Loss of THCCOOH from Urine Specimens Stored in Polypropylene and Polyethylene Containers at Different Temperatures*

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Abstract

The loss of Δ^9-tetrahydrocannabinol (THCCOOH) from urine specimens stored in polypropylene and polyethylene containers at 4°C and 25°C was examined. All specimens were analyzed by GC–MS after sampling at various times over a one-week period. Data were analyzed by one-way analysis of variance and fitted with a first order kinetic equation. Rapid loss of THCCOOH was seen at 4°C for both polypropylene (14% maximal loss, $t_{1/2}$ = 0.53 min) and polyethylene (17% maximal loss, $t_{1/2}$ = 5.77 min) bottles. At 25°C, a small loss (< 5%) was observed in polypropylene and no significant loss was seen for urine in polyethylene. All losses stabilized within 1 h, and no further losses were seen over one week. The results indicate that THCCOOH binding may be due to decreased solubility of THCCOOH at lower temperatures and subsequent association of THCCOOH with the more lipophilic plastic. The results also indicate that polypropylene and polyethylene do not bind THCCOOH to such an extent as to compromise the integrity of specimens.

Introduction

It is common practice for forensic urine drug-testing laboratories to receive urine specimens that have been transported from the collection site to the laboratory at room temperature. Additionally, it is common practice to refrigerate specimens in temporary storage during the analysis period. Several authors have raised concerns about the potential for THCCOOH loss from specimens because of its interaction with the sample container (1–10). A consensus does not exist regarding the extent of THCCOOH loss occurring in different types of containers and/or at different temperature storage conditions.

Paul et al. (4) reported a loss of up to 34% for THCCOOH from frozen specimens stored in polypropylene. Roth et al. (1) reported a decreased concentration of THCCOOH up to 46% from specimens stored at room temperature and at 2–8°C in polyethylene and reported significant differences between two types of polypropylene tested. Fraga et al. (2) observed a 22% decreased concentration at room temperature and lowered concentration (8%) in refrigerated samples. Conversely, Giardino (3) reported insignificant changes of concentration of THCCOOH stored in polypropylene at 2–8°C for up to 42 days. Blanc et al. (4) and Joern (6,7) both reported significantly decreased concentrations for THCCOOH from urine stored in borosilicate glass. Roth et al. (1) also reported a decreased concentration for THCCOOH from solutions stored in borosilicate glass though the concentration decreases were less than those observed in silanized glass. Christophersen (9) reported no change in THCCOOH concentration in blood samples stored in borosilicate glass and almost a complete loss of THCCOOH in blood specimens stored in polystyrene.

The Navy and the Department of Defense (DOD), in an effort to continue to improve the efficiency of their urine drug-testing program, have been exploring the use of an automated accessioning system that would require changing the type and configuration of the urine specimen collection bottles. The proposed new bottles are fabricated using polypropylene rather than polyethylene as the current bottles are. This provided a need to examine differences in the behavior of THCCOOH in two different bottle material systems.

We examined the time course of THCCOOH concentration change under conditions in which specimens are received and stored during testing. Specimens were examined over a period of a week (to simulate maximal time in shipping) at both 25°C and 4°C in two different specimen collection bottles (polypropylene and polyethylene).

Methods

Prototype polypropylene collection bottles were obtained from Battelle Corp. (Columbus, OH). The manufacture of these collection bottles was by injection molding using Pro-fax 6433

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polypropylene homopolymer (Montell North America Inc.) resin with silicone as the mold-releasing agent. The contact area of the urine in the bottle was 55 cm^2. The polyethylene collection bottles, currently used by the DOD, were manufactured by injection blow-molding by Wheaton USA Inc. (Millville, NJ) using Phillips Marlex 5502 high-density polyethylene resin. Zinc stearate was added at a rate of 113 g/100 lbs of resin as a lubricant for production. The resin formulation contained BHT as an antioxidant. The contact area of the urine in the bottle was 53 cm^2.

