [*2,3*]. Such methods suffer from the disadvantage of being only semispecific and, thus, are somewhat limited in their application to forensic science cases. Nevertheless, fluorescence photometry, employing the ion-pairing dye, can play an important role when applied as a preliminary testing device.

The presence of the two quaternary amines in pancuronium would appear to prohibit its assay by gas chromatography (GC). However, it has been reported that the molecule undergoes alteration in the injection port if the temperature is sufficiently high.[2] The resulting product or products are amenable to GC and detection by mass spectrometry

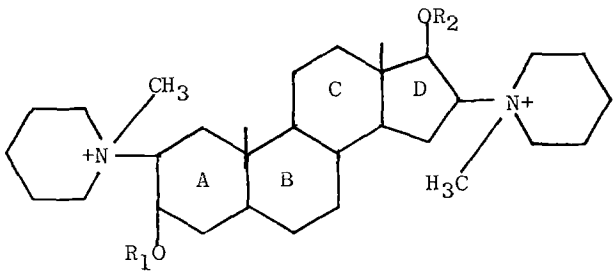

FIG. 1—*Structures of pancuronium and metabolic (hydrolysis) products:*

(A) $R_1 = R_2 =$ —C(=O)—CH_3 = pancuronium

(B) R_1 = —H; R_2 = —C(=O)—CH_3 = 3-hydroxypancuronium

(C) R_1 = —C(=O)—CH_3; R_2 = —H = 17-hydroxypancuronium

(D) $R_1 = R_2$ = —H = 3,17-dihydroxypancuronium

[2]Aron, R. and Quenzer, C., FBI Laboratories, Washington, DC, personal communication, 1988.

(MS). Other quaternary amine compounds have been discovered to undergo similar injection-port pyrolysis [*4*].

The present paper describes the analytical scheme developed for toxicologic evaluation of this case. The system consists of initial testing by ion-pair fluorometry, isolation of the drug and its metabolites from a separate aliquot of the urine, thin-layer chromatographic (TLC) cleanup of the extract, and, finally, confirmation by GC/MS.

Experimental Procedure

Reagents

A carbonate-bicarbonate buffer solution was prepared by adding 0.2 *M* aqueous sodium bicarbonate to 0.2 *M* aqueous sodium carbonate until a pH of 10.5 was obtained.

Rose Bengal reagent was prepared by dissolving 5 mg of Rose Bengal dye in 10 mL 0.45*M* aqueous dibasic potassium phosphate. Prior purification of the Rose Bengal was necessary [*5*] and was achieved by washing the dye six times with ethyl acetate, followed by three washings with chloroform. The residue was transferred to a vacuum desiccator and dried at room temperature.

Acidified iodoplatinate was prepared by dissolving 1 g of platinic chloride in 10 mL of water and adding this to a solution containing 10 g of potassium iodide in 200 mL of water. The final volume was adjusted to 250 mL by the addition of water. Prior to use as a spray reagent, the mixture was acidified by adding 2 drops of concentrated hydrochloric acid.

Preliminary Testing Procedure

Two millilitres of the urine specimen were acidified with 0.5 mL of 20% aqueous trichloroacetic acid and extracted with 10 mL of diethyl ether to remove neutral and acidic solvent-soluble compounds. After aspiration of the ether to waste, the aqueous layer was alkalized with 1 mL of 10% sodium hydroxide and again extracted with 10 mL of ether to eliminate solvent-soluble basic substances. The ether was again discarded. The aqueous layer was treated with 1 mL of the buffer to provide a pH of 9.5 to 10.0 and 250 μL of the Rose Bengal solution was added. The mixture was extracted with 7 mL of chloroform and centrifuged, and the aqueous layer was discarded. The fluorescence intensity of the chloroform phase was determined at 570 nm in a spectrophotofluorometer with the excitation monochromator set at 546 nm. Duplicate, 2-mL aliquots of blank urines and blank urines fortified with 2 and 4 μg of pancuronium were concurrently assayed.

