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Stability of Diltiazem in Whole Blood: Forensic Implications

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ABSTRACT: The stability of diltiazem (DTZ) in whole blood and in postmortem samples was investigated. In the first study, an aliquot of outdated Red Cross blood with sodium fluoride added as a preservative was spiked with DTZ and stored for one year under three separate conditions: room temperature, 4°C, and −20°C. DTZ and one of its major metabolites, desacetyldiltiazem (DAD), were quantitated at given intervals during this period. In the second study, case postmortem blood samples ($n = 36$) that exhibited different degrees of putrefaction were spiked in a similar fashion and the stability of DTZ was determined after storage at 4°C for 92 days. DTZ and DAD were extracted as bases, using mild pH conditions to prevent the hydrolysis of DTZ, and quantitated by an HPLC system equipped with a diode array detector and a Supelcosil LC-DP column, 5 μm, 250 mm × 4.6 mm inside diameter. Approximately 50% of DTZ was lost in the Red Cross blood stored at room temperature and 4°C, after 19 and 124 days, respectively. This was associated with concomitant appearance and comparable increase in DAD concentration, presumably due to the in vitro hydrolysis of DTZ to DAD. No significant loss of DTZ was observed in the −20°C samples. Similar changes in DTZ and DAD concentrations were seen in postmortem blood samples stored at 4°C for 92 days, though notably, the extent of loss of DTZ varied from complete to negligible. The data suggest that the potential for in vitro conversion of DTZ to DAD should be considered for proper interpretation of postmortem DTZ/DAD findings. Several cases examined in this laboratory will be used to discuss other forensic implications.

KEYWORDS: forensic science, forensic toxicology, diltiazem, stability, postmortem blood

Diltiazem (DTZ) is a calcium ion antagonist which has been shown to be useful in the treatment of angina pectoris, supra ventricular arrhythmia, and hypertension (1). The pharmacokinetics of DTZ have been studied by several authors (2–11) and the pharmacology and therapeutic efficacy of the drug reviewed (12,13). Diltiazem is metabolized extensively in humans via various pathways including O-deacetylation, N-demethylation, O-demethylation, and O-deamination to form acidic and basic metabolites (12,14). The chemically basic metabolites, N-monodesmethyldiltiazem (M_A) and desacetyldiltiazem (DAD), have been shown to accumulate in plasma during chronic DTZ treatment and are pharmacologically active (15–18). Previous studies have demonstrated that DTZ and M_A were unstable in different biological fluids under various storage conditions in vitro, and hydrolyzed to DAD and desacetyl-N-desmethyldiltiazem (M_2), respectively (19–24). The stability of DTZ, however, in postmortem blood stored at 4°C and over a period likely to be encountered in forensic toxicology has not been reported.

Therefore, the objectives of this work were:

1. To assess the degradation of DTZ in artificially-aged Red Cross blood at 25°, 4°, and −20°C from day one to one year;
2. To study DTZ stability in spiked postmortem blood samples stored at 4°C for three months;
3. To quantitate DTZ and DAD in forensic cases in order to assist in the interpretation of toxicological findings; and
4. To reanalyze selected positive DTZ cases and quantitate the decreasing and increasing concentrations of DTZ and DAD, respectively.

Experimental Procedure

Chemicals and Reagents

Diltiazem hydrochloride was supplied by Novopharm Ltd. (Ontario, Canada). Desacetyldiltiazem was synthetized in this laboratory, and the pure reference standards for identification and confirmation of DAD, M_A, and M_2 were generously donated by Marion Merrell Dow Co. (Quebec, Canada). Their chemical structures and chromatographic separations are shown in Figs. 1 and 2, respectively. Stock solutions of diltiazem and three of its metabolites were prepared in methanol at a concentration of 0.4 mg/mL as free base and stored at −20°C. Outdated Red Cross blood was obtained from a blood bank and artificially aged at room temperature for approximately three to four weeks; after the addition of 10 g of sodium fluoride preservative (NaF)/L, the blood was stored at 4°C for several weeks. This ensured that the sodium fluoride concentration in the Red Cross blood would be the same as that normally encountered in postmortem blood samples. Each batch of aged blood was screened and found to be drug-free before it was used either for the calibration standards or the stability study.

All other chemicals were analytical reagent grade. Solvents were distilled in glass quality. Purified water was generated using a Millipore Milli-Q Plus water purification system (Millipore, Mississauga, Canada). The triethylamine (TEA) buffer was prepared by adding 4.5 mL of concentrated phosphoric acid and 10 mL of TEA to 900 mL of water. After the pH was adjusted to 3.4 with diluted phosphoric acid, the volume was made up to 1 L with water and stored at 4°C. Before it was used to make up the mobile phase, it was kept at room temperature overnight and filtered through a Millipore 0.45 μm HA filter.

Extraction Procedure and HPLC Method

In 1992 we reported on a method for the detection and quantitation of basic drugs in postmortem blood using HPLC-DAD (25). Briefly, basic drugs were back-extracted into sulfuric acid after the initial extraction from 2.0 mL of blood with toluene under

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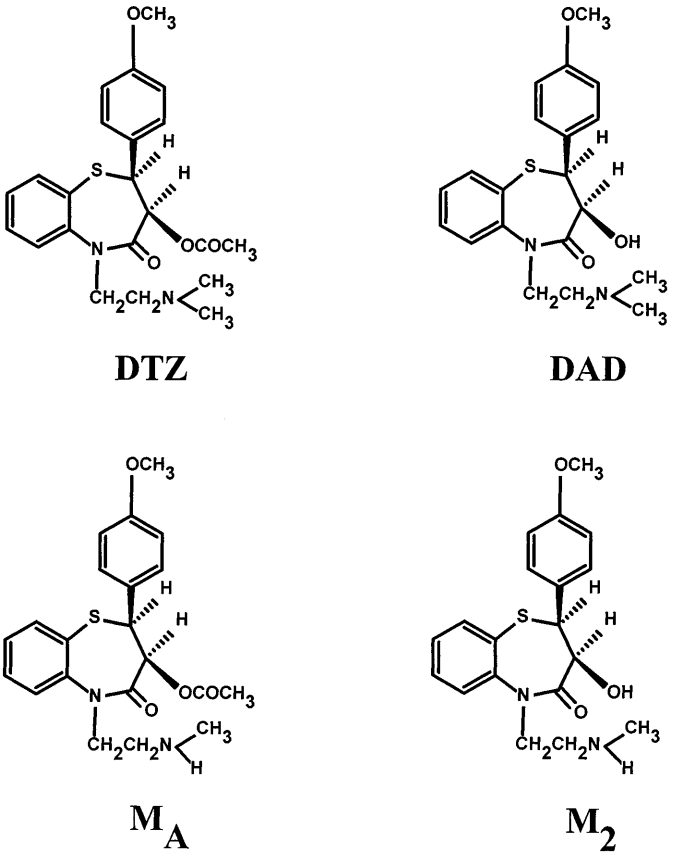

FIG. 1—*Chemical structure of diltiazem (DTZ), desacetyldiltiazem (DAD), N-monodesmethyldiltiazem (M_A) and desacetyl-N-monodesmethyldiltiazem (M_2).*

basic conditions. Only a minor modification of the method was necessary for the quantitation of DTZ and DAD. Back-extraction was performed using 0.01 M H_2SO_4 instead of the 0.1 M and the acid fraction made alkaline with 0.5 M K_2CO_3 before it was re-extracted with toluene. The extraction procedure is outlined in Fig. 3. Chromatographic separation was achieved isocratically at ambient temperature on Supelcosil LC-DP (5 μm, 250 mm × 4.6 mm inside diameter) reversed-phase column. The mobile phase consisted of acetonitrile: 3.7 mM H_3PO_4:pH 3.4 TEA buffer (25: 10:5, v/v/v). The flow rate was 0.6 mL/min and the detection was performed at 229 nm. Injection was 25 μL out of a final volume of 300 μL.

