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# Studies on the Stability and Detection of Cocaine, Benzoylecgonine, and 11-Nor-Delta-9-Tetrahydrocannabinol-9-Carboxylic Acid in Whole Blood Using Abuscreen® Radioimmunoassay

**REFERENCE:** McCurdy, H. H., Callahan, L. S., and Williams, R. D., **"Studies on the Stability and Detection of Cocaine, Benzoylecgonine, and 11-Nor-Delta-9-Tetrahydrocannabinol-9-Carboxylic Acid in Whole Blood Using Abuscreen® Radioimmunoassay,"** *Journal of Forensic Sciences*, JFSCA, Vol. 34, No. 4, July 1989, pp. 858-870.

**ABSTRACT:** A study was undertaken to assess the stability and the radioimmunoassay (RIA) detection of cocaine, benzoylecgonine (BZE), and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in whole blood while stored in 4 different kinds of blood collection tubes for up to 30 days at refrigeration and room temperatures. At various intervals, the tubes were sampled and analyzed using Abuscreen® RIA. Also, semi-quantitative data derived from RIA analysis of forensic blood specimens were compared with quantitative data acquired using gas chromatography (GC) or GC/mass spectrometry (GC/MS) on the same specimens. RIA and chromatographic studies revealed that BZE and THC-COOH were stable in blood under all conditions studied. Cocaine, however, was found not to be stable in blood, especially when stored at room temperatures. Despite cocaine's instability in blood, RIA was able to detect the presence of cocaine and its breakdown products in blood under all conditions studied.

**KEYWORDS:** toxicology, radioimmunoassay, blood, cocaine, benzoylecgonine, 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid, chemical analysis

In addition to the detection of drugs in urine, radioimmunoassay (RIA) has been applied to the detection of drugs of abuse in other biological samples, particularly whole blood. For example, Spiehler and Sedgwick [*1*] have examined postmortem blood specimens for cocaine/benzoylecgonine, opiates, phencyclidine, and barbiturates using RIA. Childs and McCurdy [*2*] reported the successful application of RIA to the detection of cannabinoids in whole blood. Others have used RIA for the detection of cocaine [*3*] and amphetamines [*4*] in bloodstains. These studies suggest that many drugs are sufficiently stable in blood to allow RIA detection of these drugs in blood for significant lengths of time under a variety of conditions.

Presented at the 40th Annual Meeting of the American Academy of Forensic Sciences, 15-20 Feb. 1988, Philadelphia, PA. Received for publication 3 Aug. 1988; revised manuscript received 11 Oct. 1988; accepted for publication 1 Nov. 1988.

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A study was undertaken to examine the stability and RIA detection of three toxicologically significant compounds, cocaine, benzoylecgonine (BZE), and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), in whole blood while being stored in 4 kinds of blood collection tubes at room and refrigeration temperatures for up to 30 days. Also, semiquantitative RIA data derived from forensic blood specimens were compared with data obtained from gas chromatography/mass spectrometry (GC/MS) or gas chromatographic/nitrogen-sensitive detector (GC/NPD) analysis or both performed on the same specimens.

The four kinds of commonly used blood collection tubes used in the study were as follows: purple-stoppered tubes (containing ethylenediaminetetraacetate [EDTA]), green stoppered tubes (containing heparin), gray-stoppered tubes (containing sodium fluoride), and red-stoppered tubes (containing no anticoagulant or preservative). The influence of the various blood collection tubes could have an effect on the RIA detection of the compounds in at least two different ways. The first is the influence of various anticoagulants or preservatives on the stability of the compounds in blood and thus on RIA detection of the compounds. The second effect is possible absorption of the compounds onto the tube's stoppers. To study these and other possible effects, we added measured quantities of cocaine, BZE, and THC-COOH to blood stored in the four kinds of tubes at both refrigeration and room temperatures for a period of up to 30 days. At specified intervals, the contents of the tubes were sampled and analyzed by RIA.

## Experimental Procedure

### *Radioimmunoassay*

Abuscreen® RIA kits for cocaine metabolite (BZE) and cannabinoids (THC-COOH) were provided by Roche Diagnostics Systems, Nutley, New Jersey. The procedure for analysis of cocaine or BZE or both in whole blood was generally the same as that described in the package insert for urine analysis, except that the sample sizes used were 50 $\mu$L of blood and RIA reagent volumes were reduced by half. It was noted during the course of this study that the RIA cocaine metabolite assay had a slightly greater sensitivity for cocaine than for BZE. The procedure used for RIA analysis of THC-COOH in blood was the same as that previously described [2]. Pure cocaine, BZE, and THC-COOH standards were obtained from Applied Science, State College, Pennsylvania. Outdated blood obtained from the Red Cross was used to prepare the various blood reference solutions. The blood collection tubes (Vacutainer®, Rutherford, New Jersey) used in the study were the 7-mL purple- (or lavender-) stoppered tubes containing 10.5 mg of EDTA, 7-mL gray-stoppered tubes containing 30 mg of sodium fluoride, 7-mL green-stoppered tubes containing 143 USP units of sodium heparin, and 7-mL red-stoppered tubes containing only a silicone-coated stopper.

