

TECHNICAL NOTE

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Improved Long-Term Stability of Blood Cocaine in Evacuated Collection Tubes

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ABSTRACT: A study was undertaken to determine if a relatively minor modification of our existing specimen collection tubes could enhance the long-term stability of blood cocaine. We added cocaine, benzoylecgonine (BE) and ethanol to whole sheep blood in glass tubes that were prepared to contain one of several combinations of preservatives and anticoagulant.

On day 1 and at intervals of up to one year, the drugs were measured by gas chromatography-mass spectrometry (cocaine and BE) or headspace gas chromatography (ethanol). Storage of blood containing 200 ng/mL cocaine at 4°C for one year resulted in 100% loss of the drug using our normal 10 mL specimen collection tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate. The substitution of oxalic acid for potassium oxalate reduced this loss to 76% without any significant effect on the benzoylecgonine or ethanol concentrations. Further addition of 10 mg echothiophate iodide, a quaternary ammonium compound, brought the cocaine loss down to 60% of the original concentration by one year. Further work will be required to determine if oxalic acid and/or echothiophate iodide could be used in blood collection vials intended for forensic toxicological purposes without any detrimental effect on other assays.

KEYWORDS: toxicology, blood, cocaine, collection tubes

Whole blood specimens collected from living individuals for forensic purposes frequently require testing for drugs many weeks or months later. The blood concentration of parent cocaine is important in assessing the effects of this frequently abused drug on individuals, yet cocaine is one of the most problematic of toxicological analytes due to its well-recognized in vitro lability [1-3].

In the western United States, whole blood specimens for forensic drug and ethanol analysis are generally obtained using evacuated glass tubes, 7 to 10 mL in volume, containing sodium fluoride as preservative and potassium oxalate as anticoagulant. Such containers have been found to stabilize blood ethanol levels for up to 3 years at 25°C [4].

A study was undertaken to determine if a relatively minor modification of our existing collection tubes could enhance the long-term stability of blood cocaine. We added cocaine,

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benzoylecgonine (BE) and ethanol to whole sheep blood in glass tubes that were prepared to contain one of several combinations of preservatives and anticoagulant. The specimens were refrigerated and the drugs measured by chromatographic means at intervals for up to one year.

Experimental Procedure

Sterile 10 mL glass collection tubes with silicone-coated interior and silicone-lubricated red synthetic rubber stoppers (#6530, Becton-Dickinson & Co., Rutherford, NJ) were obtained commercially. Sodium fluoride, potassium oxalate and oxalic acid were purchased from Fisher Scientific Co. (Santa Clara, CA). Echothiophate iodide was a gift of Wyeth-Ayerst Research (Princeton, NJ). These chemicals were added to the opened tubes in powder form according to the schedule in Table 1 and the tubes were re-evacuated in a vacuum chamber at 25 in Hg.

The tubes were sent to Antibodies, Inc. (Davis, CA), where they were filled with blood from live sheep by venipuncture on day -2. They were shipped at approximately 4°C to our laboratory, where they were received on day -1. On day 0, cocaine, BE and ethanol were added to each tube at target concentrations of 200 ng/mL, 500 ng/mL, and 0.075% w/v, respectively, and the specimens were stored in a refrigerator at 4°C. On day 1 and at intervals of up to one year, the drugs were measured by gas chromatography-mass spectrometry (GC-MS) (cocaine and BE) or headspace gas chromatography (GC) (ethanol) using previously described procedures [5]. The pH of the blood specimens was measured on day 1 and after one year using a pH meter and glass electrode.

Results and Discussion

The pH of the blood specimens in the three types of collection tubes is shown in Table 1. It is apparent that the substitution of oxalic acid for potassium oxalate had a significant effect in lowering the pH of the blood below neutrality. It has been previously demonstrated that cocaine displays greater stability in an acid relative to an alkaline environment [6].

The concentrations of cocaine, BE and ethanol in the stored blood specimens over time are shown in Table 2. Cocaine stability was enhanced by the use of oxalic acid in place of potassium oxalate; some further benefit was seen with the addition of echothiophate iodide, a known cholinesterase inhibitor [7]. Benzoylecgonine, believed to be both a metabolite and metabonate of cocaine, was relatively unaffected by the passage of time in any of the three containers, as was ethanol.

TABLE 1—Chemical content and blood pH for experimental blood collection tubes.^a

Tube no.	Chemical content	Blood pH	
		Day 1	1 Year
1	100 mg sodium fluoride 20 mg potassium oxalate	7.55	7.20
2	100 mg sodium fluoride 20 mg oxalic acid	6.70	6.85
3	100 mg sodium fluoride 20 mg oxalic acid 10 mg echothiophate iodide	6.52	6.53

^aTubes were 10 mL silicone-treated glass with red rubber stoppers (Becton-Dickinson #6530) that were re-evacuated after addition of chemicals.

TABLE 2—Drug concentrations (ng/mL or % w/v) in blood collection tubes over one-year refrigerated storage.^a

Tube no.	Drug	1 Day	1 Month	3 Months	6 Months	1 Year
1	Cocaine	186	109	21	8	0
	BE	482	517	531	536	534
	Ethanol	0.074	0.074	0.066	0.065	0.060
2	Cocaine	165	149	149	73	39
	BE	422	437	484	507	551
	Ethanol	0.073	0.070	0.065	0.070	0.067
3	Cocaine	156	154	156	107	62
	BE	426	417	495	512	587
	Ethanol	0.072	0.070	0.065	0.072	0.067

^aSee Table 1 for chemical content of collection tubes. Analysis by GC-MS (cocaine and BE) or GC (ethanol).

Conclusions

In summary, storage of blood containing 200 ng/mL cocaine at 4°C for one year resulted in 100% loss of the drug using our normal 10 mL specimen collection tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate. The substitution of oxalic acid for potassium oxalate reduced this loss to 76% without any significant effect on the benzoylecgonine or ethanol concentrations. Further addition of 10 mg echothiophate iodide, a quaternary ammonium compound, brought the cocaine loss down to 60% of the original concentration by one year.

The blood cocaine concentrations in both our modified tubes remained relatively stable for up to three months, while the unmodified tube lost 89% of its original cocaine content. Further work will be required to determine if oxalic acid and/or echothiophate iodide could be used in blood collection vials intended for forensic toxicological purposes without any detrimental effect on other assays.

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