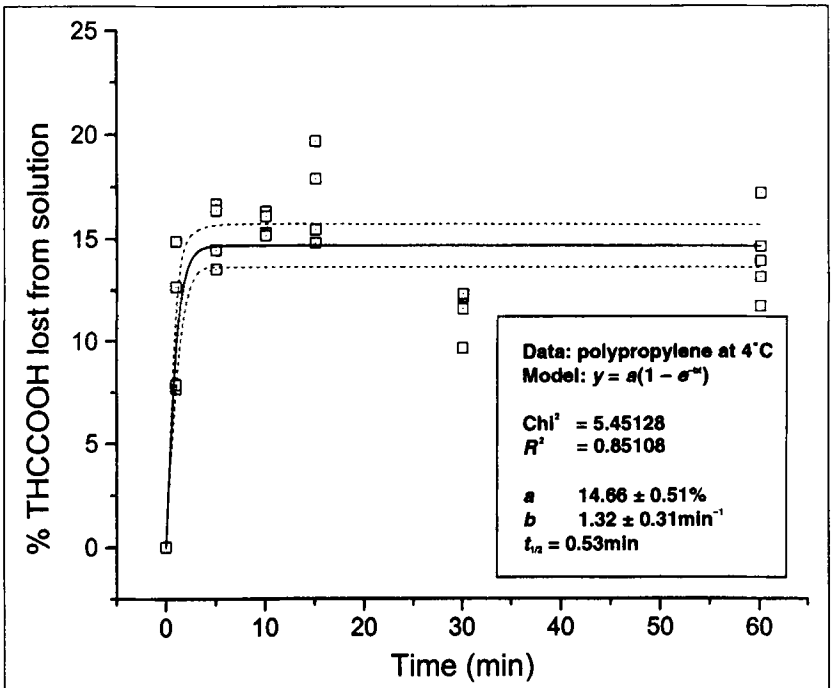

Figure 1. Loss of THCCOOH from urine stored in polypropylene at 4°C as a percentage of the initial measured concentration. The curve fit results and statistics are presented. The dashed lines represent a 95% confidence interval on the modeled line. A significant time effect was observed by one-way ANOVA ($p < 0.0001$).

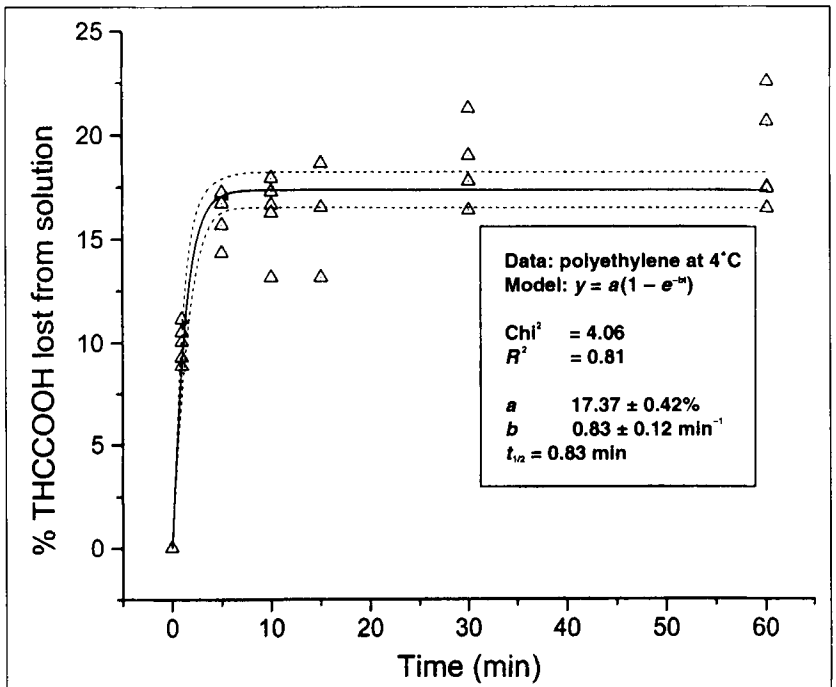

Figure 2. Loss of THCCOOH from urine stored in polyethylene at 4°C as a percentage of the initial measured concentration. The curve fit statistics are presented. The dashed line represents a 95% confidence interval on the modeled line. A significant time effect was seen by one-way ANOVA ($p < 0.0001$). The maximum loss of THCCOOH and the $t_{1/2}$ were both higher than the results of the polypropylene bottles at the same temperature.