Blood containing 200 ng/mL of cocaine was stored in ten each of the four different kinds of Vacutainer tubes. Benzoylecgonine in blood (200 ng/mL) and THC-COOH in blood (100 ng/mL) were also stored in ten of each of the four different kinds of Vacutainer tubes. The tubes were divided such that one half ($n = 5$) was kept at room temperature and one half ($n = 5$) was stored at refrigerated temperature (Table 1). The tubes were analyzed by RIA on Days 0, 1, 3, 5, 10, or 12 (depending upon the analyte), 21 and 30 (hereafter referred to as the "stored blood samples"). For comparison purposes, freshly prepared blood reference solutions were also analyzed just before the analysis of the stored blood samples. The cpm data for each set of five tubes was averaged and their respective $B/B_0$ values calculated.

In a parallel, but somewhat more limited study, blood standard curves for cocaine, BZE, and THC-COOH were prepared in duplicate and analyzed over the same 30-day period. Thus, 6-point blood standard curves for cocaine and BZE were prepared at concentrations of 0, 50, 100, 200, 300, and 400 ng/mL and were stored in gray- and red-stoppered tubes at refrigerated temperatures. No RIA studies were conducted on blood samples stored in green-

TABLE 1—*Preparation of spiked, whole blood samples for room-temperature study. The same series of blood samples was prepared for the refrigeration temperature study.*[a]

| | Purple (EDTA) | Green (Heparin) | Gray (NaF) | Red (None) |
|---|---|---|---|---|
| Cocaine, 200 ng/mL | $n = 5$ | $n = 5$ | $n = 5$ | $n = 5$ |
| BZE, 200 ng/mL | $n = 5$ | $n = 5$ | $n = 5$ | $n = 5$ |
| THC-COOH, 100 ng/mL | $n = 5$ | $n = 5$ | $n = 5$ | $n = 5$ |

[a] Analyzed on Days 0, 1, 3, 5, 10, 21, and 30.

and purple-stoppered tubes. Also, no room temperature studies were conducted. Cocaine and BZE standard curves were analyzed on Days 0, 1, 3, 5, 12, 21, and 30 (hereafter referred to as the "stored blood standard curves"). For comparison purposes, on each day of analysis freshly prepared cocaine and BZE standard curves were analyzed along with the stored blood standard curves.

Likewise, an eight-point THC-COOH blood standard curve was prepared at concentrations of 0, 10, 25, 50, 100, 150, 200, and 250 ng/mL. Standard curves for THC-COOH were analyzed in the same manner with the only exception that the analyses were performed on Day 10 instead of Day 12. Also, for comparison purposes, on each day of analysis a freshly prepared THC-COOH standard curve was analyzed along with the stored blood standard curves.

Forensic blood specimens were analyzed by RIA for cocaine or metabolites or both and for cannabinoids employing the same procedures used for the analysis of the stored blood samples. For cocaine or metabolites or both, RIA semi-quantitative data from forensic science specimens were obtained using blood cocaine reference solutions prepared at concentrations of 80, 200, and 300 ng/mL. The RIA data thus obtained were then compared with quantitative analyses on the same forensic blood specimens using GC/NPD. In a similar manner, RIA semi-quantitative data for total cannabinoids in forensic blood specimens were derived from THC-COOH reference solutions prepared in blood at concentrations of 25, 50, 100, and 250 ng/mL. At concentrations higher than 250 ng/mL, total cannabinoids in blood were determined by extrapolation. Cannabinoid RIA data were then compared with GC/MS quantitative analyses on the same forensic specimens.

### *Gas Chromatographic Studies on Cocaine and Benzoylecgonine*

Mepivacaine internal standard (5 μg) was added to 5 mL of blood in a 125-mL separatory funnel and extracted with approximately 80 mL of methylene chloride/isopropanol (8:2). The solvent was removed and evaporated to dryness. Benzoylecgonine was derivatized using 200 μL of 0.2 *M* tetramethylammonium hydroxide/dimethyl sulfoxide (1:1) and 100 μL of 1-iodoethane. Cocaine and ethyl derivatives of BZE were then extracted and analyzed according to a previously reported procedure [5]. Appropriate cocaine and BZE reference blood solutions were processed simultaneously.

Forensic blood specimens were analyzed for cocaine and BZE using a Varian 6000 capillary GC equipped with a nitrogen-sensitive detector (thermionic specific detector). The capillary column used was a DB-5, 25 m by 0.32 mm, on a programmed run from 120 to 275°C at 15°C per minute with helium as the carrier gas. Analysis of cocaine and the ethyl ester of BZE was by peak height ratios.