Procedure for Stability Study

Stability of DTZ in Preserved Red Cross Blood

An aliquot of 3.0 mL of aqueous diltiazem hydrochloride was added to 147 mL of blood to obtain a concentration of 8.0 mg/L. Aliquots of 2.0 mL of blood were transferred to 18 screw-cap culture tubes (16 by 125 mm, Kimax® with a trifluoroethylene (Teflon®) resin faced rubber liner in the screw cap) and stored at −20°C. The remaining blood sample was divided into equal amounts and stored in corked Erlenmeyer flasks in the dark at room temperature, or at 4°C. Duplicate samples were analyzed after 24 h, 1, 2, 3, and 4 weeks then 2, 3, 6, and 12 months of storage.

Stability of DTZ in Spiked Postmortem Blood

The stability of DTZ in postmortem blood stored at 4°C was also investigated. Thirty-six previously screened, drug-free postmortem blood samples (usually preserved with sodium fluoride) from forensic cases were spiked at a concentration of 8.0 mg/L. The spiked blood samples were equilibrated overnight and the initial

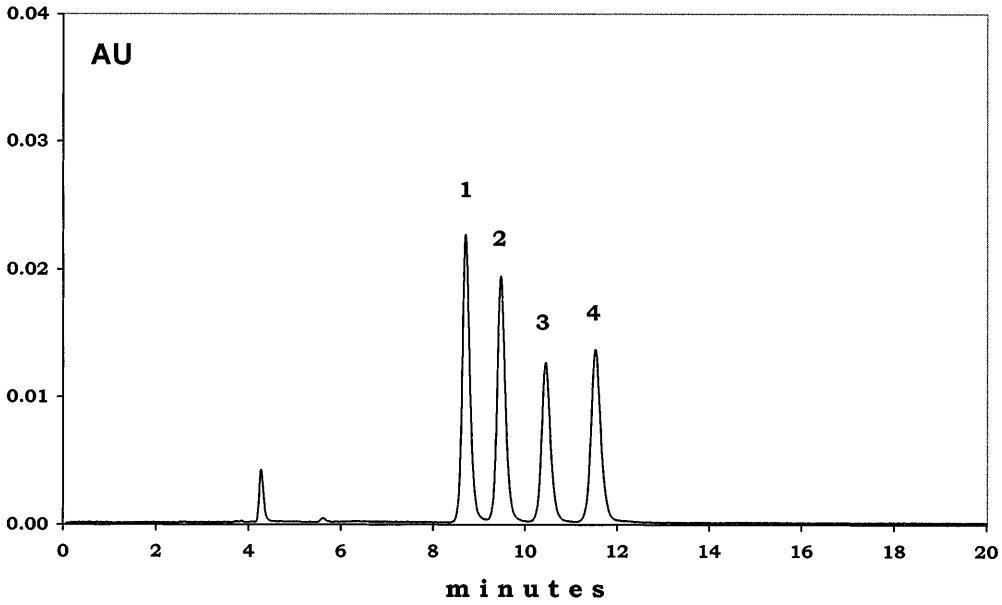

FIG. 2—*Chromatogram of a standard solution containing 4.0 mg/L of desacetyl-N-monodesmethyldiltiazem (1), desacetyldiltiazem (2), N-monodesmethyldiltiazem (3), and diltiazem (4).*

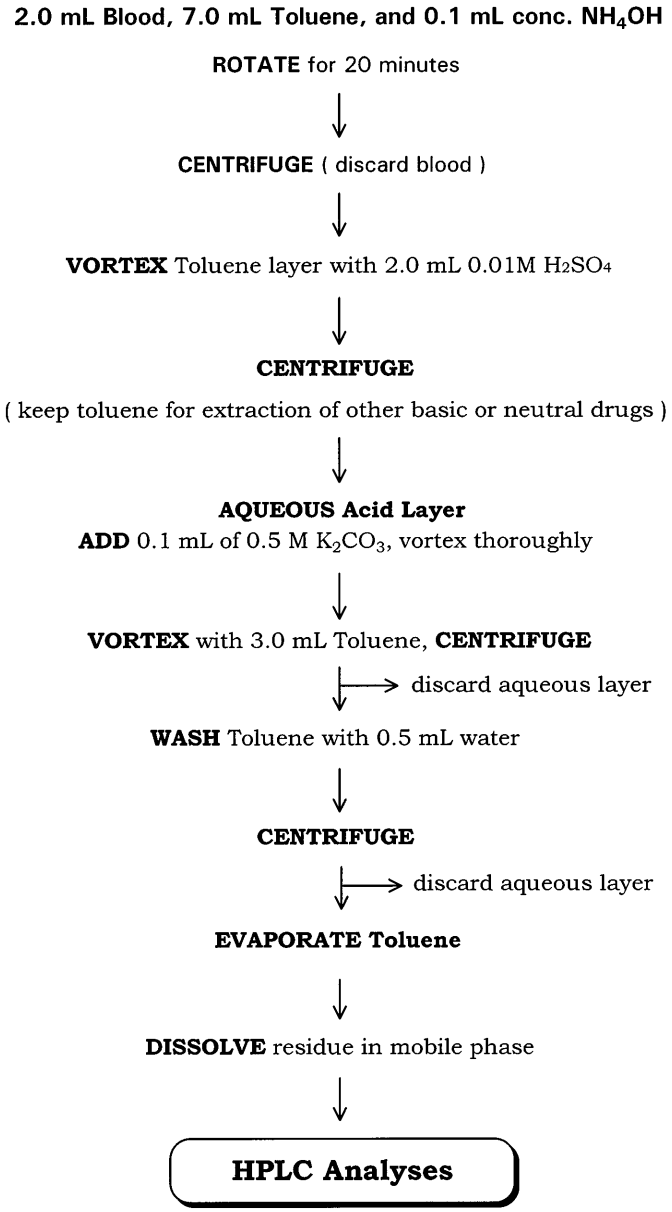

FIG. 3—*Flow diagram of method for DTZ and DAD extraction from whole blood.*

concentration of DTZ was determined in duplicate analyses for each case blood. Three months later, blood samples were reanalyzed for DTZ and DAD and the values obtained were compared with those measured on the day following preparation.