GC/MS Procedure

Duplicate 5-mL aliquots of the urine specimen were acidified with 1 mL of 0.67*N* sulfuric acid and extracted with 10 mL of diethyl ether. The organic layers were discarded. One millilitre of 10% sodium hydroxide and 10 mL of ether were added to each aqueous phase and the mixtures were thoroughly shaken. Again, the ether layers were aspirated to waste. The pH of each aqueous layer was adjusted to 9.5 to 10 by the addition of 1 mL of the carbonate-bicarbonate buffer, and excess solid potassium iodide (KI) was added. The mixtures were extracted with 10 mL of methylene chloride/methanol (4:1) for 5 min. After centrifugation, the aqueous layers were discarded, the organic phases transferred to 30-mL beakers, and the solvent was carefully evaporated just to dryness. The residues were reconstituted with 100 μL of the same solvent and the entire solutions were spotted at two points of origin on a Merck Kieselgel 60 F254, 20 by 20-cm, thin-

layer chromatography plate 250-μm in thickness. Duplicate 5-mL aliquots of a blank urine and a blank urine fortified with 25 μg of pancuronium were identically and concurrently treated and spotted on the same plate. Calibrators, containing 5 μg of pancuronium were also applied at the origin, and the plate was developed to a distance of 10 cm in a solvent system consisting of methanol/acetonitrile/concentrated hydrochloric acid (75:25:1.5).[3] Half the plate, containing a blank, an enriched blank, a calibrator, and one of the duplicate unknowns, was covered and the remainder of the plate was sprayed with acidified potassium iodoplatinate. The silica gel in the unsprayed areas, corresponding to the retardation factor (Rf) of pancuronium, was scraped off the plate into separate 12-mL screw-capped centrifuge tubes. Two millilitres of water, 1 mL of the buffer, excess solid KI, and 8 mL of methylene chloride were added, and the mixtures were placed on a mechanical shaker for 15 min. After centrifugation, the aqueous layers were aspirated to waste, and the organic phases were evaporated just to dryness at room temperature under a stream of nitrogen. The residues were reconstituted with 100 μL of ethanol, and 2-μL aliquots were injected into the gas chromatograph/mass spectrometer.

The Hewlett-Packard GC/MSD, Model 5890/5970, was operated in the selected-ion monitoring mode, with ions of 322, 292, 323, 338, 396, and 397 *m/z* scanned. The capillary column was 25 m long and 0.2 mm in inside diameter, with a 5% phenylmethyl stationary phase. Helium was the carrier gas at a flow rate of 1.0 mL/min. The injection port and detector temperatures were 300°C and 280°C, respectively. The initial column temperature was 125°C and was ramped at a rate of 25°C/min to the final temperature of 280°C and held for 8 min.

Results and Discussion

Both specimens from the victim were positive upon preliminary testing with Rose Bengal. Using the described procedure, succinylcholine, tubocurarine, vecuronium, metocurine, atracurium, and bretylium were tested for Rose Bengal cross-reactivity. All proved essentially negative. Of these substances, vecuronium is of special interest. It is the monoquaternary amine analog of pancuronium, in which only the piperidine nitrogen on Ring D is quaternized. Despite their close structural similarity, vecuronium was found to exhibit cross-reactivity of less than 2% relative to pancuronium. Even this may be attributable to impurities of the pancuronium, de-esterified pancuronium, or both, which are suspected to be present in vecuronium as synthesis by-products. The three metabolites of pancuronium, on the other hand, were observed to yield Rose Bengal cross-reactivity with fluorescence intensities comparable to that for the parent compound.

Fluorescence emission values at 570 nm observed for the blank and the 1 and 2-μg/mL enriched blanks were 1, 47, and 82, respectively ($n = 2$). Duplicate analyses of the unknown urine specimens yielded intensities of 127 and 267. While these data may appear to permit quantitative determinations of total pancuronium and its metabolites, such calculations were not made because they would be of little value, and perhaps even misused, particularly in attempts to apply them to estimate pharmacologic effects or to establish the amount, time, and route of administration of the drug. Moreover, in cases such as this, the question of utmost importance is qualitative; that is, is the drug (or metabolite) present in the urine of an individual who has not received it for legitimate purposes?

Pancuronium has a retardation factor (Rf) of approximately 0.5 in the TLC method described. The acidic developing solvent may cause some distortion in the shapes of TLC spots and some streaking in the lower one third of the plate. However, this does not

[3]Rieders, F., National Medical Services, Willow Grove, PA, personal communication, 1988.

seriously impair the usefulness of the system when it is employed as a cleanup step prior to GC/MSD confirmation.