Stored blood samples containing cocaine and BZE were analyzed using a Varian 6000 gas chromatograph interfaced to a Model 700 Ion Trap Detector (ITD, Finnigan MAT, San Jose, California). The 25-m by 0.32-mm HP-5 capillary column was operated from 120° to

280°C at a 15°C per minute programmed run. The gas chromatograph was operated in the splitless mode. The capillary column was passed through the heated transfer line directly into the ITD, bypassing the use of an open-split interface. Helium was used as the carrier gas. Analysis of cocaine was by total ion current.

### *GC/MS Studies on THC-COOH*

The GC/MS analyses for THC-COOH and delta-9-THC were performed by Elsohly Labs, Inc., Oxford, Mississippi. Briefly, the procedure was to add 100 ng each of d3-THC-COOH and cannabinol (CBN) internal standards to 1 mL of blood and extract the sample with 3.0 mL of hexanes/ethyl acetate (9:1). The residue resulting from evaporation of the organic solvent contained delta-9-THC and CBN which were dissolved in 100 μL of *N*-(tert-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (TBDMS) and heated for 15 min at 75°C. Excess derivatizing reagent was removed by evaporation, and *iso*octane (20 μL) was added to the tube before GC/MS analysis. The aqueous layer from the first extract was then acidified and extracted with hexanes/ethyl acetate (9:1). The organic extract containing THC-COOH and d3-THC-COOH was dried under nitrogen. The residue was then derivatized with 200 μL of 25% tetramethylammonium hydroxide/dimethyl sulfoxide (1:20) and 50 μL of iodomethane. After 5 min, the reaction was quenched by the addition of 0.4 mL of hydrochloric acid and the mixture extracted with 1.0 mL of *iso*octane. The organic layer was transferred to a clean tube and evaporated to dryness. Twenty microlitres of *iso*octane was added to the residue just before GC/MS analysis. Appropriate blood reference solutions were carried simultaneously through the same procedure.

The GC/MS system used was a Hewlett-Packard 5890 gas chromatograph interfaced to an 5790A mass selective detector in the selected ion monitoring (SIM) mode. A 15-m by 0.25-mm DB-1 capillary column was operated isothermally at 250°C using helium as the carrier gas. The TBDMS derivatives of delta-9-THC and CBN were monitored at ions 371.25, 428.30, 374.25, and 431.30. The ions monitored for the methyl derivatives of THC-COOH and d3-THC-COOH were 313.20, 357.25, 372.25, 316.20, and 375.25, respectively.

## Results and Discussion

### *Stored Blood Samples Containing Cocaine, BZE, and THC-COOH*

Excellent RIA detection of THC-COOH in blood was achieved for the 4 types of Vacutainer tubes stored at room temperature for 30 days, as demonstrated by Fig. 1. All 4 curves demonstrated relatively constant $B/B_0$ values for the entire 30-day analysis period. The data indicate remarkably good stability of THC-COOH in blood at room temperature. These findings are in agreement with Johnson et al. [6], who noted little differences in the stability of THC-COOH in blood when stored at room temperature for periods of up to 6 months and then analyzed by GC/MS.

As might be expected under refrigerated storage conditions, excellent RIA detection for THC-COOH in blood was also obtained for the 4 Vacutainer tubes, as shown in Fig. 2. Nearly constant $B/B_0$ values were obtained during the 30-day analysis period.

Figures 3 and 4 show the detection by RIA for BZE in blood at room temperature and refrigerated temperature, respectively, for the 4 kinds of Vacutainer tubes during the 30-day study period. Relatively constant $B/B_0$ values were also obtained indicating good stability for BZE in blood with the exception of a slight, but unexplained rise in the $B/B_0$ value at Day 21 in the room-temperature study.

RIA detection for cocaine under refrigerated storage temperatures showed relatively constant $B/B_0$ values, as shown in Fig. 5. Cocaine stability in blood was confirmed by analysis of the blood samples after Day 12 of storage, which demonstrated that significant quantities

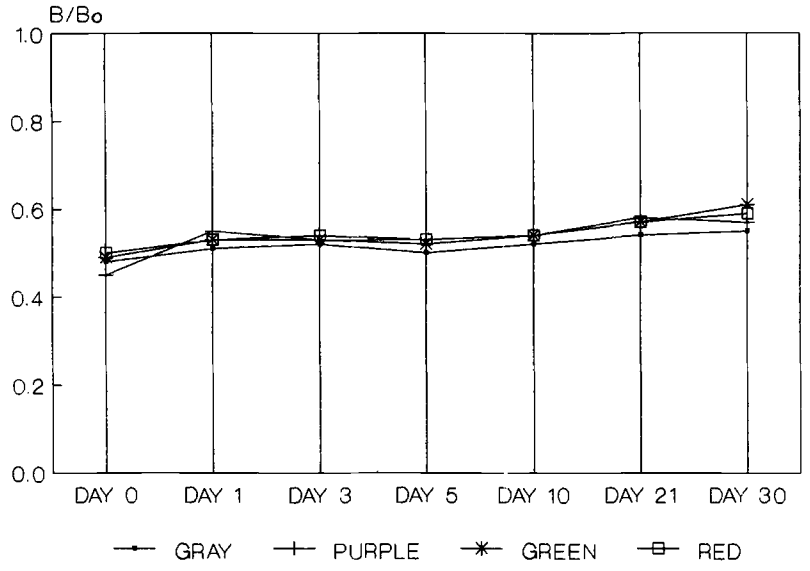


FIG. 1—$B/B_0$ *values for THC-COOH in each of the 4 Vacutainer blood tubes kept at room temperatures for 30-day analysis period. Every point on all curves is the average of 5 determinations.*

