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The Storage of Forensic Urine Drug Specimens as Dry Stains: Recovery and Stability

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ABSTRACT: The feasibility of storing forensic urine drug specimens as dry stains on Whatman #3 paper was studied by evaluating the stability of the drugs and recovery from the stains. Drug stains prepared from urine (3 mL) were stored at − 20°C, 4°C, and at room temperature for a period of 12 weeks. The study included: amphetamine, benzoylecgonine, 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), morphine, and phencyclidine (PCP) as examples of the HHS regulated drug classes. Drugs were eluted from the stains as follows: methanol:saline (1:1) for PCP and THC-COOH, saline for benzoylecgonine and carbonate/bicarbonate buffer pH 9.2 for amphetamine and morphine. Stains were eluted from the support matrix (Whatman #3 filter paper), extracted and analyzed by gas chromatography/mass spectrometry. All drugs were stable under all of the storage conditions except the THC-COOH urine stain stored at room temperature that degraded to zero after 12 weeks. Therefore, drug stains when kept frozen or refrigerated appear to provide a viable means for storing positive urine specimens.

KEYWORDS: forensic science, forensic toxicology, substance abuse detection, urine, dry stain, storage amphetamine, benzoylecgonine, 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid, morphine, phencyclidine

With the growth of job-related urine drug testing has come the practical problem of the long-term storage of those specimens that are confirmed as positive. The HHS SAMHSA (NIDA) mandatory guidelines 53FR11970 (1) require the remaining urine specimen to be retained in its original container at −20°C for a period of one year. A number of states have statutes and regulations with similar requirements (2–4). A large segment of the private work force is mandated to participate in drug testing programs because they are regulated by the Department of Transportation under 49*CFR* Part 40(5) or the Nuclear Regulatory Commission under 54FR24468 (6). These regulations adopt the HHS SAMHSA (NIDA) requirement that all positive specimens be stored frozen for one year. This is extremely expensive, generally necessitating a walk-in freezer with back-up emergency generator capability.

The storage of physiological fluids in the dry state (as stains) has regularly been applied in the forensic sciences. Forensic serologists typically store known blood specimens that are received in the laboratory from the victim of a crime or the defendant in a criminal case. Once the liquid blood specimen has been analyzed, the remaining sample is placed onto a suitable support matrix (either clean cotton cloth or filter paper), dried, and stored frozen. The frozen stain is then available for re-analysis if such becomes necessary by the laboratory or by a defense expert. The original container is maintained in a secure storage evidence room that is kept at room temperature. If, for chain of custody purposes, it becomes necessary to produce the original container at the time of trial, the evidence would contain all of the appropriate initials, identifying numbers, and seals. As long as a definitive link is made between the original container and the stain prepared from its contents, an effective chain of custody can be maintained. Some previous reports on detecting drugs in blood stains, semen, and other body fluids have appeared (7–11); however, these studies were limited to radioimmunoassay (RIA) testing. It has been well established that lyophilized drug controls are more stable than similar liquid preparations (12). Further, a number of recent reports have described the trapping of drugs from sweat onto dry patches (13,14).

This paper describes a method for storing positive urine specimens in the dry state as stains. The elution and recovery for a characteristic group of drugs were optimized, and the stability of the drugs in urine stains stored over time under varying conditions was determined. All analysis were conducted by quantitative gas chromatography/mass spectrometry (GC/MS).

Materials and Methods

Materials

The following drug standards were obtained and solutions in drug-free urine prepared: phencyclidine (PCP) (U.S.P.C., Inc.), 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) (AllTech), morphine sulfate (U.S.P.C., Inc.), d-amphetamine sulfate (Sigma), and benzoylecgonine tetrahydrate (AllTech).

Other materials used in this study included the following: disposable weight boats, 100 mL, medium-polystyrene (American Scientific Products); culture tubes 16 × 100 (Sarstedt); nonsterile gauze sponges, 4 × 4 (CIDA); Varian Bond Elut Certify tubes, standard, 3 mL reservoir (Varian); Whatman #1 qualitative filter paper, 9.0 cm; Whatman #3 qualitative filter paper, 9.0 cm; isotonic buffered saline (American Scientific Products); carbonate/bicarbonate buffer pH 9.2 (Biochemical Diagnostics, Inc.); potassium phosphate, monobasic, primary standard certified (Fisher Scientific). The following were all reagent grade: n-propanol, isopropanol, ethanol, methanol, sodium hydroxide, hydrochloric acid, trichloroacetic acid, hexane, and ethyl acetate.