Results and Discussion

Assay Validation for DTZ and DAD

The between-day coefficient of variation (CV) for the assay was less than 6% ($n = 7$) for DTZ and DAD at concentrations of 0.1 and 8.0 mg/L. In spiked Red Cross blood (over a year), the apparent recoveries of DTZ and DAD were greater than 80%, compared with unextracted standards in methanol. This was seen over the concentration range of the blood standard curve (0.125 to 2.0 mg/L) and at concentration of 8.0 mg/L. The accuracy of quantitation of DTZ and DAD in blood, using separately spiked blood as reference, was within 15% of the target concentrations of 1.0 and 8.0 mg/L. The limit of quantitation, which allowed spectral confirmation, was set at half of the lowest concentration of the standard curve used for analysis, i.e., 0.06 mg/L. The limit of detection with spectral confirmation was approximately 0.02 mg/L for both DTZ and DAD.

Degradation of DTZ in Preserved Red Cross Blood

In the first study, the stability of DTZ was investigated in spiked Red Cross blood. The decreasing concentrations of DTZ and increasing concentrations of DAD are illustrated in Fig. 4. Blood samples stored at room temperature showed a significant decrease of DTZ concentration with time. The losses of DTZ were 8.3, 20.3, and 33% after 1, 7, and 14 days, respectively. Within 19 days, 50% of DTZ hydrolyzed to DAD. By contrast, no significant degradation of DTZ was observed at 4°C until day 14, where only 10% of the initial concentration of DTZ hydrolyzed to DAD. The loss of DTZ was 33% and 50% after 72 and 124 days, respectively, when stored at 4°C. Unlike the results for room temperature and 4°C, DTZ appeared to be stable in frozen blood samples. A degradation of less then 15% of DTZ was noticed when blood samples were stored at −20°C for 1 year.

The hydrolysis of DTZ was associated with concomitant appearance of a comparable increase in DAD concentrations. The combined concentrations of DTZ and DAD, detected at each testing interval, averaged to a total of 9.0 mg/L ($n = 54$, CV 3.8%). As the initial spiked DTZ concentration was confirmed through analysis to be 8.5 mg/L, there is a support for the argument that all of the detected DAD originated from the added DTZ.

For the statistical analysis, however, four data points were left out. These were the data obtained at 6 and 12 months for blood samples stored at room temperature. After DAD reached a maximum concentration (8.8 mg/L) in the third month, it underwent further degradation. It appears that DAD was degraded to other breakdown products (similar in spectral properties) which were not accounted for in this experiment. The final course for the disappearance of DAD at room temperature can be seen in Fig. 4.

Degradation of DTZ in Spiked Postmortem Blood

The stability of DTZ in 36 postmortem blood samples was also examined (Fig. 5). The storage temperature and the length of time were chosen to reflect common laboratory situations. In many forensic laboratories, case samples would normally be stored at 4°C under refrigeration, until a full drug screening and quantitative drug analysis is completed (usually, two to three months or more).

Similar changes in DTZ and DAD concentration were seen in postmortem blood samples stored for three months at 4°C, although notably, the extent of loss of DTZ varied from complete to negligible. In three of the cases, DTZ was not detected and concentrations of DAD were 7.8, 8.5, and 8.2 mg/L, respectively. The loss of DTZ was less than 10% in only one of the case blood samples. Out of the 36 cases, 11 blood samples had a ratio of 0.66 (DAD/DTZ), similar to Red Cross blood when it is stored under the same conditions. All others were higher.

It is noteworthy that the initial average concentration of DTZ for the 36 cases was 8.4 mg/L with a CV of 10.6%. After three months of storage at 4°C, the average concentration decreased to 3.1 mg/L with an extremely high CV of 58.7%. The average of the combined concentrations of DTZ and DAD, however, was 8.1 mg/L with CV of 6.91%.

Data on the stability of DTZ in postmortem blood have not been

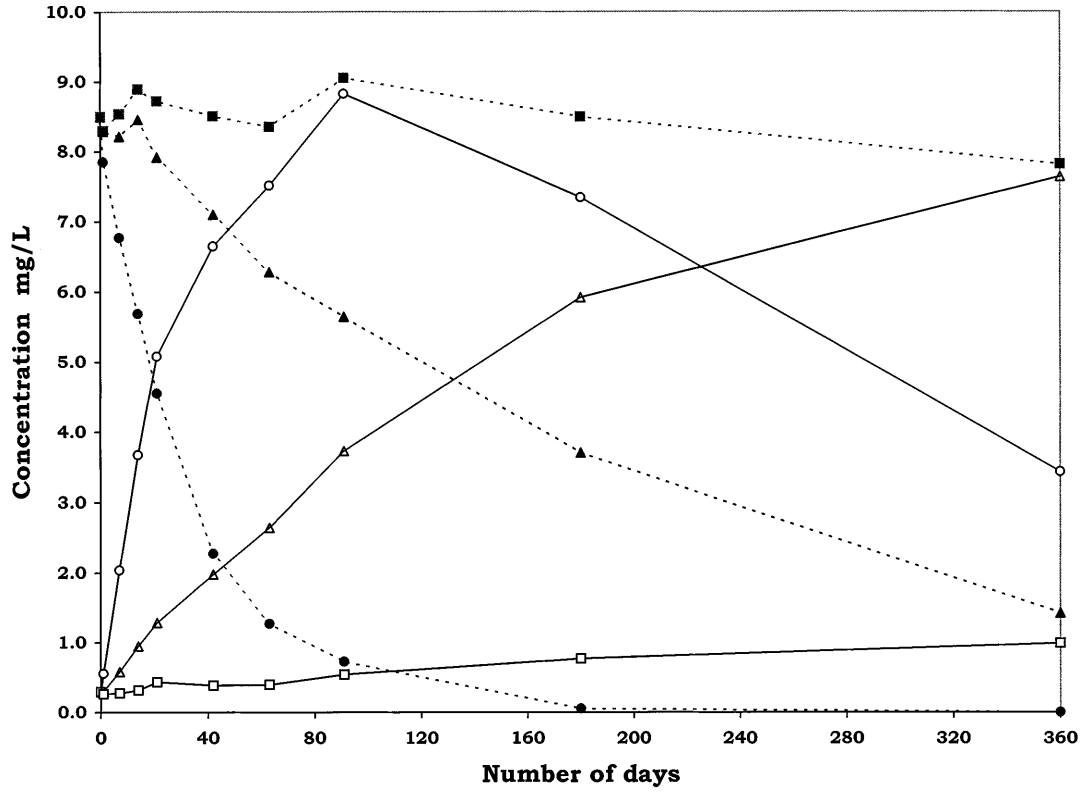

FIG. 4—*Mass balance plot of diltiazem hydrolysis to desacetyldiltiazem in preserved Red Cross blood stored at room temperature: (···●···) DTZ and (—○—) DAD, at 4° C: (···▲···) DTZ and (—△—) DAD and at −20° C: (···■···) DTZ and (—□—) DAD from day 1 to 360 days.*