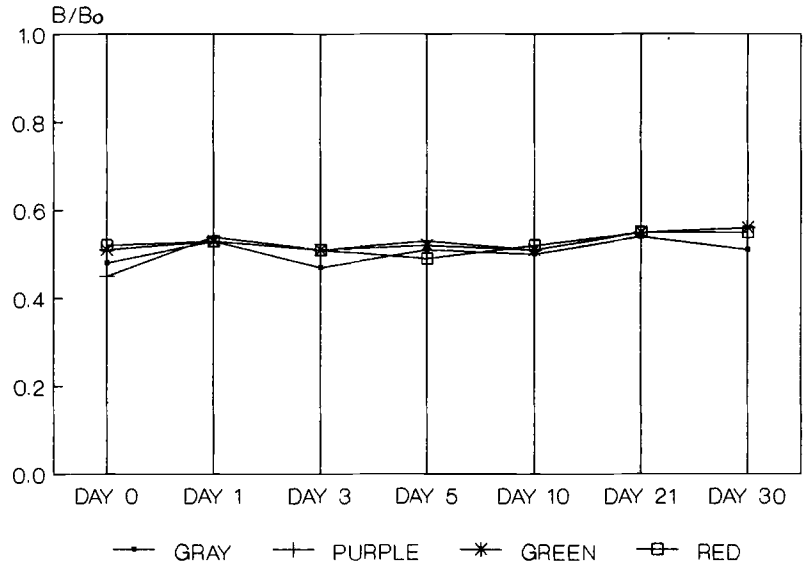


FIG. 2—$B/B_0$ *values for THC-COOH in each of the 4 Vacutainer blood tubes kept at refrigeration temperatures for 30-day analysis period. Every point on all curves is the average of 5 determinations.*

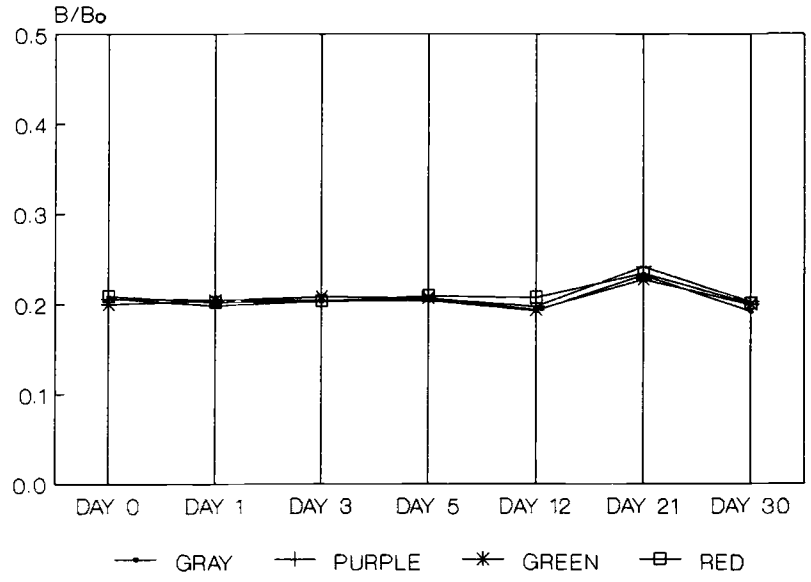


FIG. 3—$B/B_0$ *values for BZE in each of the 4 Vacutainer blood tubes kept at room temperatures for 30-day analysis period. Every point on all curves is the average of 5 determinations.*

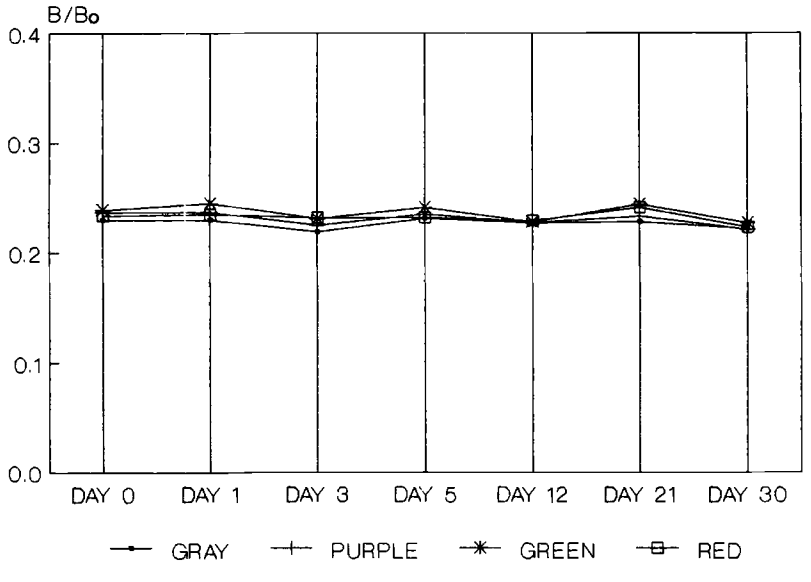


FIG. 4—$B/B_0$ *values for BZE in each of the 4 Vacutainer blood tubes kept at refrigeration temperatures for 30-day analysis period. Every point on all curves is the average of 5 determinations.*