Equipment

Hewlett-Packard 5890A gas chromatographs with Hewlett-Packard 5970B mass selective detectors were used for the analysis.

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Additional equipment used in this study: Fisher scientific touch mixer-Model 231, Eberbach shaker, gas vacuum pump, and Analytichem Vac Elut SPS 24.

Gas Chromatography/Mass Spectrometry

THC-COOH was analyzed following hydrolysis with 6*N* sodium hydroxide, extraction into hexane/ethyl acetate (9:1) and silyl derivatization using *N*-methyl-*N*-trimethylsilyltrifluoroactamide, 97% (MSTFA). The internal standard was D^3-THC-COOH (Research Triangle Institute). Ion peak areas monitored: THC-COOH (371,473,474), and D^3-THC-COOH (374,476,477) (15).

For the analysis of benzoylecgonine, the specimen was extracted and derivatized using *N,N*-dimethylformamide (Sigma) and *N,N*-dimethylformamide dipropyl acetal, 97% (Aldrich) to n-propyl cocaine. After derivatization, the analyte was back extracted and then injected on the GC/MSD. The internal standard used is D^3-benzoylecgonine tetrahydrate (MSD Isotopes). Ion peak areas monitored were: BE (210,331,272) and D^3-BE (213,334,275) (16).

For the analysis of morphine, the specimen was hydrolyzed with acid, adjusted to pH 6.0 to 7.5, and extracted with 2% ammonium hydroxide in methylene chloride:isopropanol solution (8:2). The product was then derivatized using *N*-methyl-*bis*-(trifluoracetamide) (MBTFA) (Pierce). The internal standard used was D^3-morphine (Radian). Ion Peak areas monitored: morphine (364,477,478) and D^3-morphine (367,480,481) (17).

For the analysis of phencyclidine, the specimen was alkalinized using carbonate/bicarbonate buffer pH 9.2 and extracted with methylene chloride. The organic layer was evaporated to dryness, the residue was reconstituted with methanol then injected onto the GC/MS. The internal standard used was D^3-phencyclidine (Radian). Ion peak areas monitored were: PCP (200,242,243) and D^5-PCP (205,248,246) (15).

For the analysis of amphetamine, the specimen was alkalinized, extracted, and after evaporation, the compounds were acetylated. The derivatizing agent was pentafluoropropionic anhydride, 99% (Aldrich). The internal standard used was D^5-amphetamine (Radian). Ion peak areas monitored were: amphetamine (190,118, 91) and D^5-amphetamine (194,123,93) (18).

Preparation of Fortified Urine Drug Specimens

Drug-free urine was obtained from volunteers. The urine pool was verified negative by GC/MS for each of the five drugs studied. The drug free urine was fortified with pure drug standards to concentrations at the HHS SAMHSA (NIDA) screening cut-off, the HHS SAMHSA (NIDA) confirmation cut-off and one-half of the HHS SAMHSA (NIDA) confirmation cut-off. The target concentrations for the fortified urine specimens in ng/mL are as follows: THC-COOH 100, 15, 7.5; PCP 25, 25, 12.5; morphine 300, 300, 150; amphetamine 1,000, 500, 250; and benzoylecgonine 300, 150, and 75.

Staining Procedure

The fortified urine was dropped onto one piece of Whatman filter paper and allowed to air dry overnight at room temperature. The placement of one piece of filter paper on a disposable weight boat facilitated the drying process and was a convenient method for preparing large numbers of stains efficiently. All stains were identified by code numbers written in pencil on the filter paper before staining.

Stain Elution Procedure

The dried filter paper was cut into a series of strips, approximately 25 strips per piece. The cut strips were then placed in a disposable 15-mL plastic tube. The solvent as described below was then added to the tube. The tube was vortexed for 15 s and then placed on a shaker for 20 min. Previously used empty Bond Elut columns were found to be ideal for recovering all of the solvent from the filter paper (the solid phase was removed and the columns were thoroughly cleaned and dried.) A piece of gauze was inserted into the column to act as a plug to prevent any pieces of filter paper from being sucked through the column. The plugged column was then placed on a Vac Elut SPS 24 apparatus that was attached to a vacuum pump. Beneath the empty Bond Elut column was placed a labeled 15-mL test tube. The filter paper slurry was placed into the empty Bond Elut column, and all liquid from the slurry was poured into the column. The slurry was further macerated and pressed down into the column. The vacuum pump was turned on and this very efficiently drew the solvent through the filter paper and removed all solvent from the filter paper slurry. An additional volume of solvent was then added to the filter paper in the empty Bond Elut column and the vacuum assisted aspiration through the column/filter paper slurry was allowed to continue. The vacuum remained on for 10 min. The recovered solvent solution containing drug was then subjected to the GC/MS extraction analysis for the specific drug as previously described.