Reference pancuronium exhibits a GC retention time (Rt) of approximately 11 min. The electron impact mass spectrum we obtained of the putative desdimethyl product of injection-port pyrolysis has been confirmed by an independent laboratory. It is characterized by a base peak of 322 *m/z*, with additional masses of analytical usefulness of 292, 323, 338, 396, and 397 *m/z*. The total ion chromatogram (TIC) and the spectrum are shown in Fig. 2. To our knowledge, this mass spectrum appears nowhere else in the literature.

Figure 3 displays the selected ion chromatograms and the derived TIC produced by a blank (negative) urine which was carried through the entire procedure. The chromato-

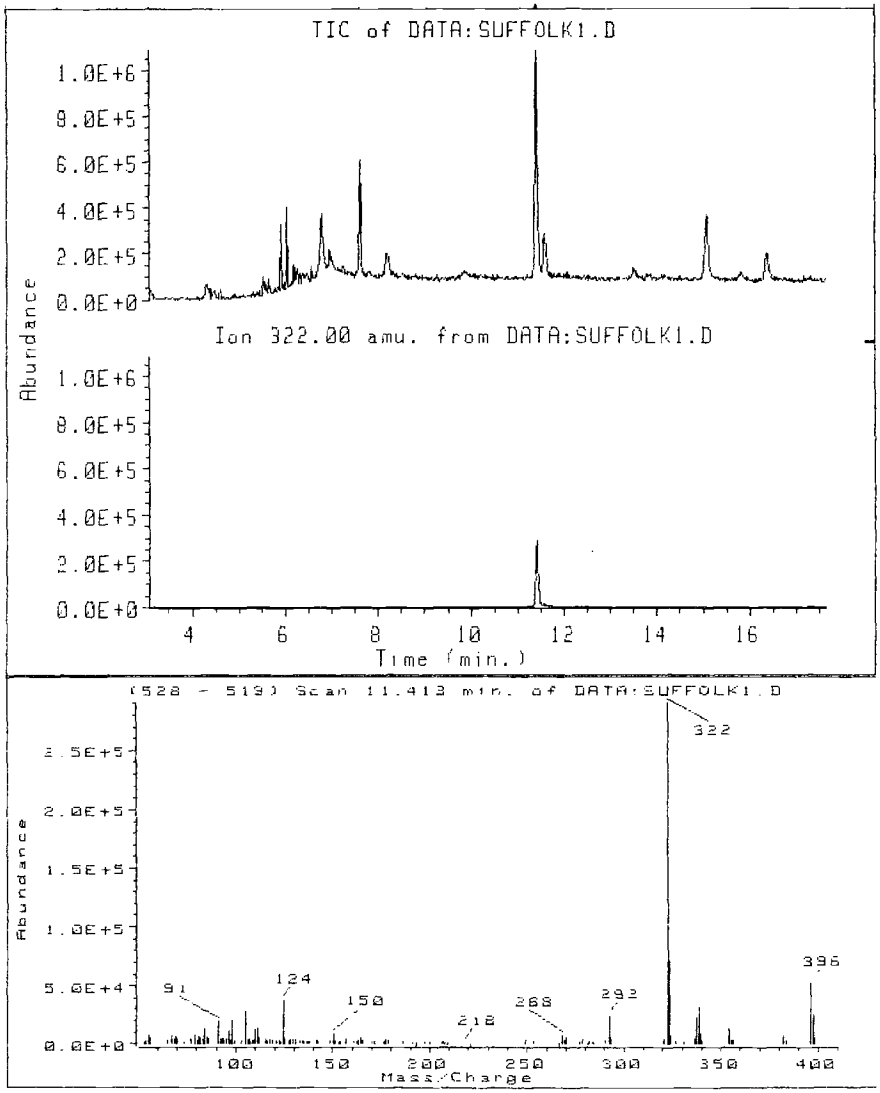

FIG. 2—*Ion chromatograms and mass spectrum of pancuronium.*

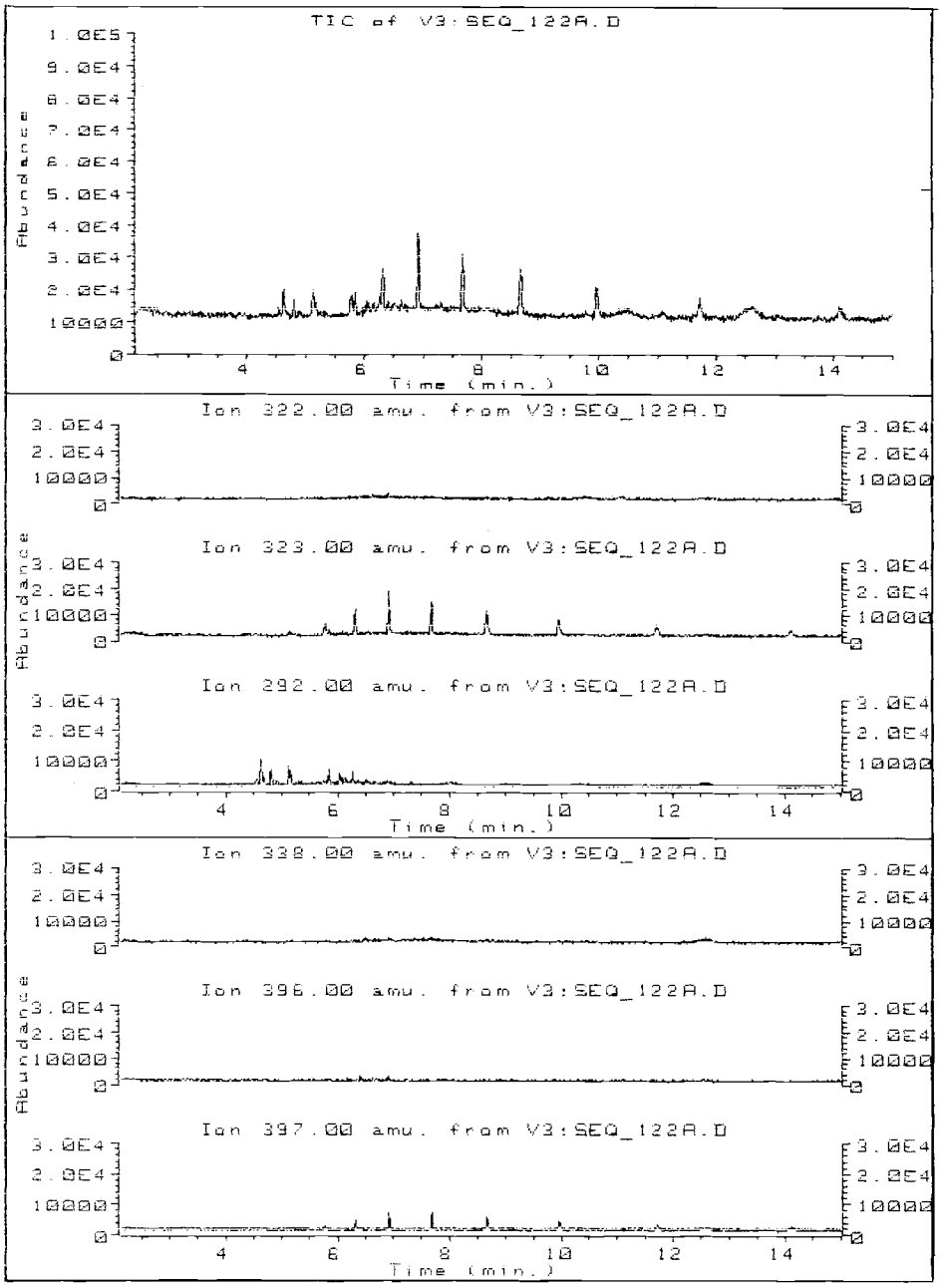

FIG. 3—*Ion chromatograms of blank (negative) urine.*

grams obtained when the same urine was fortified with 5μg/mL of pancuronium appear in Fig. 4.

Both urines from the victim provided ion chromatograms which were qualitatively identical to those for the fortified urine. Figure 5 shows the ion chromatograms furnished by the urine obtained shortly after the first alleged assault. These data are also representative of the observations made for the second urine.