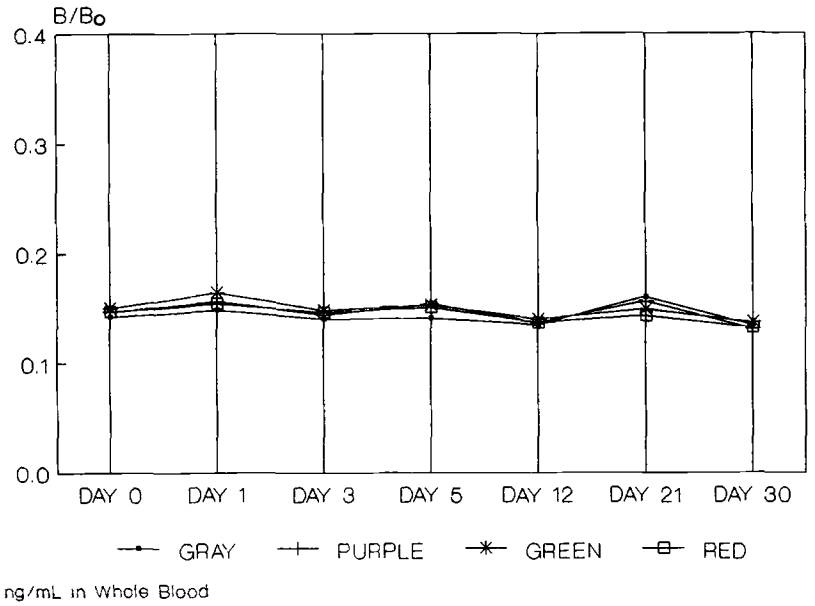


FIG. 5—$B/B_0$ *values for cocaine in each of the 4 Vacutainer blood tubes kept at refrigeration temperatures for 30-day analysis period. Every point on all curves is the average of 5 determinations.*

of cocaine were still present. Based on these results, the stability of cocaine under refrigerated conditions would seem largely unaffected by the presence or absence of anticoagulants or preservatives. This is in contrast to a study by Baselt [7], which noted that even under refrigeration conditions, the concentration of cocaine in blood preserved with sodium fluoride declines rapidly after about 8 days of storage, and without preservative, declines sharply after only 1 day of storage. Liu and others [8] also reported at refrigerated conditions, there was a 7% loss of cocaine after 1 day and 30% loss after 36 days of storage. Although no such rapid declines in cocaine concentrations were observed using RIA under refrigerated conditions, studies conducted on cocaine standard curves did reveal cocaine instability (see Standard Curves).

Figure 6 shows the $B/B_0$ values versus time for cocaine in blood while stored at room temperature in the four kinds of Vacutainer tubes. As might be expected, cocaine was observed to be unstable when stored under these conditions. This is consistent with two facts: cocaine is unstable even in water at pH values greater than neutrality and cocaine in blood is hydrolyzed to BZE at physiological pH by non-enzymatic means, a process that could certainly be exacerbated by storage at room temperatures [9].

As shown in Fig. 6, the purple-, green-, and red-stoppered tubes begin to exhibit dramatic increases in their respective $B/B_0$ values after Day 3 which continued to increase up to Day 30. GC/MS analysis of these blood samples confirmed that there were 50% or more losses of cocaine in each tube after 12 days of storage. Analysis of the gray-stoppered tubes, as evidenced by the relatively constant $B/B_0$ values during the 30-day period, appears to exhibit a high degree of cocaine stability. GC/MS analysis, however, revealed that there were significant losses of cocaine in the gray-stoppered tubes stored after 12 days of storage as well. The conversion of cocaine to ecgonine methyl ester (EME), but not BZE, is known to be solely a function of liver and plasma cholinesterases, which can be inhibited by freezing or by the addition of cholinesterase inhibitors, such as sodium fluoride, or both [10]. It is not known