TABLE 1—*Recovery of drugs from stains (3 mL) on Whatman No. 3 paper using various elution reagents.*

Drug	Target Concentration in Urine (ng/mL)		Elution Reagent*	Total Drug Recovered, ng	Percent Recovered
PCP	25	(22.7)	A	15.4	22.6
PCP	25	(19.6)	A	14.4	24.5
PCP	25	(19.6)	A	12.9	21.9
PCP	12.5	(9.8)	A	8.7	29.4
PCP	12.5	(9.8)	E	9.4	31.8
AMP	1000	(973.5)	D	2070.0	70.9
AMP	500	(462.2)	D	1096.0	79.1
AMP	250	(220.4)	E	653.0	98.9
AMP	250	(220.4)	E	626.0	94.7
MOR	300	(278.4)	D	217.0	26.0
MOR	300	(265.1)	D	217.0	27.3
MOR	150	(133.3)	D	99.0	24.8
MOR	150	(133.3)	E	90.6	22.7
BE	300	(314.6)	A	212.0	22.5
BE	150	(139.7)	A	97.7	23.3
BE	75	(74.8)	A	24.3	10.8
BE	75	(74.8)	E	15.5	6.9
THC	100	(79.5)	A	13.3	5.6
THC	15	(11.1)	A	1.3	3.9
THC	15	(15.3)	E	7.9	17.4
THC	100	(80.9)	E	39.8	16.4
THC	100	(80.9)	F	30.3	12.5
THC	100	(80.9)	G	19.0	7.8
THC	100	(80.9)	A	20.6	8.5
THC	7.5	(7.7)	E	1.7	7.3
THC	7.5	(7.7)	H	0	0
THC	7.5	(7.7)	I	0	0
THC	7.5	(7.7)	J	0	0

*Elution reagents: A—Saline, B—phosphate buffer pH 5.0, C—TCA followed by saline, D—carbonate/bicarbonate buffer pH 9.2, E—methanol:saline (1:1), F—methanol:phosphate buffer (1:1), G—hexane:ethyl acetate (85:15), H—ethanol:saline (1:1), I—isopropanol:saline (1:1), J—n-propanol:saline (1:1).

