Determination of Five Abused Drugs in Nitrite-Adulterated Urine by Immunoassays and Gas Chromatography–Mass Spectrometry

S.-C.J. Tsai[1], M.A. ElSohly[2], T. Dubrovsky[1], B. Twarowska[1], J. Towt[1], and S.J. Salamone[1]

[1]International Drug Monitoring Business Unit, Roche Diagnostic Systems, Inc., 1080 US Highway 202, Somerville, New Jersey 08876 and [2]ElSohly Laboratories, Inc., 5 Industrial Park Drive, Oxford, Mississippi 38655

Abstract

The adulteration of urine specimens with nitrite ion hasseen shown to mask the gas chromatography–mass spectrometry (GC–MS) confirmation testing of marijuana use. This study was designed to further investigate the effect of nitrite adulteration on the detection of five commonly abused drugs by immunoassay screening and GC–MS analysis. The drugs tested are cocaine metabolite (benzoylecgonine), morphine, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH), amphetamine, and phencyclidine. The immunoassays evaluated included the instrument-based Abuscreen ONLINE assays, the on-site Abuscreen ONTRAK assays, and the one-step ONTRAK TESTCUP-5 assay. Multianalyte standards containing various levels of drugs were used to test the influence of both potassium and sodium nitrite. In the ONLINE immunoassays, the presence of up to 1.0M nitrite in the multianalyte standards had no significant effect for benzoylecgonine, morphine, and phencyclidine assays. With a high concentration of nitrite, ONLINE became more sensitive for amphetamine (detected more drug than what was expected) and less sensitive for THCCOOH (detected less drug than what was expected). No effects of nitrite were observed on the results of the Abuscreen ONTRAK assays. Similarly, no effects were observed on the absolute qualitative results of the TESTCUP-5 when testing the nitrite-adulterated standards. However, the produced intensities of the signals that indicate the negative test results were slightly lowered in the THC and phencyclidine assays. The presence of 1.0M of nitrite did not show dramatic interference with the GC–MS analysis of benzoylecgonine, morphine, amphetamine, and phencyclidine. In contrast, nitrite ion significantly interfered with the detection of THCCOOH by GC–MS. The presence of 0.03M of nitrite ion resulted in significant loss in the recovery of THCCOOH and its internal standard by GC–MS. The problem of nitrite adulteration could be alleviated by sodium bisulfite treatment even when the specimens were spiked with 1.0M of nitrite ion. Although bisulfite treatment decomposed all nitrite ions in the sample to recover the remaining THCCOOH by GC–MS, the net recovery of THCCOOH depended on urinary pH and time and conditions of sample storage. The presence of nitrite concentrations that might arise from all possible natural sources, including microorganisms, pathological conditions, and medications, did not interefere with the GC–MS analysis of THCCOOH.

Introduction

Several surveys and studies to support the premise that drug-of-abuse testing effectively deters and detects drug abuse problems have been published (1). Well-established in its role in curbing and monitoring criminal offenders (2), drug testing also serves as an indispensable diagnostic aid and effectual treatment tool of drug abuse and addiction. Over the last decade, employee drug testing has become a common business practice in the American workplace (3,4). A study by the American Management Association showed that the rate of positive drug-test results among employees has dropped from 8.1% in 1989 to 1.9% in 1994 since the inception of the SAMHSA workplace drug-testing program (5). As urine drug testing gains in popularity in both scheduled and random tests in society, there is a growing number of new products and methods designed to defeat the test and mask drug use or abuse (6).

A battery of commercial adulterants and household chemicals have been used for the purposeful adulteration of urine (7–11). Most of the in vitro adulterants were used to skew the initial immunoassay drug screening and hence bring about a false-negative result, which in turn will avoid the gas chromatographic–mass spectrometric (GC–MS) confirmation test. The mechanisms or impact of these adulterants on several instrument-based immunoassays have been well documented (7–11). Several laboratories have recently encountered increased incidences of specimens that screened positive for 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) by immunoassay but failed the GC–MS confirmation test (12–15). Further investigations revealed that a new adulterant was presented in these urine specimens (12). ElSohly et al. (12) identified this adulterant, "Klear," as potassium nitrite (KNO_2) and showed a procedure to overcome its interference in the GC–MS analysis of THCCOOH. Although it is not clear how widely Klear has been used for urine adulteration, the product is still actively advertised on the internet and in trade magazines with claims such as "undetectable urine purifier", "mask all foreign materials from the body", "a proven solution", or "the best product available if you are not subject to supervised testing". Moreover, it was also not clear if nitrite adulteration could

interfere with the detection of other abused drugs besides marijuana.