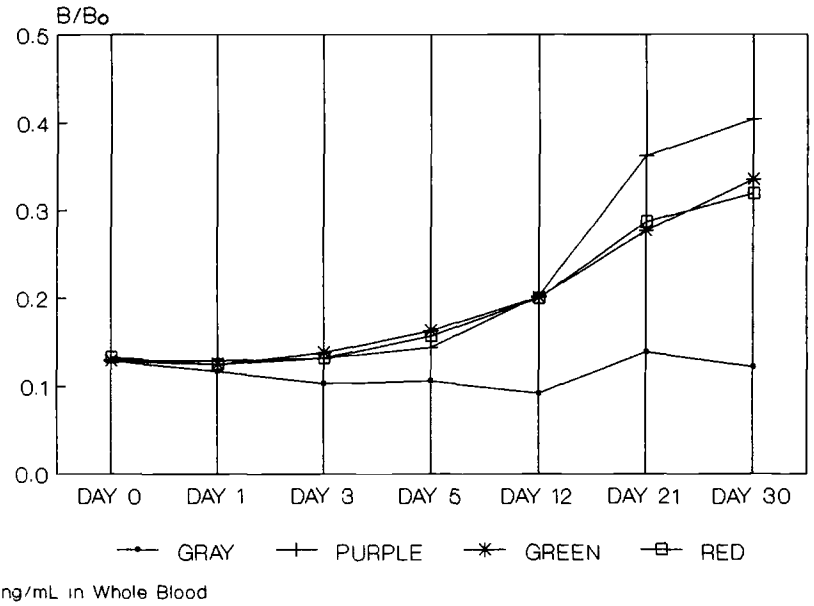


FIG. 6—$B/B_0$ *values for cocaine in each of the 4 Vacutainer blood tubes kept at room temperatures for 30-day analysis period. Every point on all curves is the average of 5 determinations.*

at present if decreased levels of EME due to the presence of sodium fluoride could account for the seemingly stable $B/B_0$ values observed for the 30-day period.

### *Blood Standard Curves for Cocaine, BZE, and THC-COOH*

Figure 7 shows the results of three standard curves for THC-COOH in gray-stoppered tubes that were obtained over the 30-day analysis period. For the purposes of clarity, curves generated on Days 0, 12 (or 10), and 30 only are shown, which are representative of all the curves obtained. The $B/B_0$ values for the THC-COOH blood standard curves are generally very consistent, which infers a high degree of stability. Standard curves generated for THC-COOH in blood stored in the red-stoppered tubes gave essentially identical results. Therefore, THC-COOH standard curves retain good stability in blood and do not appear in any way to be dependent on the type of blood collection container used.

The same results were obtained for BZE standard curves in blood stored in red-stoppered tubes at refrigeration temperatures, as shown in Fig. 8. As indicated by the relative sameness of the curves generated on Days 0, 12, and 30, the stability of BZE in blood is again demonstrated. The standard curves generated from BZE in the gray-stoppered tubes gave virtually identical results, again showing that the type of blood collection tube is obviously not a factor in BZE stability.

Figure 9 shows the cocaine standard curve stored in gray-stoppered tubes at refrigerated temperatures. As can be seen, the curves are not identical. Blood cocaine standard curves stored in red-stoppered tubes at refrigerated temperatures show even less consistency (Fig. 10) beginning around Day 12. Thus, data from RIA and other analytical techniques would suggest that cocaine in blood should not be considered stable for more than a few days even when stored in sodium fluoride-containing tubes and kept at refrigerated temperatures. However, it has been clearly demonstrated that under all storage conditions studied, degra-

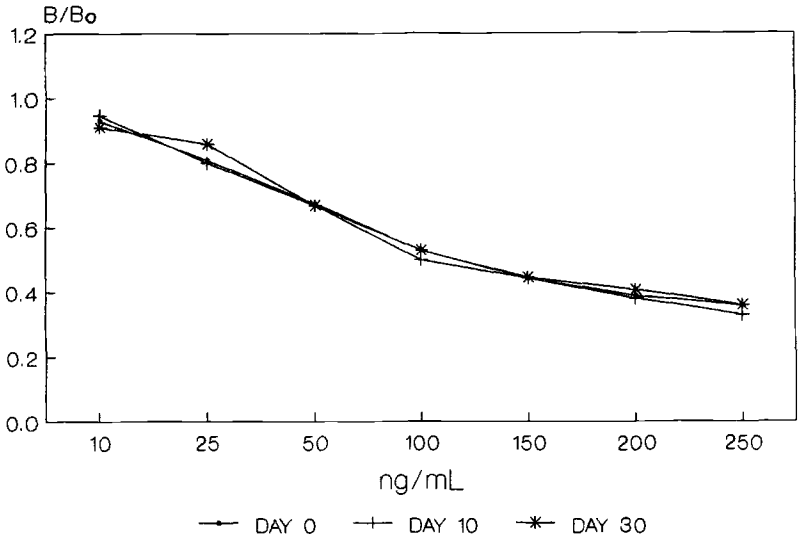


FIG. 7—*Standard curves for THC-COOH in whole blood stored in gray-stoppered tubes for 30 days. Shown are the curves generated on Days 0, 10, and 30.*