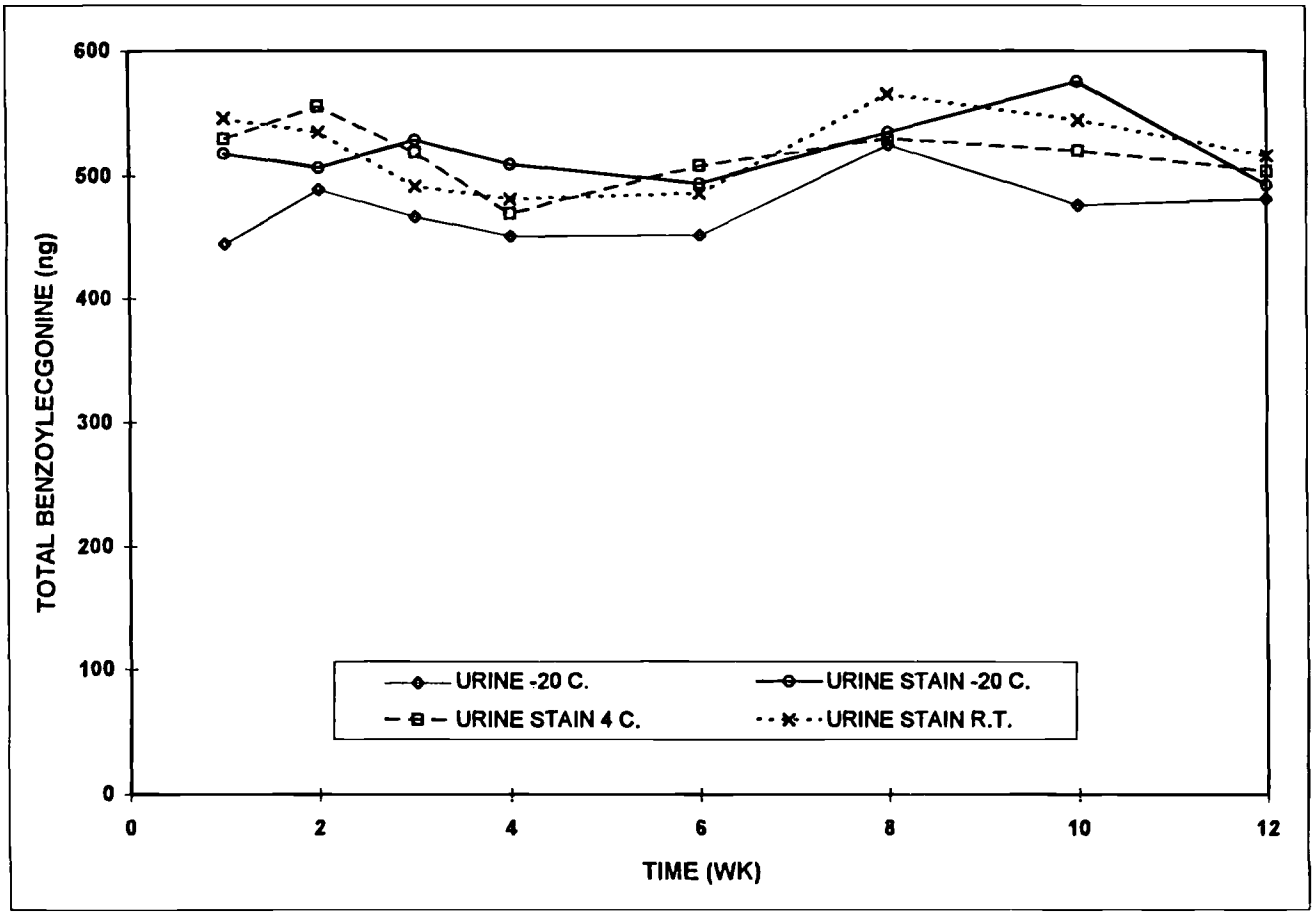

FIG. 1—*Stability of benzoylecgonine in paper stains stored at −20°C, 4°C, and room temperature compared to urine stored at −20°C.*

Characterization of Method

Support Matrix

Whatman #1 filter paper and Whatman #3 filter paper were evaluated as a support matrix for the fortified urine.

Eluting Solvent

Urine stains that were prepared on both Whatman #1 filter paper and Whatman #3 filter paper were eluted and subjected to GC/MS analysis. The eluting reagents used were isotonic buffered saline, phosphate buffer pH 5.0, trichloroacetic acid followed by saline, carbonate/bicarbonate buffer pH 9.2 and methanol:saline (1:1). The stains were subjected to the elution procedure that has previously been described.

Volume Study

Once it had been established that the drug could be easily recovered from the urine stain by use of the appropriate eluting reagent, a study was conducted to determine the maximum quantity of urine that could be held by Whatman #1 and Whatman #3 filter paper.

Elution—Recovery Optimization

The Drug Recovery Study used 3 mL of fortified urine on Whatman #3 filter paper. In addition to the elution reagents that were used in the first recovery study (Whatman #1), the following reagents were used in this study: (a) methanol:phosphate buffer (1:1), (b) hexane:ethyl acetate (85:15), (c) ethanol:saline (1:1), (d) isopropanol:saline (1:1), and (e) n-propanol:saline (1:1).

Stability and Recovery Study

The stability of the drugs/metabolites in dry urine stains under various storage conditions was evaluated. Blank urine was fortified with the designated drug at the HHS SAMHSA (NIDA) confirmation cut-off concentrations. Stains were prepared from 3 mL of fortified urine onto Whatman #3 filter paper. The stains were allowed to air dry overnight at room temperature. After drying, stains were stored at −20°C, 4°C, and room temperature. Additionally, aliquots of the fortified urine for each drug were stored at −20°C.

The urine drug stains for each drug at each storage condition were assayed by GC/MS in duplicate every week for the first four weeks and then every other week for the next eight weeks. The exception to this was THC-COOH stored at room temperature that was assayed every week in duplicate. The elution reagents used to recover each drug before GC/MS analysis were those previously determined to give the greatest drug recovery.