Certified drug-free urine was obtained from Hydrocarbons Inc. (Virginia Beach, VA). THCCOOH and THCCOOH-d_3 standards were obtained from Research Triangle Institute (Research Triangle Park, NC). All solvents used were ACS grade.

A 15-ng/mL THCCOOH solution was prepared in borosilicate glass using drug-free urine just prior to beginning the experiments. All urine specimens, pipettes, and collection bottles were equilibrated at 4°C or 25°C prior to adding urine to the bottles and sampling of urine from the bottles. Thirty milliliters of urine was added to each type of bottle (polypropylene and polyethylene) at 4°C or 25°C using sterile, disposable, borosilicate serological pipettes (Fisher Scientific). There were five replicates ($n = 5$) for each set of conditions (temperature and type of collection). Three-milliliter aliquots were removed at 1 min, 5 min, 10 min, 15 min, 30 min, and 60 min. A separate set of five collection bottles contained 30 mL of the same stock urine, and 3-mL samples were removed at 1 day, 5 days, and 7 days. Collection bottles were gently mixed prior to sampling to prevent foaming, which has been associated with decreased concentration of THCCOOH (6). The aliquots were placed in 50-mL polypropylene tubes (VWR) for further processing. The THCCOOH-d_3 internal standard concentration was 40 ng/mL.

Five additional 3-mL aliquots were taken from the stock bottle at the time urine was added to the sample vials to serve as the starting value and control for any loss in concentration of THCCOOH in the stock bottles. Aliquots taken directly from the stock bottles were treated in the same manner as all other samples to control for loss during the sample preparation and extraction steps.

Samples were extracted using a solid-phase extraction (SPE) method based on the method of Paul et al. (11). The pH of each sample was adjusted to between 2 and 4 and extracted using an automated extraction routine on Zymark Rapid Trace SPE workstations. The method used a C8 column matrix (Isolute, 100 mg, International Sorbent Technology, Mid-Glamorgan, U.K.). After conditioning, sample application, and washing steps, the columns were eluted with methanol.

The eluate was dried down under a stream of

nitrogen at 60°C. The residue in each tube was reconstituted with 100 µL tetramethylammonium hydroxide/DMSO (1:20). After 2 min, 10 µL of iodomethane was added to each tube and allowed to react for 5 min. Adding 0.2 mL of 0.1N HCl and extracting into 1.2 mL of isooctane removed excess derivitizing reagent. The isooctane was decanted and dried under a stream of nitrogen. Residual was then reconstituted in 100 µL of isooctane.