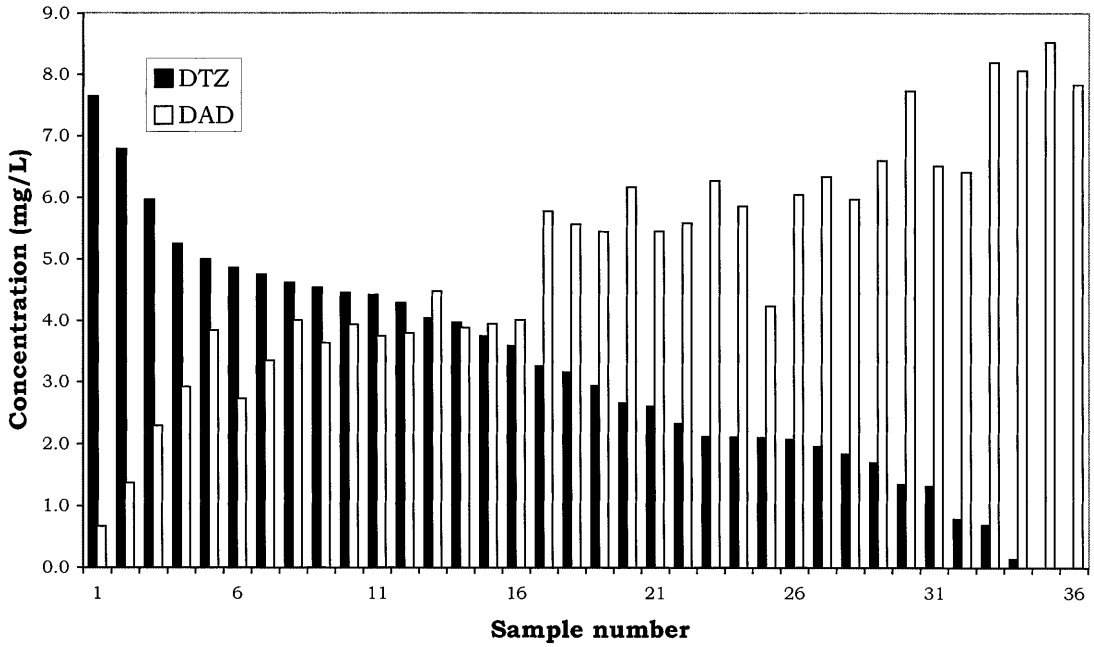

FIG. 5—*Stability of diltiazem in spiked (8.0 mg/L) postmortem blood samples stored at 4°C and analyzed after 92 days. N = 36.*

reported previously, and this study shows that degradation of DTZ is more rapid in postmortem blood at 4°C than in Red Cross blood. It also suggests that a portion of the measured concentration of DAD in postmortem blood may originate from the initial concentration of DTZ, if quantitation of DTZ is not performed properly or blood samples are not stored in the freezer until assayed for DTZ.

Forensic Cases

Possible combinations of DTZ and its metabolites in postmortem blood are illustrated in Figs. 6 and 7. Diltiazem concentration in Fig. 6*A* was 0.21 mg/L and three of its metabolites (M_A, DAD, and M_2) were approximately the same concentrations. In Fig. 6*B*,

concentrations of DTZ and DAD were 0.68 and 0.22 mg/L, respectively. Neither M_A nor M_2 were detected. Other finding for this case included lorazepam 156 ng/mL, ethyl alcohol 0.26 g/dL, and sertraline 0.1 mg/L.

The concentrations of DTZ and DAD in Fig. 7*C* were 8.5 and 14.0 mg/L, respectively. In Fig. 7*D*, the concentrations of DTZ and DAD were 22.0 and 6.5 mg/L, respectively. Other finding for this case included 6.4 mg/L diphenhydramine.

It is interesting to note that one of the cases (Fig. 7*C*) was analyzed after 92 days, the other case (Fig. 7*D*) after 10 days. It is obvious that the high concentration of DAD (Fig. 7*C*)—16 times greater than the concentration of the parent drug—is the result of hydrolysis of DTZ in vitro. It is also evident that the high concentration of M_2 is the hydrolysis product of M_A in vitro.

The authors' laboratory reported 55-positive DTZ cases in the past three years. The concentrations of DTZ for 25 cases were within the accepted therapeutic range. The median for DTZ was 0.14 mg/L and ranged from 0.01 to 0.45 mg/L. The combined concentrations of DTZ and DAD ranged from 0.14 to 0.76 mg/L.

Blood concentrations of DTZ and DAD that were in excess of therapeutic or possibly toxic are listed in Table 1. Based on our stability study, it is reasonable to assume that a portion of DAD has originated from DTZ during the storage period at 4°C. Desacetyldiltiazem is detectable in the plasma within 30 min after