Results and Discussion

It was determined that the five drugs studied could be recovered from urine drug stains and quantitated by GC/MS. Table 1 lists the results of the drug recovery study. The ideal matrix was found

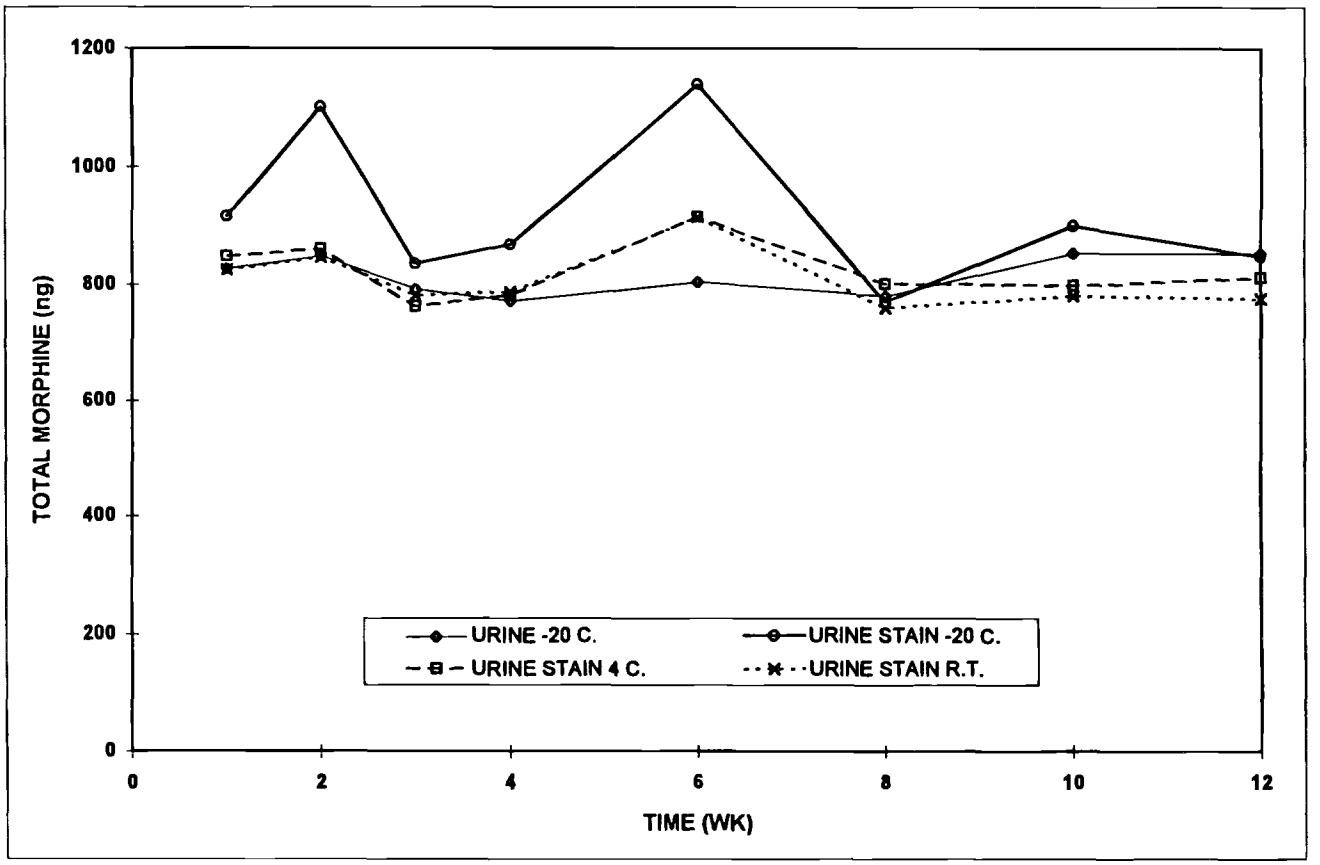

FIG. 2—*Stability of morphine in paper stains stored at −20°C, 4°C, and room temperature compared to urine stored at −20°C.*