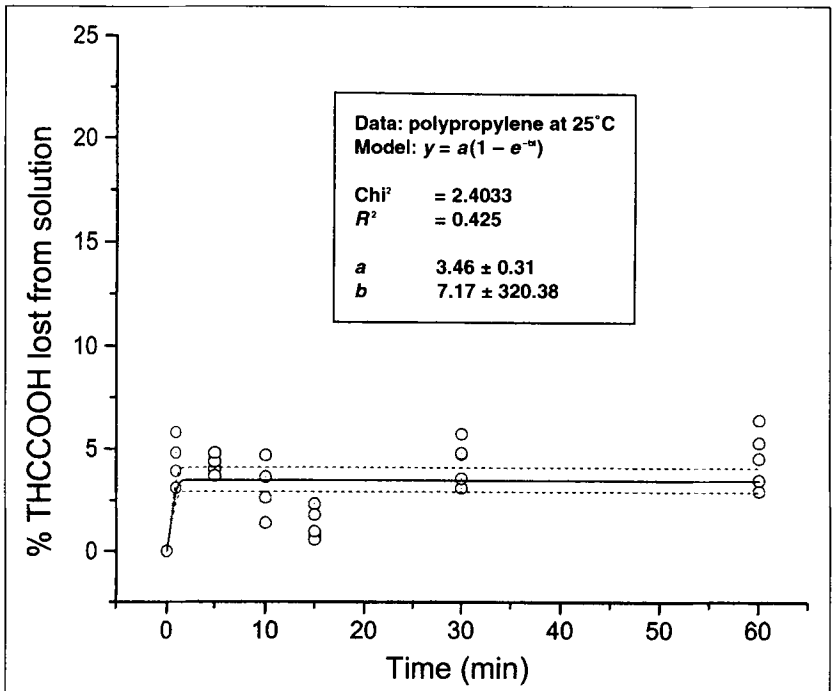

Figure 3. Loss of THCCOOH from urine stored in polypropylene at 25°C as a percentage of the initial measured concentration. The curve fit statistics are presented. The dashed line represents a 95% confidence interval on the modeled line. Although a significant time effect was seen by one-way ANOVA ($p = 0.0001$), no clear trend was observed and the curve fit was poor. A significant, but small (< 5%), amount of THCCOOH was lost from solution over the course of an hour.

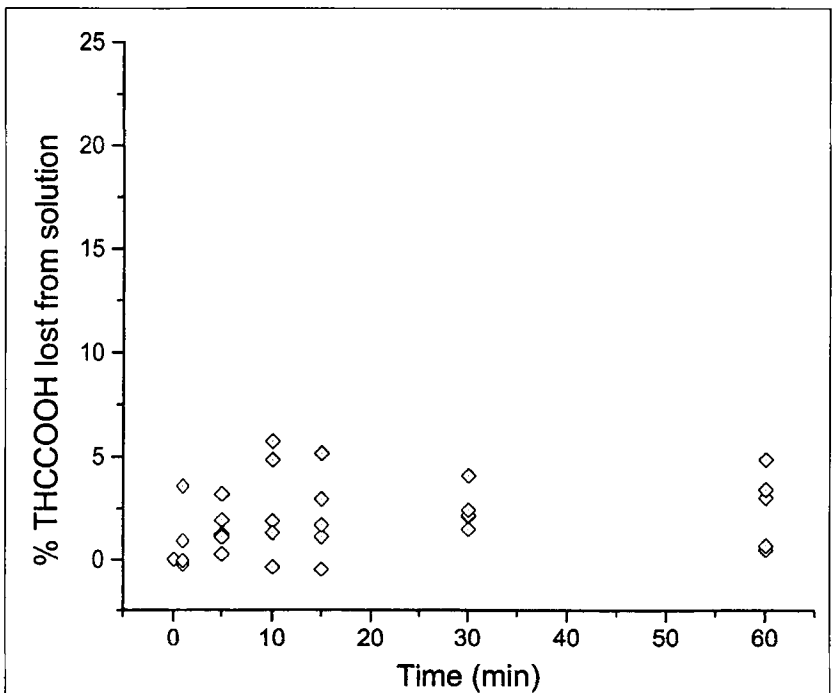

Figure 4. Loss of THCCOOH from urine stored in polyethylene at 25°C as a percentage of the initial measured concentration. No significant time effect was seen, and the data did not significantly differ from the initial concentration. No curve fit was obtained for the data.

All samples were analyzed using an HP 6890 GC coupled to a 5973 mass selective detector (MSD) with a 6890 series autoinjector. The column was an HP 5 (15 m × 250 µm × 0.25 µm) using helium as a carrier gas. Samples were injected (3 µL) in splitless mode, and gas flow was maintained at 1.0 mL/min through out the run. The oven temperature started at 180°C and ramped to 280°C at 25°C/min. The injection port was maintained at 245°C, the transfer line at 285°C, the MS source at 230°C, and the MS quadrupole at 150°C. This profile resulted in a 4.5-min retention time for THCCOOH.