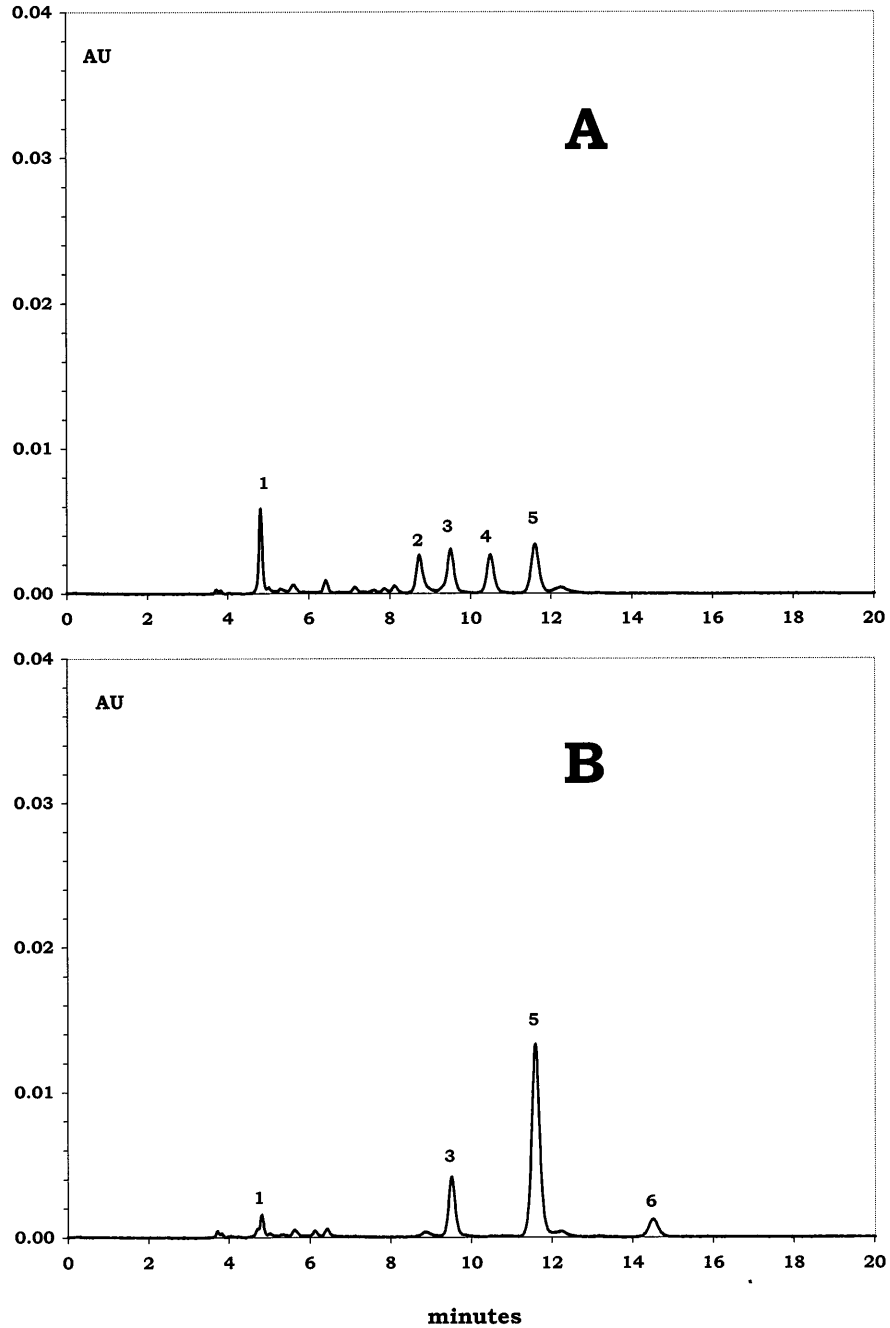

FIG. 6—*Chromatograms obtained from extracts of two forensic blood samples (A and B). Peaks: 1 = caffeine; 2 = M_2; 3 = DAD; 4 = M_A; 5 = DTZ; and 6 = sertraline.*

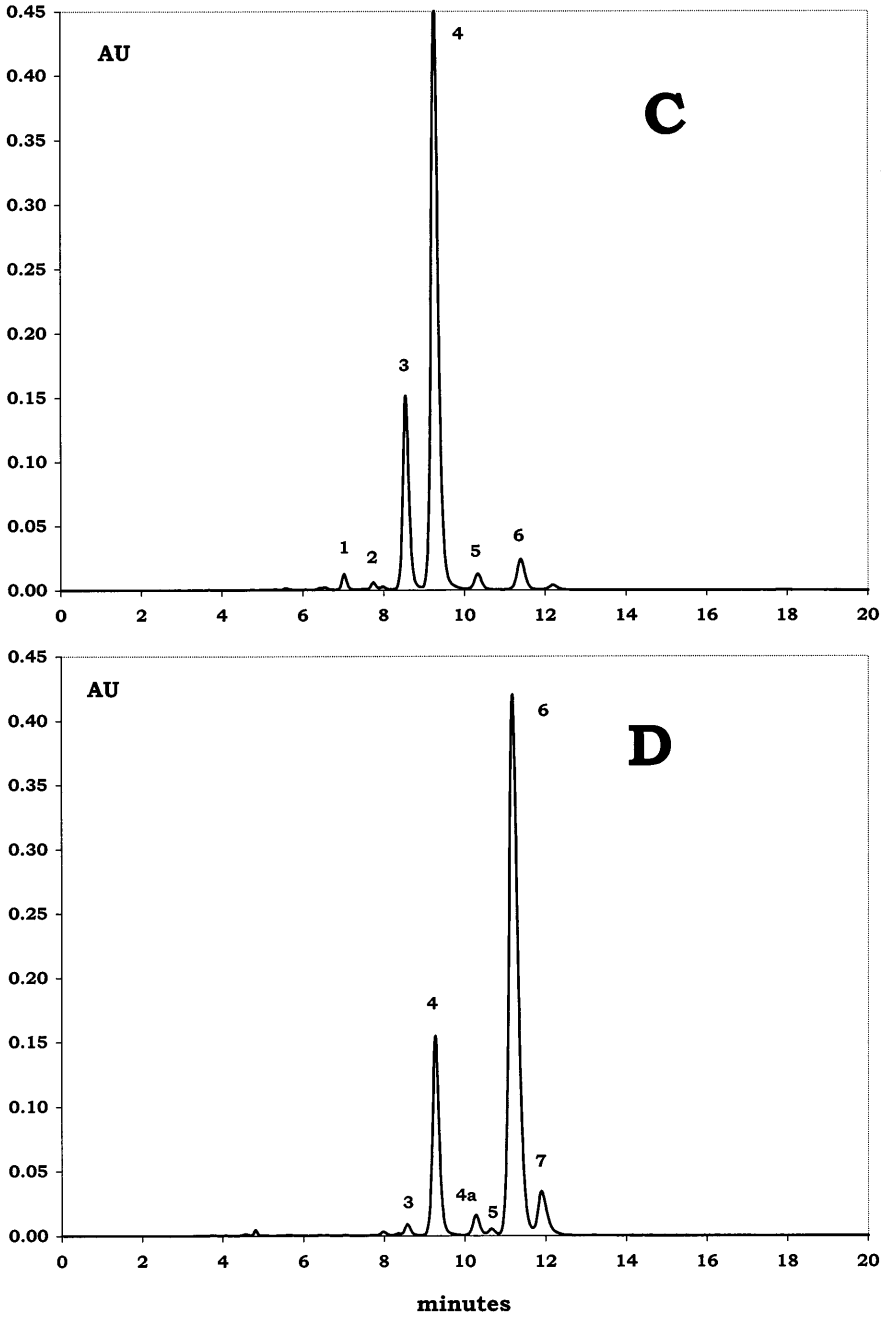

FIG. 7—*Chromatogram obtained from extracts of two forensic blood samples (C and D). Peaks: 1 and 2 = DTZ metabolites (not identified); 3 = M_2; 4 = DAD; 4a = Nordiphenhydramine; 5 = M_A; 6 = DTZ; and 7 = diphenhydramine.*

oral ingestion, reaching concentrations of 10% to 30% of the parent drug in the first 6 to 8 h (13,26). However, there are only three cases, numbers 1, 6, and 10 in Table 1, where DAD concentrations are within this range. All others are significantly higher.

Reported DTZ concentrations in nonfatal overdoses ranged from 0.56 to 6.1 mg/L (27). Fatalities involving DTZ have been reported with postmortem blood concentrations in the range of 1.5 to 15.0 mg/L (27–31).

Currently there are two published reports of DTZ overdose where DAD was also analyzed. One of the cases involved a 51-year-old male who took an overdose of 150 sixty-mg DTZ tablets. The patient survived. Concentrations of DTZ and DAD 2.5 h after ingestion of DTZ were 4.5 and 0.9 mg/L, respectively (32). In the other report, postmortem blood concentrations of DTZ and DAD were 15.0 and 12.0 mg/L, respectively (31).