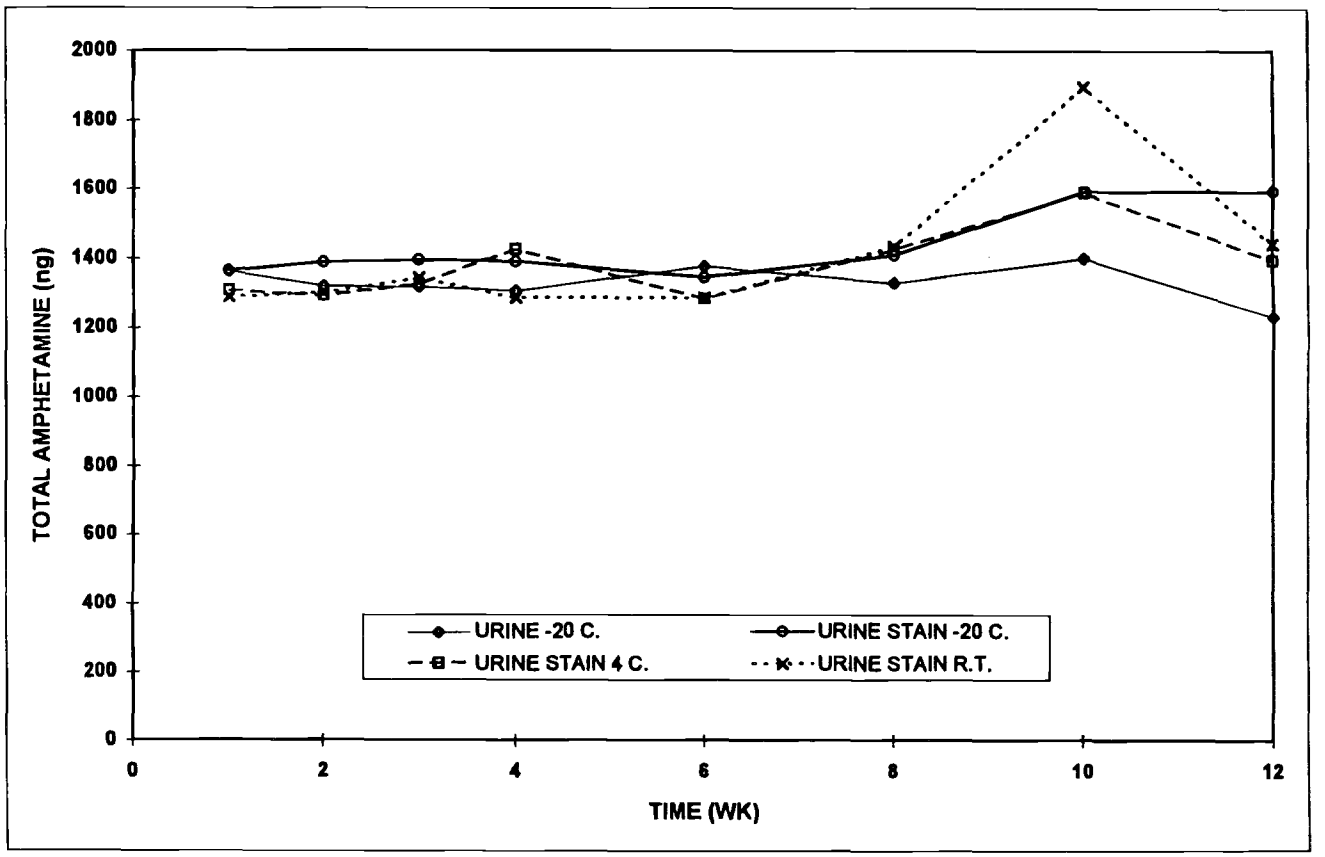

FIG. 3—*Stability of amphetamine in paper stains stored at −20°C, 4°C, and room temperature compared to urine stored at −20°C.*