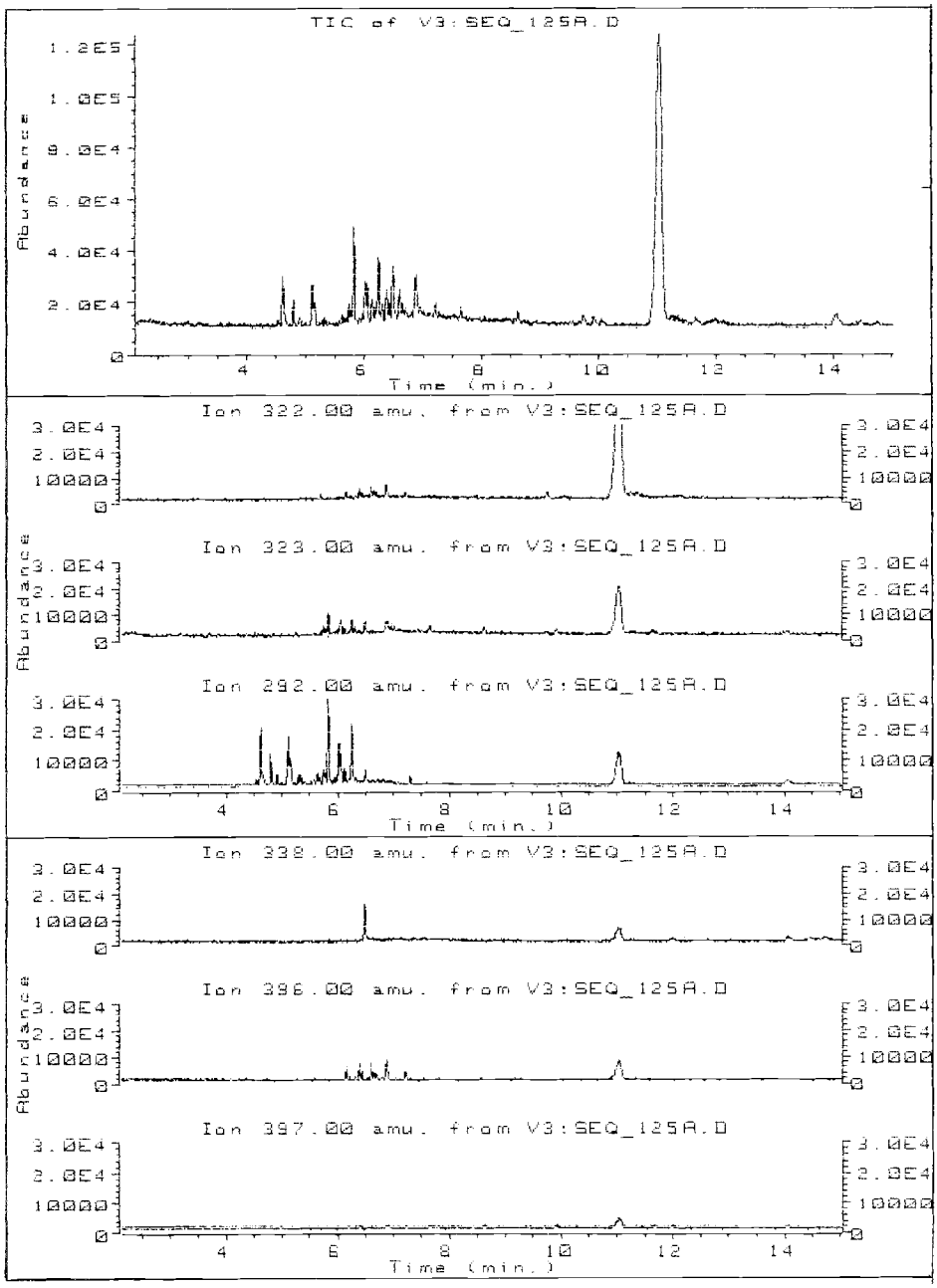

FIG. 4—*Ion chromatograms of blank urine fortified with pancuronium (5 μg/mL).*

Table 1 lists the ion ratios for reference pancuronium and the two unknowns. In all instances the ratios for the unknowns lie well within the customary 20% range for acceptability.

The combination of factors, namely, positive Rose Bengal response, extractability as a KI ion-pair, appropriate Rf and Rt, and the presence of all six monitored fragments,