This study was conducted to investigate the effect of high concentrations of nitrites on the detection of five commonly abused drugs by immunoassay screening and GC–MS analysis. In addition to evaluating the instrument-based Abuscreen ONLINE assays, we also examined the on-site Abuscreen ONTRAK assays and the one-step, multianalyte ONTRAK TESTCUP-5 assay (16–19). Although most of the reports on urine adulteration were on instrument-based immunoassays, the study of adulteration on on-site drug tests are important because there has been an ever-increasing application of on-site assays in workplace drug testing (20,21). Further, recent immunochromatographic-based on-site assays such as ONTRAK TESTCUP do not require sample manipulation and use the whole urine sample matrix for the testing (18). Thus the study of adulterant issues is especially important for the on-site tests.

It has been demonstrated that the interference in the GC–MS confirmation of marijuana use by up to 0.12M of nitrite can be overcome by a bisulfite-treatment step (12). The effect of bisulfite treatment on urine specimens that were adulterated with high concentration of nitrite (1.0M) was investigated in this study. Because nitrite can be present in urine from conditions such as urinary tract infection or medications (13,22), the effect of low nitrite concentrations on the GC–MS analysis of THC-COOH was also examined.

Experimental

Drugs and chemicals

Benzoylecgonine-HCl, morphine sulfate, phencyclidine-HCl, amphetamine-HCl, sodium nitrite, and potassium nitrite were purchased from Sigma (St. Louis, Mo). THCCOOH was obtained from Research Triangle Institute (Chapel Hill, NC).

Instrumentation and reagents

Abuscreen ONLINE reagents and calibrators, Abuscreen ONTRAK reagents, and ONTRAK TESTCUP were obtained from Roche Diagnostic Systems, Inc. (Somerville, NJ). ONLINE assays were performed on a COBAS MIRA analyzer in a semi-quantitative mode according to the manufacturer's instructions (23). Instrument calibration is performed using the five-level Abuscreen ONLINE Cannabinoids calibrators for THCCOOH assay. A four-point calibration curve was generated for each of the other four assays based on the Abuscreen ONLINE Calibration Pack (23). ONLINE technology is based on the kinetic interaction of microparticles in a solution as measured by changes in light transmission. The calibration curves were used to convert the response of the instrument (optical densities measured from the sample) to units of concentration.

The qualitative ONTRAK assays and TESTCUP assay were performed according to the manufacturer's instructions (24). The CHEMSTRIP urine test strips were purchased from Boehringer Mannheim Corp. (Indianapolis, IN).

Urine controls

Controls were prepared in pooled, filtered, normal human urine containing 0.09% sodium azide. The urine pool was determined to be drug free for the five assays examined in this study by both the Abuscreen ONLINE assays and GC–MS analysis. Multi-drug containing control was made at the assay cutoff levels (benzoylecgonine, 300 ng/mL; morphine, 300 ng/mL; THC-COOH, 50 ng/mL; amphetamine, 1000 ng/mL; and phencyclidine, 25 ng/mL), and at twice the assay cutoff levels by addition of drug stock solutions (1 mg/mL) to this urine pool. Drug concentrations in these multianalyte standard (MAS) controls (cutoff and 2× cutoff) determined to be within ± 10% of their targets by GC–MS (ElSohly Laboratories, Oxford, MS). MAS control-containing drugs at 0.5× the cutoff was prepared by dilution of the cutoff control with the pooled normal human urine. Drug concentrations in all MAS controls were also determined to be within ± 10% of their target concentrations by ONLINE. Nitrite-containing urine specimens were prepared by direct spiking of sodium nitrite or potassium nitrite to each of the MAS controls containing 0, 0.5×, 1×, or 2× of the drug cutoff levels, respectively. The final concentrations of nitrites in urine controls were as follows: 0.1M, 0.5M, or 1.0M (85 mg/mL KNO_2 or 69 mg/mL $NaNO_2$). Following the dissolution of nitrite salts, all the urine specimens and control standards were stored at room temperature and all of the assays were performed 2 days after nitrite spiking.

Conductivity of urine specimens

The conductivity of urine specimens containing nitrites was measured using a CDC 641T platinum conductivity cell and a CDM83 conductivity meter (radiometer) at room temperature.

Table I. Characteristics of the GC–MS Confirmation Assays

Drug	Extraction Method	Derivative	Limit of detection LOD (ng/mL)	Limit of quantitation LOQ (ng/mL)	Upper limit of linearity ULOL (ng/mL)
THCCOOH	liquid–liquid	Methyl	1	4	1000
Benzoylecgonine	liquid–liquid	TMS*	15	30	2000
Amphetamine	liquid–liquid	HFB†	25	50	10000
Morphine	liquid–liquid	TMS*	50	50	2000
Phencyclidine	liquid–liquid	None	1	2	400

* TMS: trimethyl silyl
† HFB: heptafluoro butyrate

GC–MS analysis

Urine specimens containing an appropriate amount of internal standard (hexadeutero-Δ^9-THCCOOH, ElSohly Laboratories, Inc., Oxford, MS) were hydrolyzed and extracted for GC–MS analysis according to the procedure published by ElSohly et al. (25). For specimens used for the bisulfite study, 250 mg sodium bisulfite was added to the specimen following the addition of internal standard, and the mixture was allowed to stand for 5 min prior to extraction (12).