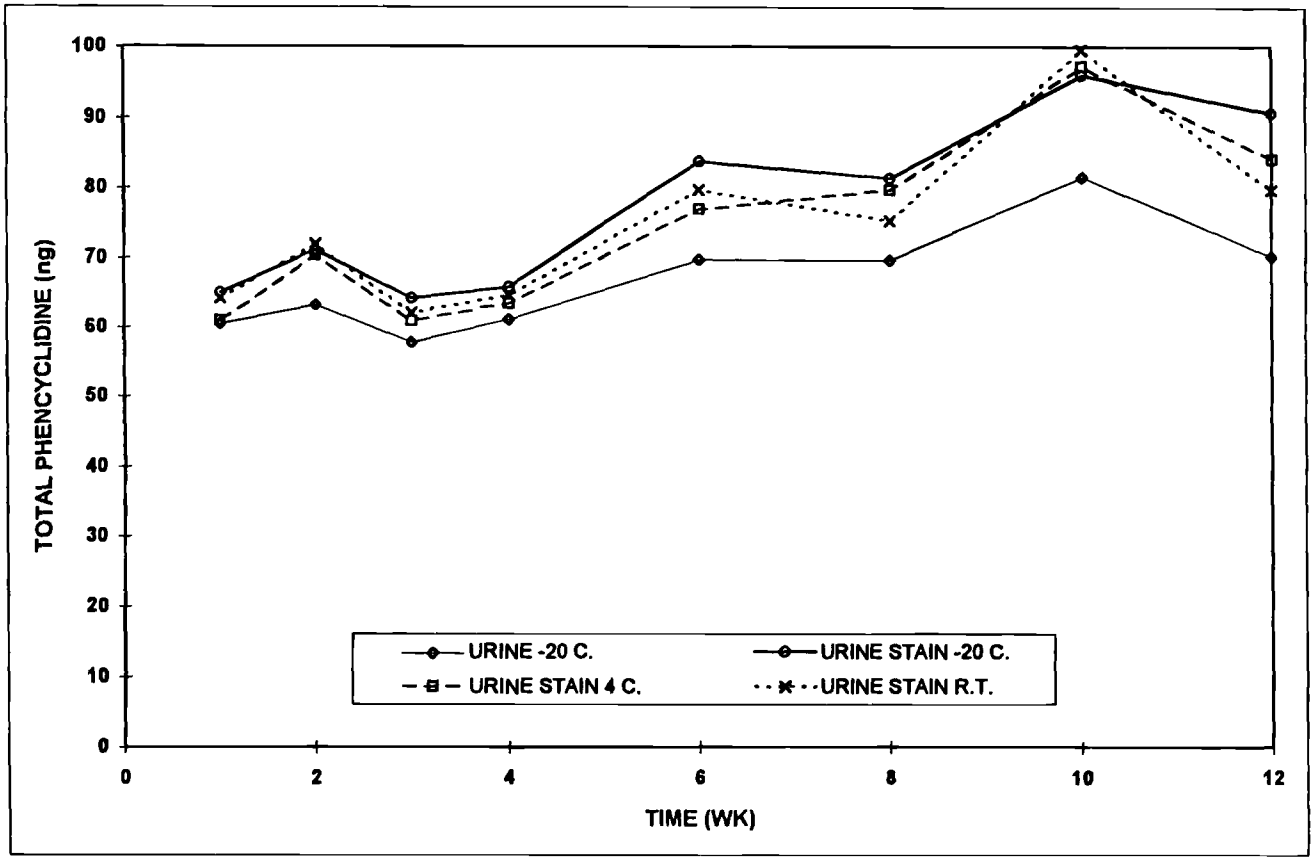

FIG. 4—*Stability of phencyclidine in paper stains stored at −20°C, 4°C, and room temperature compared to urine stored at −20°C.*