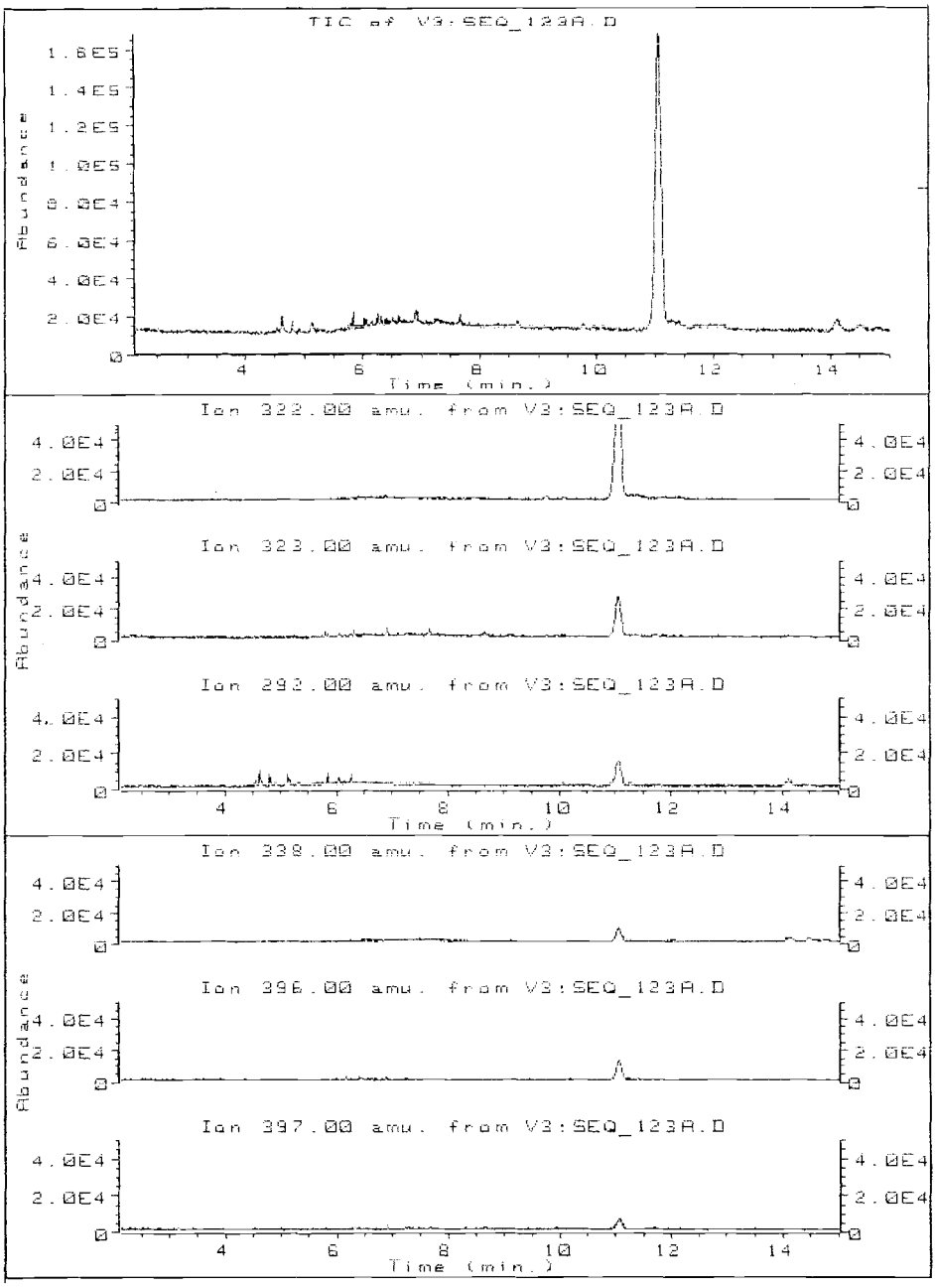

FIG. 5—*Ion chromatograms of unknown urine.*

each within target ion ratios, led to the inescapable conclusion that both urine specimens from the victim contained pancuronium. These results were instrumental in the final resolution of the case, in which the assailant ultimately pleaded *nolo contendere* to the charges.

During the course of this work and since its completion, several other cases of suspected

TABLE 1—*Mass spectral ion ratios for pancuronium and unknowns.*[a]

			Urine	
m/z	Pancuronium	Target Range	No. 1	No. 2
292	15.87	(12.70–19.04)	15.06	16.03
322	100.00		100.00	100.00
323	26.88	(21.50–32.26)	28.17	28.99
338	8.18	(6.54–9.82)	9.43	8.90
396	10.93	(8.74–13.12)	12.97	12.29
397	5.14	(4.11–6.17)	5.82	5.81

[a]Averages of two trials.

assault, murder, and suicide with pancuronium have been referred to our laboratory. In connection with these cases, we have conducted additional studies on the physicochemical characteristics of the drug and its metabolites. These data will be reported in a future paper.

Acknowledgment

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