Two of the most unusual cases we had were numbers 25 and 26 in Table 1. The case history in case number 25 involved a 57-year-old male who was found unresponsive in bed and who had left a suicide note. The other case involved a 24-year-old female who took an overdose of 52 Cardizem® SR90 (Diltiazem) and 60 Zantac® (Ranitidine) at approximate 4:00 a.m. On admission to hospital, at 9:18 a.m., she was alert but with a blood pressure of 70/32 mm Hg. Despite the extensive treatment, she developed grand mal seizures and died at 10:00 a.m. on the following morning with cardiac arrhythmia. DTZ concentration in case number 25 was 0.85 mg/L, and altogether not detected in case number 26.

However, DAD concentrations were 14.0 and 17.0 mg/L in case numbers 25 and 26, respectively. A general screening procedure revealed no other significant findings in either case. The coroner concluded that based on the toxicological findings, death was due to cardizem intoxication in both cases.

It is noteworthy that the presence of DTZ was detected in the initial gas chromatographic screening and confirmed by gas chromatography/mass spectrometry (GC/MS) for case number 26. The quantitative assay, however, was done three months later by HPLC and DTZ was not detected. It is also unlikely that a DTZ concentration of 0.85 mg/L in case number 25 was the cause of death. The only reason that could be accepted was that DTZ hydrolyzed to DAD during the three-month storage at 4°C.

TABLE 1—*Diltiazem (DTZ) and desacetyldiltiazem (DAD) concentrations in forensic blood samples.*

No.	DTZ (mg/L)	DAD (mg/L)	DTZ + DAD (mg/L)	Ratio DAD/DTZ	Days*
1	22.00	6.50	28.50	0.30	10
2	2.30	4.40	6.70	1.91	20
3	2.10	2.10	4.20	1.00	24
4	0.48	0.37	0.85	0.77	25
5	1.90	2.60	4.50	1.37	25
6	0.68	0.22	0.90	0.32	26
7	4.30	na	—	—	26
8	2.10	1.60	3.70	0.76	29
9	2.50	5.30	7.80	2.12	30
10	3.90	1.30	5.20	0.33	34
11	2.40	3.50	5.90	1.46	34
12	29.00	44.00	73.00	1.52	35
13	11.00	42.00	53.00	3.82	35
14	0.36	0.55	0.91	1.53	43
15	19.10	9.70	28.80	0.51	46
16	1.60	5.70	7.30	3.56	46
17	1.10	9.20	10.30	8.36	48
18	5.70	4.40	10.10	0.77	55
19	3.70	5.00	8.70	1.35	55
20	0.41	0.49	0.90	1.20	58
21	0.92	2.60	3.52	2.83	61
22	1.10	1.70	2.80	1.55	62
23	2.40	7.40	9.80	3.08	62
24	0.11	0.72	0.83	6.55	76
25	0.85	14.00	14.85	16.47	92
26	nd	17.00	17.00	—	92
27	0.19	0.84	1.03	4.42	106
28	1.30	9.30	10.60	7.15	109
29	10.30	10.00	20.30	0.97	116
30	0.23	0.72	0.95	3.13	159

*Time elapsed between analysis and arrival of sample, stored at 4°C in refrigerator; na = not analyzed due to co-eluting peak (proparanolol); nd = not detected.

Stability of DTZ in Forensic Cases After Repeated Analysis

The results of five forensic cases are listed in Table 2 and illustrated in Figs. 8–10. Concentrations of DTZ were found to be 29.0 mg/L on the first analysis and 8.6 mg/L in the second (Fig. 8). The concentration of DAD increased from 44.0 to 62.8 mg/L during the two-month storage at 4°C. Based on the results of the stability study, it is not surprising that the combined concentration of DTZ and DAD remained the same for both analyses: 73.0 mg/L on the first and 71.4 mg/L on the second. Several metabolites were also detected based on similar spectral properties as DTZ. Two of these metabolites were identified as N-desmethyldiltiazem (M_A), peak number 7 and deacetyl-N-desmethyldiltiazem (M_2), peak number 5. It is noteworthy that the ratio of M_2/M_A increased after two months probably due to the deacetylation of M_A in vitro. In the second case that was repeated (Fig. 9), the concentration of DTZ was found to be 5.6 mg/L in the first analysis and 2.7 mg/L after three months. The concentration of DAD increased from 4.4 to 7.4 mg/L and the combined concentrations of DTZ and DAD were 10.0 and 10.1 mg/L in the first and second analyses, respectively. The ratio of M_2/M_A (peak numbers 3 and 5) also increased during the three-month storage.

These two cases were similar with respect to deacetylation of DTZ and M_A and the increase in blood concentration of DAD and M_2. The difference in storage time between the first and second analyses, however, provided some interesting results. The loss of DTZ was 29% in case one (Fig. 8), which was reanalyzed after two months, and 48% in the second case (Fig. 9), which was repeated after three months.

Unless reopened, forensic cases are generally not analyzed after one year. To confirm our observations, however, the quantitation of DTZ was repeated for a case blood following storage at 4°C and −20°C for one year (Fig. 10). Diltiazem was detected in the blood samples regardless of the storage temperature at the end of one year. The loss of DTZ, however, was 83% when stored at 4°C and 29% at −20°C. The reduction in DTZ concentration in blood was associated with an increase in concentration of DAD. The combined blood concentrations of DTZ and DAD after one year, at both storage temperatures, did not differ significantly from the value obtained in the initial analysis; they were 3.7, 4.2 and 3.4 mg/L. The deacetylation of M_A to M_2 (peak numbers 5 and 3, respectively) was more pronounced at 4°C compared with that at −20°C. This is shown in chromatograms B and C in Fig. 10. The M_2/M_A peak area ratio was 10.7 for 4°C and 0.98 for the −20°C storage conditions.

The present data do not permit extensive conclusions regarding the toxicological interpretation in death investigations where diltiazem is implicated. Our findings, however, demonstrate that quantitative results for DTZ and DAD in postmortem blood should be

TABLE 2—*Concentration of diltiazem (DTZ) and desacetyldiltiazem (DAD) in forensic cases: repeated analysis.*

No.	DTZ	DAD (mg/L)	Total*	T2—T1†	DTZ	DAD (mg/L)	Total*	T3—T2‡
1	2.10	1.60	*3.70*	29 days	0.36	3.88	*4.24*	> 1 year
2	2.30	4.40	*6.70*	20 days	1.05	5.38	*6.43*	6 months
3	5.60	4.40	*10.00*	55 days	2.70	7.40	*10.10*	3 months
4	11.00	42.00	*53.00*	35 days	1.10	48.80	*49.90*	3 months
5	29.00	44.00	*73.00*	35 days	8.60	62.80	*71.40*	2 months

†T2—T1 = time elapsed between first analyses and arrival of sample.
‡T3—T2 = time elapsed between second and first analyses.
*Combined concentration of diltiazem and desacetyldiltiazem.

reviewed carefully and assessed against the possible conversion of DTZ to DAD in vivo and in vitro. Although different combinations of anticoagulants and preservatives, or lack thereof, were not studied in this work, it is most likely that lengthy postmortem interval or long storage time even at 4°C would still result in the increased conversion of DTZ to DAD in postmortem blood. It is beyond the scope of this study to suggest precise mechanisms for this conversion.