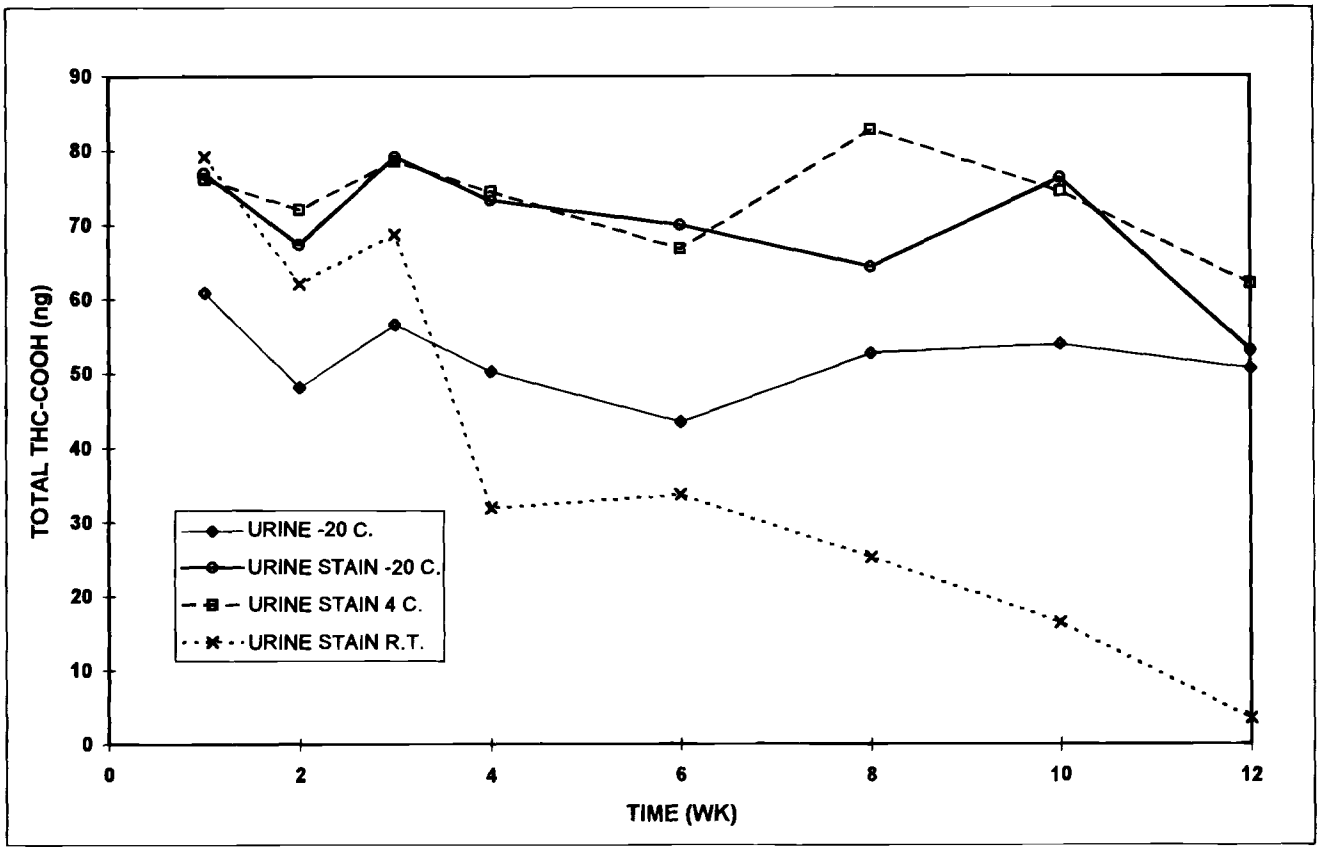

FIG. 5—*Stability of THC-COOH in paper stains stored at −20°C, 4°C, and room temperature compared to urine stored at −20°C. THC-COOH (11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid).*

to be Whatman #3 filter paper with a maximum of 3 mL of urine. Drug recovery was optimum with the use of the following elution solvents: carbonate/bicarbonate buffer pH 9.2 for amphetamine and morphine, saline for benzoylecgonine and methanol:saline (1:1) for phencyclidine and THC-COOH. Recovery for THC-COOH was increased by recycling the recovered eluting reagent containing drug through the Bond Elut tube containing the filter paper slurry four times by means of vacuum aspiration.

Figures 1 through 5 show the results of the drug stability and recovery studies. For neat urine the concentration is the actual assayed result. For the urine stains the concentration is the assayed result corrected for the experimentally determined percent recovery for each drug.

Benzoylecgonine was found to be stable in dry urine stains stored at −20°C, 4°C, and at room temperature. The frozen aliquot stored at −20°C showed no degradation after 12 weeks.

Morphine was found to be stable in dry urine stains stored at −20°C, 4°C, and at room temperature. The frozen aliquot stored at −20°C showed no degradation after 12 weeks.

Amphetamine was found to be stable in dry urine stains stored at −20°C, 4°C, and at room temperature. The frozen aliquot stored at −20°C degraded 16% after 12 weeks.

PCP was found to be stable in dry urine stains stored at −20°C, 4°C, and at room temperature. The frozen aliquot stored at −20°C showed no degradation after 12 weeks.

THC-COOH was found to be stable in dry urine stains stored at −20°C and at 4°C. THC-COOH in dry urine stains stored at room temperature was found to be stable for only four weeks. The quantity of THC-COOH recovered after four weeks in the room temperature samples fell by one half and after twelve weeks was less than 1 ng/mL. The frozen aliquot stored at −20°C show no degradation after 12 weeks.

The loss in concentration of THC-COOH has been reported as due to oxidation, temperature effects, and lipophilic binding (19). Additionally, the loss of THC-COOH, observed in the room temperature stain, may be explained by microbial growth.

Summary and Conclusion

This study has demonstrated that it is possible to store urine specimens as dry stains and to recover the drugs/metabolites from these stains. With the exception of THC-COOH which degraded after four weeks at room temperature, the storage condition did not appreciably affect the drug recovery. Additional studies are required to determine stability over a longer period of time. Multiple stains may be prepared to ensure adequate specimen availability for forensic analysis. This method is presented as a possible alternative to frozen urine retention and may not be acceptable to regulatory agencies. Although this study may have implications in how urine specimens may be stored for subsequent reanalysis, it also demonstrates the feasibility of assaying for drugs of abuse on certain evidential items. These evidential items might include stains at crime scenes, in vehicles or on suspect's or victim's clothing.

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