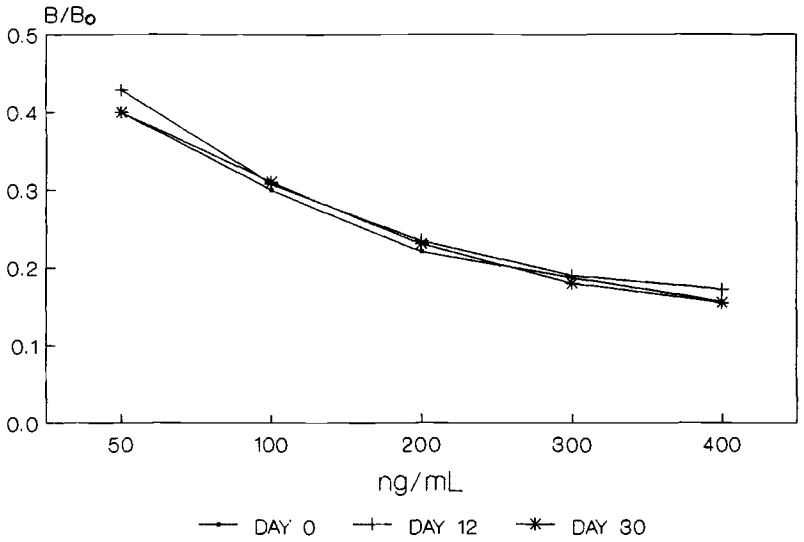


FIG. 8—*Standard curves for BZE in whole blood stored in red-stoppered tubes for 30 days. Shown are the curves generated on Days 0, 12, and 30.*

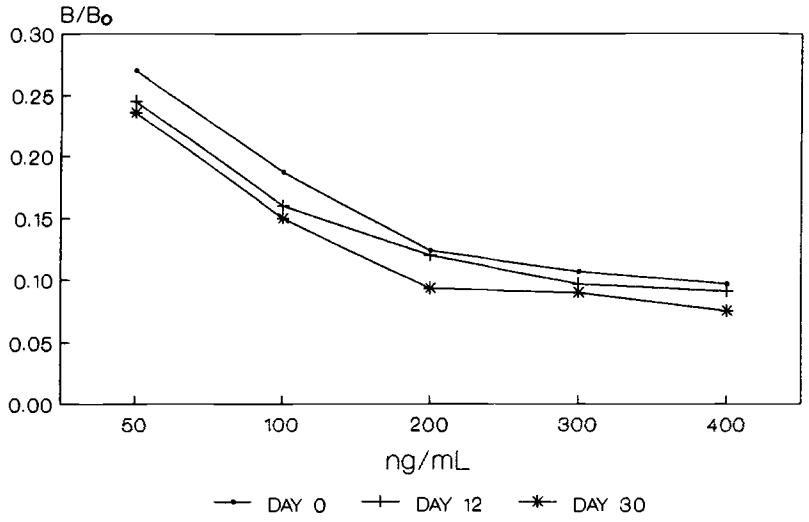


FIG. 9—*Standard curves for cocaine in whole blood stored in gray-stoppered tubes for 30 days. Shown are the curves generated on Days 0, 12, and 30.*

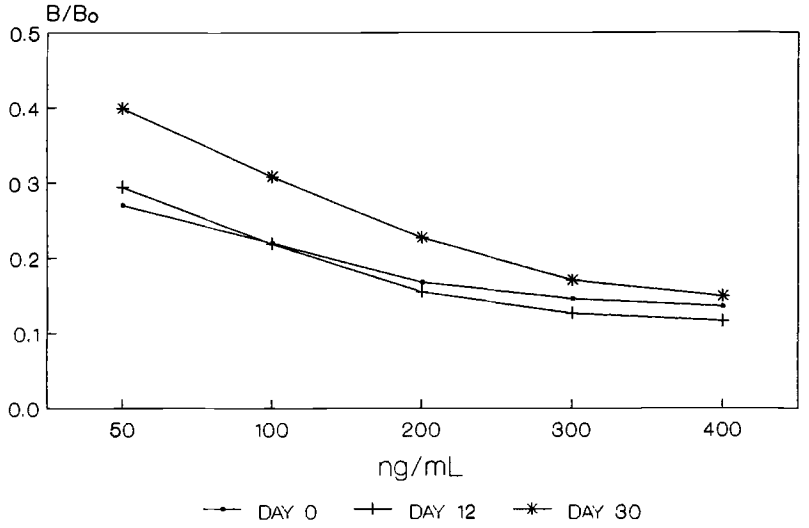


FIG. 10—*Standard curves for cocaine in whole blood stored in red-stoppered tubes for 30 days. Shown are the curves generated on Days 0, 12, and 30.*

dation products resulting from the instability of cocaine in blood (BZE, EME, and so forth) remain fully detectable by RIA even at concentrations as low as 50 ng/mL.

### *Comparison of RIA Data with Other Analytical Data*

A comparison was made between 25 forensic blood specimens analyzed by RIA to those analyzed by GC/NPD. Generally, the concentrations of cocaine and benzoylecgonine were found to be much too high to make any direct comparison between the two techniques. It

was noted, however, that if the RIA result were higher than 300 ng/mL, the quantitative results from GC/NPD were also likely to be comparably high. It was observed also that if the RIA semi-quantitative values for cocaine or its metabolites or both were less than 80 ng/mL, the probability was greatly increased that a reliable quantitative result could not be obtained by most other chromatographic techniques. Thus, RIA analysis has proven quite valuable in predicting probable success or failure for cocaine or BZE confirmations or both.