When the DAD concentration in postmortem blood is far in excess of a therapeutic concentration, postmortem conversion should be considered, in addition to any antemortem toxicokinetics that may lead to an excessive DAD concentration. When both DTZ and DAD concentrations are far in excess of therapeutic, toxicologists should consider summing both concentrations to estimate the total body load of diltiazem and its toxic impact, since the present findings suggest that the stoichiometry of in vitro conversion of DTZ to DAD is approximately one to one. By corollary, the absence of DTZ in combination with DAD may not always rule out the recent ingestion of DTZ just prior to death.

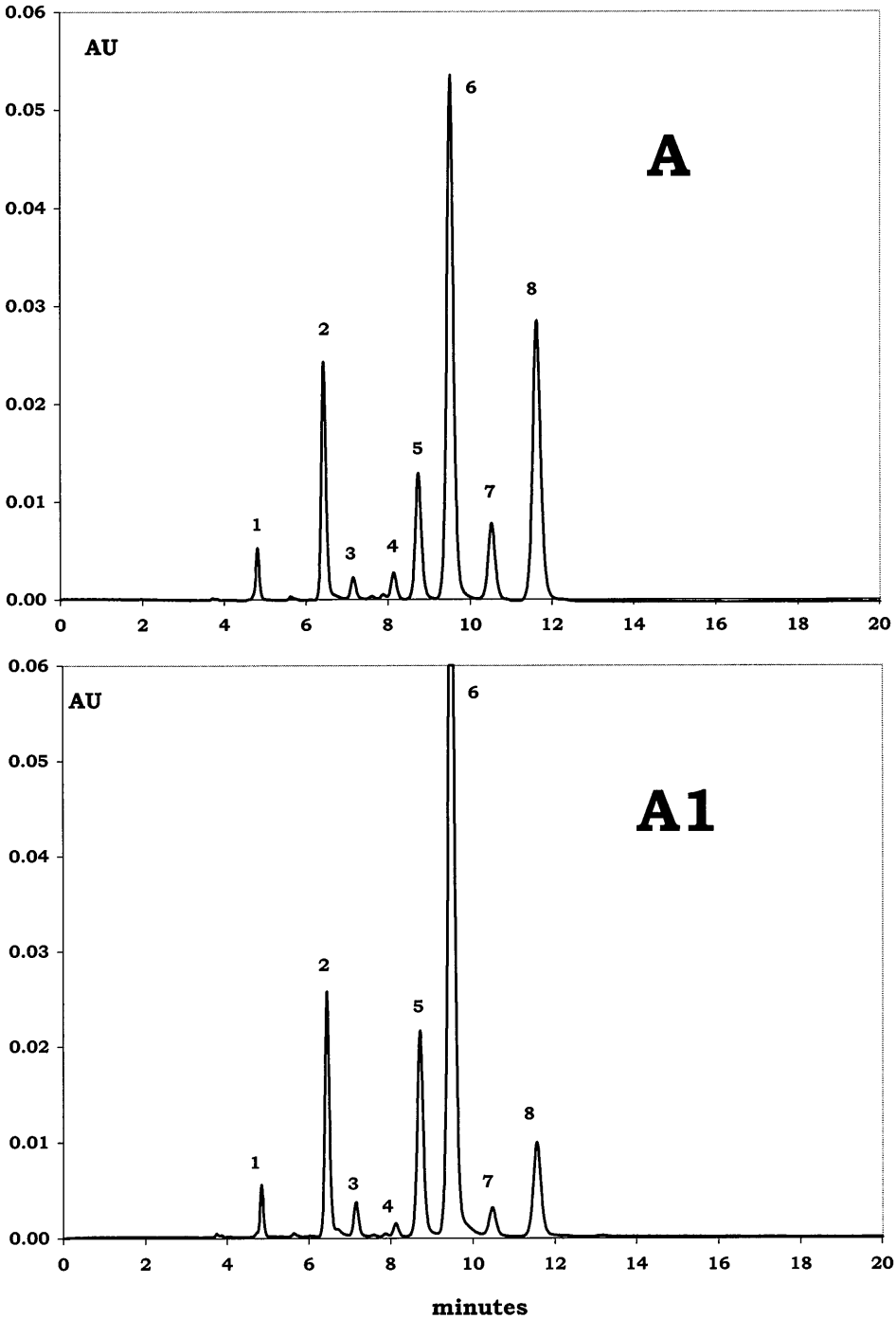

FIG. 8—*Chromatograms obtained from extracts of a forensic blood sample. Chromatogram A = first analysis and A1 = stored at 4°C for 95 days. Peaks: 1 = caffeine; 2 = codeine; 3 and 4 = DTZ metabolites (not identified); 5 = M_2; 6 = DAD; 7 = M_A; and 8 = DTZ.*