The MSD was operated in EI SIM mode. The electron multiplier was operated at 400 eV above tune value. Ions monitored were *m/z* 375 and 360 for the internal standard and *m/z* 372, 357, and 313 for the analyte. All quantitations were based on the analyte to internal standard ratio (*m/z* 372/375). Controls were made in certified-negative urine with THCCOOH concentrations at 0, 7.5, 15, 30, and 60 ng/mL. All correlation coefficients were all greater than 0.999 for all batches.

All data were compared to the samples taken directly from the stock bottle to control for any loss of analyte during sample preparation and extraction as well as variance encountered in the extraction process. Nonlinear regressions were performed on the data using Origin 6.0 (Microcal Software, Northampton, MA). All data were fit to the first order kinetic equation $y = a(1 - e - bt)$ as used by Roth et al. (1). The coefficient of determination (r^2) was calculated for each nonlinear curve fit and is presented in each of the figures. Data are presented with a 95% confidence interval for the fitted line. Only data sets with a significant time effect determined by analysis of variance (ANOVA) were fitted to the model. One-way ANOVA tests, followed by ad hoc least significant difference means testing, were performed on the data using Statgraphics 6.0 (Manugistics, Rockville, MD). Significance for ANOVA tests was assumed at the $\alpha = 0.05$ level.

Figure 1 presents the loss of THCCOOH in urine stored in polypropylene at 4°C as a percentage of the initial concentration. A significant time effect was observed for the data by one-way ANOVA ($p < 0.0001$, $f = 18.91$). No significant difference was seen between 60 min and the longer time periods by least significant difference means testing. Thus, the nonlinear

curve fitting included data up to 60 min. When the full data set was fit, the kinetic parameters were not significantly altered. A good fit was achieved using the first order model (r^2 = 0.85). The maximal percentage of THCCOOH lost was 14.66% ± 0.51 with a half-life of 0.53 min.

Figure 2 shows a higher maximal percentage loss of THCCOOH in urine stored at 4°C in polyethylene (17.37% ± 0.42). The loss of THCCOOH had a longer half-life ($t_{1/2}$ = 5.77min) than in polypropylene. As with the polypropylene data, a significant time effect was observed in the data set for times up to 60 min ($p < 0.0001$, $f = 20.82$) and no significant difference was observed between 60 min and longer time periods. Curve fitting was performed on data up to 60 min and a good fit was obtained (r^2 = 0.81). One data point was excluded from the 30-min sample group as an outliner.

In Figure 3, a significant time effect was seen for the loss of THCCOOH from solution stored in polypropylene at 25°C (p = 0.0001, f = 5.30); however, there was no clear time pattern for the loss. The 10- and 15-min time samples were not significantly different from the zero time point. Although the other time points were significantly different from the initial time point, no clear trend was evident. Other than the 10- and 15-min time points, none of the time points were significantly different from each other. The lack of a clear trend is also evident from the poor curve fit (r^2 = 0.42) obtained for the data. These results indicate a small (< 5%) loss of THCCOOH from solutions stored in polypropylene at 25°C.

Figure 4 shows the analysis of urine stored in polyethylene bottles at 25°C. No significant time effect was seen by ANOVA. No time points were significantly different from the initial time point; therefore, no curve fit was obtained for the data.

Discussion

This study indicated there was no significant loss of THCCOOH in urine stored at 25°C in polyethylene bottles, a small loss (< 5%) from urine stored at 25°C in polypropylene bottles, and significantly more THCCOOH lost from the urine specimens stored and sampled at 4°C than from those at 25°C. The maximum amount lost was 14.66% in polypropylene and 17.37% in polyethylene bottles. This is equivalent to 1.2–1.8 ng lost/cm^2 and is considerably less than the 46 ng/cm^2 calculated by Roth et al. (1) for a mono-layer of THCCOOH. The results of this study are consistent with their finding of THCCOOH binding occurring as a single layer.

The rapidity of the loss of THCCOOH in urine is consistent with several reports that indicate the observed loss occurred within the first hours after the introduction of the specimen into the container. This is also consistent with loss of THCCOOH in urine being a surface phenomenon and not diffusion of THCCOOH into the plastic matrix.