Table 2 compares RIA data for total cannabinoids obtained from 24 forensic blood specimens and also GC/MS quantitative data for THC-COOH and delta-9-THC. The actual level of THC-COOH found by GC/MS is roughly one third of the value found for total cannabinoids using RIA. This is further exemplified by comparing the 2 sets of data shown in Fig. 11. Plotting the RIA total cross-reacting cannabinoids to the corresponding GC/MS results demonstrates a definite linear relationship ($r = 0.88$). Therefore, blood analysis using RIA was also found to give reliable indications as to the quantity of cannabinoids that might be expected upon further analysis.

## Conclusions

Storage in various kinds of blood collection tubes and temperature has been shown to produce little or no significant effects on the stability and RIA detectability of benzoylecgonine and THC-COOH in either the stored blood samples or their respective stored blood standard curves. Cocaine, however, was found not to be stable in blood, particularly

TABLE 2—*Comparison of semi-quantitative RIA data for total cannabinoids in 24 forensic blood specimens with corresponding GC/MS quantitative data for THC-COOH and delta-9-THC.*

| Case | Total Cross-Reacting Cannabinoids by RIA[a], ng/mL | GC/MS THC-COOH, ng/mL | GC/MS $\Delta^9$-THC, ng/mL |
|---|---|---|---|
| 1 | 34 | 16 | 0 |
| 2 | 38 | 17 | 0 |
| 3 | 38 | 12 | 0 |
| 4 | 38 | 17 | 0 |
| 5 | 46 | 26 | 0 |
| 6 | 63 | 30 | 0 |
| 7 | 86 | 44 | 0 |
| 8 | 95 | 40 | 0 |
| 9 | 98 | 30 | 4 |
| 10 | 100 | 26 | 0 |
| 11 | 103 | 40 | 0 |
| 12 | 124 | 38 | 0 |
| 13 | 173 | 73 | 0 |
| 14 | 193 | 81 | 7 |
| 15 | 197 | 54 | 0 |
| 16 | 213 | 33 | 0 |
| 17 | 214 | 51 | 0 |
| 18 | 250 | 89 | 0 |
| 19 | 265 | 59 | 0 |
| 20 | 272 | 75 | 0 |
| 21 | 292 | 61 | 0 |
| 22 | 300 | 82 | 0 |
| 23 | 312 | 115 | 0 |
| 24 | 333 | 88 | 17 |

[a]Average THC-COOH content of RIA total cross-reacting substances is about 1/3.

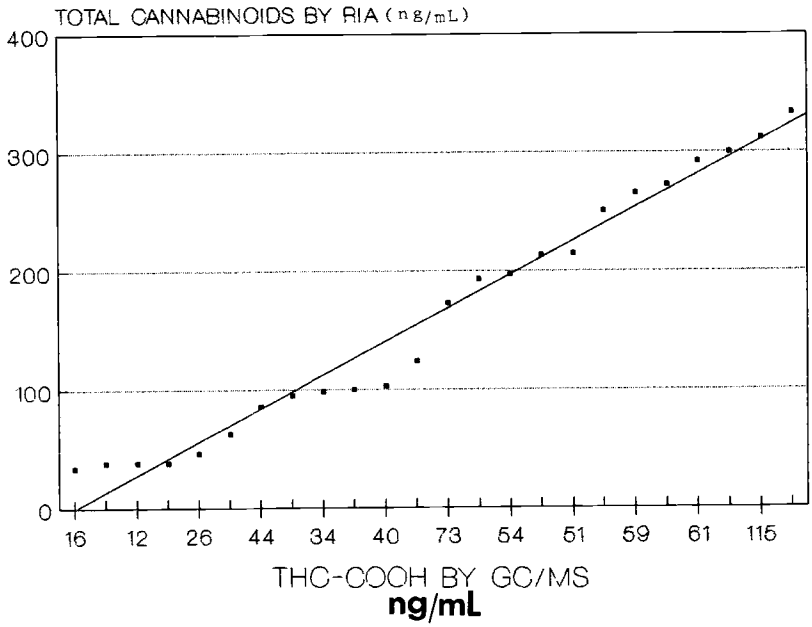


FIG. 11—*Linear plot of semi-quantitative RIA data for total cannabinoids in 24 forensic blood specimens versus GC/MS quantitative data for THC-COOH and delta-9-THC.*

when stored at room temperatures. Even at refrigeration temperatures and using sodium fluoride-containing tubes, cocaine instability was still found to be present, although not readily apparent using RIA techniques. Regardless of cocaine's instability, there was no condition observed that hindered the capacity of RIA to detect cocaine in whole blood.

*Acknowledgment*

The authors express their appreciation to Roche Diagnostic Systems, Inc., for their generous support of this project.

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