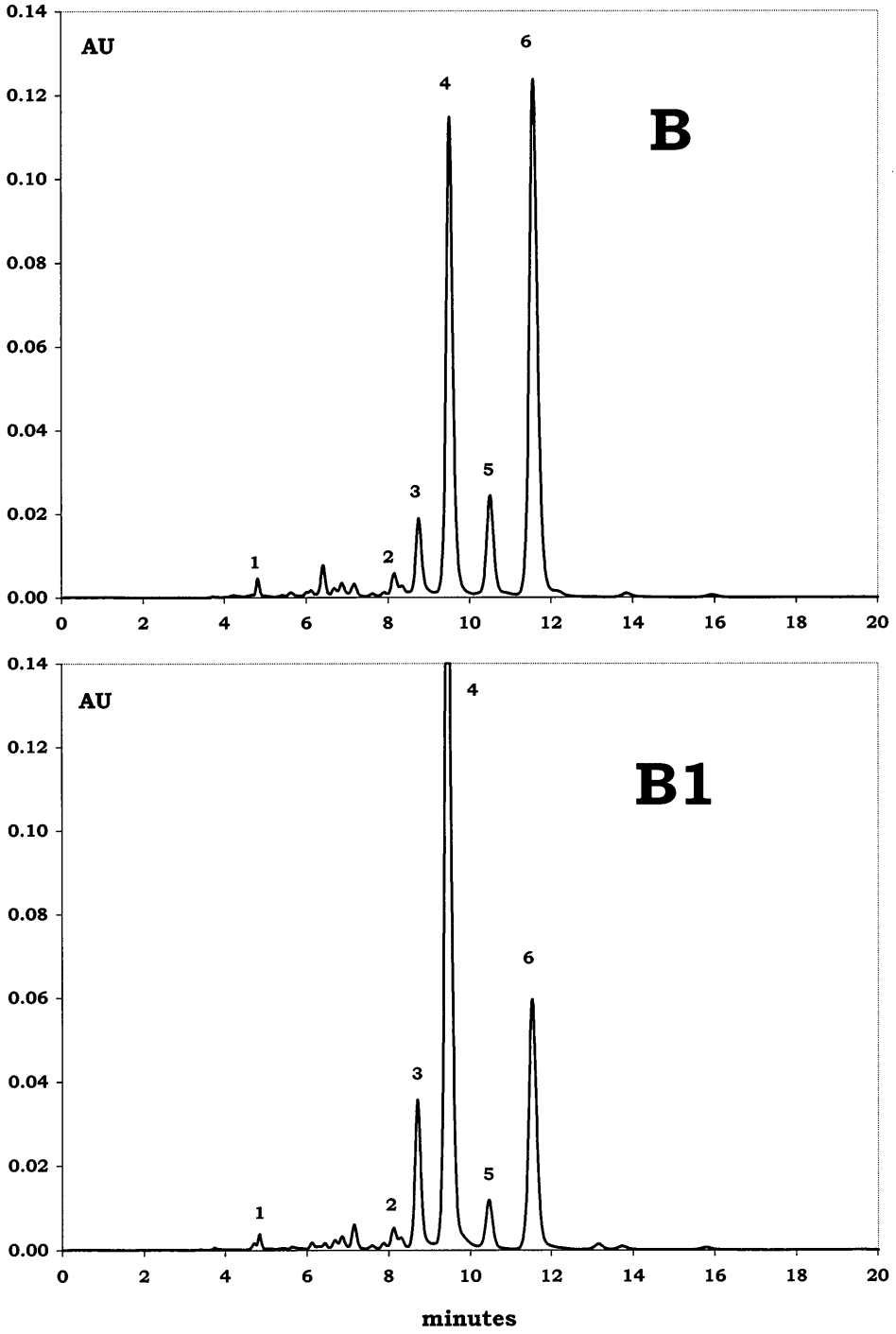

FIG. 9—*Chromatograms obtained from extracts of a forensic blood sample. Chromatogram B = first analysis and B1 = stored at 4°C for 122 days. Peaks: 1 = caffeine; 2 = DTZ metabolite (not identified); 3 = M_2; 4 = DAD; 5 = M_A; and 6 = DTZ.*

Conclusion

Diltiazem was found to be stable in whole blood over a six-month period when stored at −20°C with recovery of 98%. By contrast, approximately 50% of DTZ was lost after 19 and 124 days when stored at room temperature and 4°C, respectively. The reduction in concentration of DTZ was accompanied by a comparable increase in DAD concentrations. Similar changes in DTZ concentrations were seen in spiked postmortem blood stored for 92 days at 4°C. The extent of loss of DTZ, however, varied greatly from complete to negligible.

Repeated analyses of forensic cases stored at 4°C have proved that the initial concentrations of DTZ could rapidly decrease and concentrations of DAD will increase accordingly within two months. In addition, the results of 55 positive DTZ cases have indicated that DAD concentrations are significantly higher and more variable in postmortem blood as compared with those in plasma or whole blood obtained from healthy volunteers who received DTZ, or from patients at steady state. The rate of DTZ hydrolysis over time is probably dependent on temperature and, although not studied here, may also be dependent on the action of

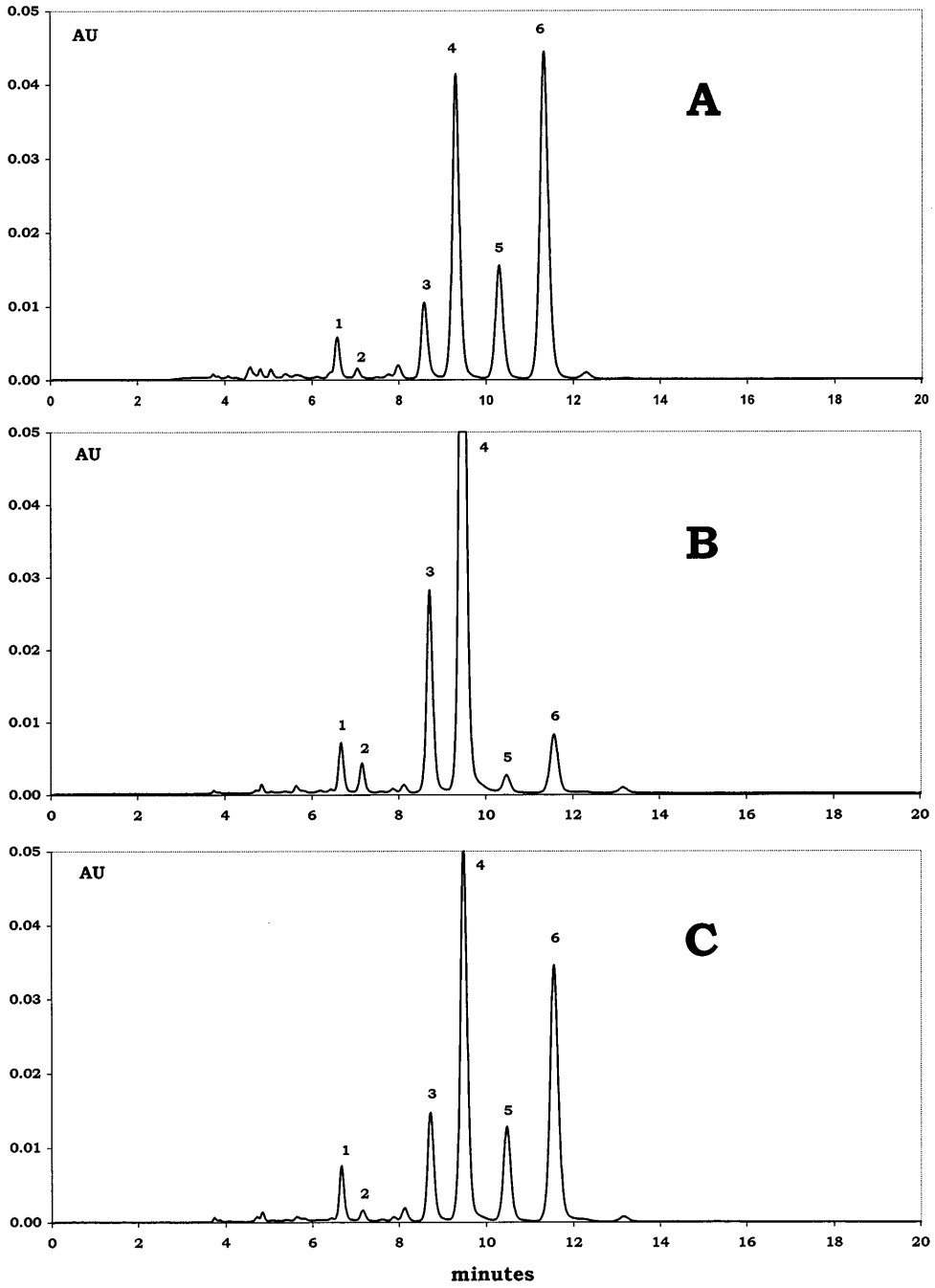

FIG. 10—*Chromatogram obtained from extracts of a forensic blood sample. Chromatogram A = first analysis, B = stored at 4°C for 1 year and C = stored at −20°C for 1 year. Peaks: 1 = acebutolol; 2 = DTZ metabolite (not identified); 3 = M_2; 4 = DAD; 5 = M_A; and 6 = DTZ.*

esterase enzymes produced by bacterial, which proliferate during the putrefactive process in postmortem blood.

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