The results of this study are contrary to several reports that found reduced loss in refrigerated samples (1,2,5). This highlights the idea that variation in the formulation of the plastic, methods of molding, and the differing combinations of plastic and mold-releasing agents may greatly alter the binding properties of the plastics. However, several studies report increased loss upon freezing and attribute this effect to the reduced solubility of THCCOOH in the aqueous phase and favoring of the interaction of THCCOOH with a more lipophilic container wall (4,5,8). This explanation is consistent with the results observed in this study in which greater loss may be attributable to decreased solubility of THCCOOH in the colder solutions.

Roth et al. (1) suggested that the more nonpolar the plastic is, the greater the loss of THCCOOH from solution. Likewise, Pearson et al. (10) suggested that the addition of Visine eye drops reduces measured THCCOOH concentrations by forming benzalkonium chloride micelles into which THCCOOH partitions. Thus, it is likely that lipophilic interactions can favor the partitioning of THCCOOH out of solution.

These results are important in highlighting the variability in THCCOOH binding to containers. Various formulations of the same polymer and various environmental conditions may impact the same plastics. From the results of this study, both the polypropylene and polyethylene bottle systems tested produced small losses of THCCOOH from urine specimens. Additionally, THCCOOH loss appears to stabilize rapidly and further loss from urine was not observed over a week's storage time. This supports other reports suggesting that the polypropylene and polyethylene bottles used by many labs and drug-testing programs do not adversely affect the reliability of THCCOOH testing.

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References

1. K.D.W. Roth, N.A. Siegel, R.W. Johnson, Jr., L. Litauszki, L. Salvati, Jr., C.A. Harriongton, and L.K. Wray. Investigation of the effects of solution composition and container material type on the loss of 11-nor-delta-9-THC-9-carboxylic acid. *J. Anal. Toxicol.* **20:** 291–300 (1996).
2. S.G. Fraga, J.D. Estevez, and C. Romero. Stability of cannabinoids in urine in three storage temperatures. *Ann. Clin. Lab. Sci.* **28(3):** 160–162 (1998).
3. N.J. Giardino. Stability of 11-nor-delta-9-THC-9-carboxylic acid in negative human urine in high density polyethylene (Nalgene). *J. Anal. Toxicol.* **20:** 275–276 (1996).
4. B.D. Paul, R.M. McKinley, J.K. Walsh, Jr., T.S. Jamir, and M.R. Past. Effect of freezing on the concentration of drugs of abuse in urine. *J. Anal. Toxicol.* **17:** 378–380 (1993).
5. J.A. Blanc, V.A. Manneh, R. Ernst, D.E. Berger, S.A. deKeczer, C. Chase, J.M. Centofanti, and A.J. De Lizza. Adsorption losses from urine-based cannabinoid calibrators during routine use. *Clin. Chem.* **39(8):** 1705–1712 (1993).
6. W.A Joern. Surface adsorption of the urinary marijuana carboxy

metabolite: the problem and partial solution. *J. Anal. Toxicol.* **16:** 401 (1992).
7. W.A. Joern. Detection of past and recurrent marijuana use by a modified GC/MS procedure. *J. Anal. Toxicol.* **11:** 49–52 (1987).
8. F.M. Moore and D. Simpson. Detection of cannabinoids in urine: a cost-effective low risk immuno-assay procedure. *Med. Lab. Sci.* **48:** 76–79 (1991).
9. A.S. Christophersen. Tetrahydrocannabinol stability in whole blood: plastic versus glass containers. *J. Anal. Toxicol.* **10:** 129–131 (1986).
10. S.D. Pearson, K.O. Ash, and F.M. Urry. Mechanism of false-negative cannabinoid immunoassay screens by Visine eyedrops. *Clin. Chem.* **35(4):** 636–638 (1989).
11. B.D. Paul, L.D. Mell, Jr., J.M. Mitchell, R.M. McKinley, and J. Irving. Detection and quantitation of urinary 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid, a metabolite of tetrahydrocannabinol, by capillary gas chromatography and electron impact mass fragmentography. *J. Anal. Toxicol.* **11:** 1–5 (1987).

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