GC–MS analysis was performed using a 10-m × 0.18-mm DB-1 column (0.4-μm film thickness) programmed from 200°C (0.5 min) to 250°C at 30°C/min and held 8 min. Helium was used as carrier gas at 45 cm/s, splitless injector at 250°C with a 0.2-min delay. The ions were monitored at m/z 372, 357, and 313 for THC-COOH and at m/z 378, 360, and 319 for the hexadeutero-THCCOOH. Concentrations of unknown specimens were calculated relative to a calibration curve prepared with 6, 15, and 50 ng/mL THCCOOH with both negative and positive controls.

Analyses of benzoylecgonine, morphine, amphetamine, and phencyclidine were performed according to the standard GC–MS operation procedures established in the ElSohly Laboratories, Inc. The characteristics of the confirmation assays are listed in Table I.

the detection of five abused drugs using control standards (MAS) and urine controls containing 0.1M, 0.5M, and 1.0M nitrite ion.

The spiking by up to 1.0M of nitrites did not cause a visually discernible change in the physical appearance of urine specimens. Neither potassium nitrite nor sodium nitrite affected the urinary pH. All nitrite-spiked urine produced strong positive reactions in nitrite tests using commercially
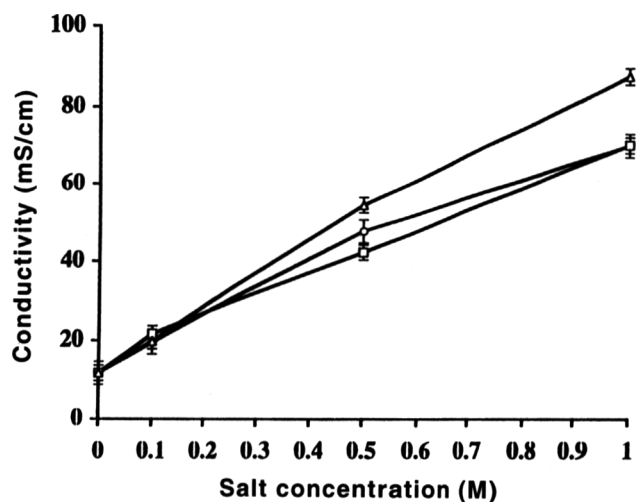

Figure 1. The relationship between urine conductivity and the salt concentrations in urine specimens. —O—, NaCl; —□—, $NaNO_2$; and —△—, KNO_3.

Results and Discussion

Nitrite-adulterated urine specimens

The adulterant Klear is generally sold by dealers as two plastic vials. Each vial is filled with a solid reagent weighing about 0.5 g that can be added directly to urine. Assuming the volume of urine collected to the specimen container is in the range of 20 to 120 mL, the nitrite concentration in the specimens (ignoring the variation of percent purity of potassium nitrite) would be in the range of 0.05 to 0.6M. Because of this assumption, we first selected to evaluate

Table II. Abuscreen ONLINE Values* for Nitrite-Free and Nitrite-Containing Multianalyte Standards

Assay	Nitrite-free standard[†]	0.5M nitrites		1.0M nitrites	
		K⁺	Na⁺	K⁺	Na⁺
Amphetamine	1008	1239	1246	1503	1481
Benzoylecgonine	302	315	309	306	316
Morphine	307	301	306	306	297
Phencyclidine	26	24	23	23	25
THCCOOH	50	48	46	40	40

* The average values from duplicate results (ng/mL) by ONLINE analysis using the COBAS MIRA automated analyzer in a semi-quantitative mode.
† Multianalyte standard

Table III. GC–MS Analysis

Assay	Concentration (ng/mL)			Percent of control standards		
	Control nitrite-free standards	Urine standards containing 1.0 M nitrite ion	Nitrite-containing urine treated with bisulfite	Control nitrite-free standards	Urine standards containing 1.0M nitrite ion	Nitrite-containing urine treated with bisulfite
Amphetamine	1055	1178	1361	100%	112%	129%
Benzoylecgonine	285	251	264	100%	88%	93%
Morphine	299	294	295	100%	98%	99%
Phencyclidine	25.6	24.2	23.6	100%	95%	92%
THCCOOH	53.1	0	47.3	100%	0%	89%

available urinalysis reagent strips that were designed to detect nitrite at concentrations 1–10,000-fold lower than 0.05M. The presence of nitrite salt also changed the conductivity of urine specimens. As shown in Figure 1, the high concentration of nitrites and other salts in the urine samples increased the ionic strength, and the conductivity of samples changed from 11 mS/cm in the control urine to 85 mS/cm in urine containing 1.0M KNO_2.

ONLINE assays

The effect of nitrites on the performance of ONLINE immunoassays was examined at three drug levels and in drug-free urine for each of the five drug assays. The average value of duplicate results from testing urine standards that contained five drugs at their specific cutoff level are shown in Table II. As compared with the nitrite-free controls, urine standards containing 0.5 or 1.0M nitrite ion showed approximately 25 or 50% higher
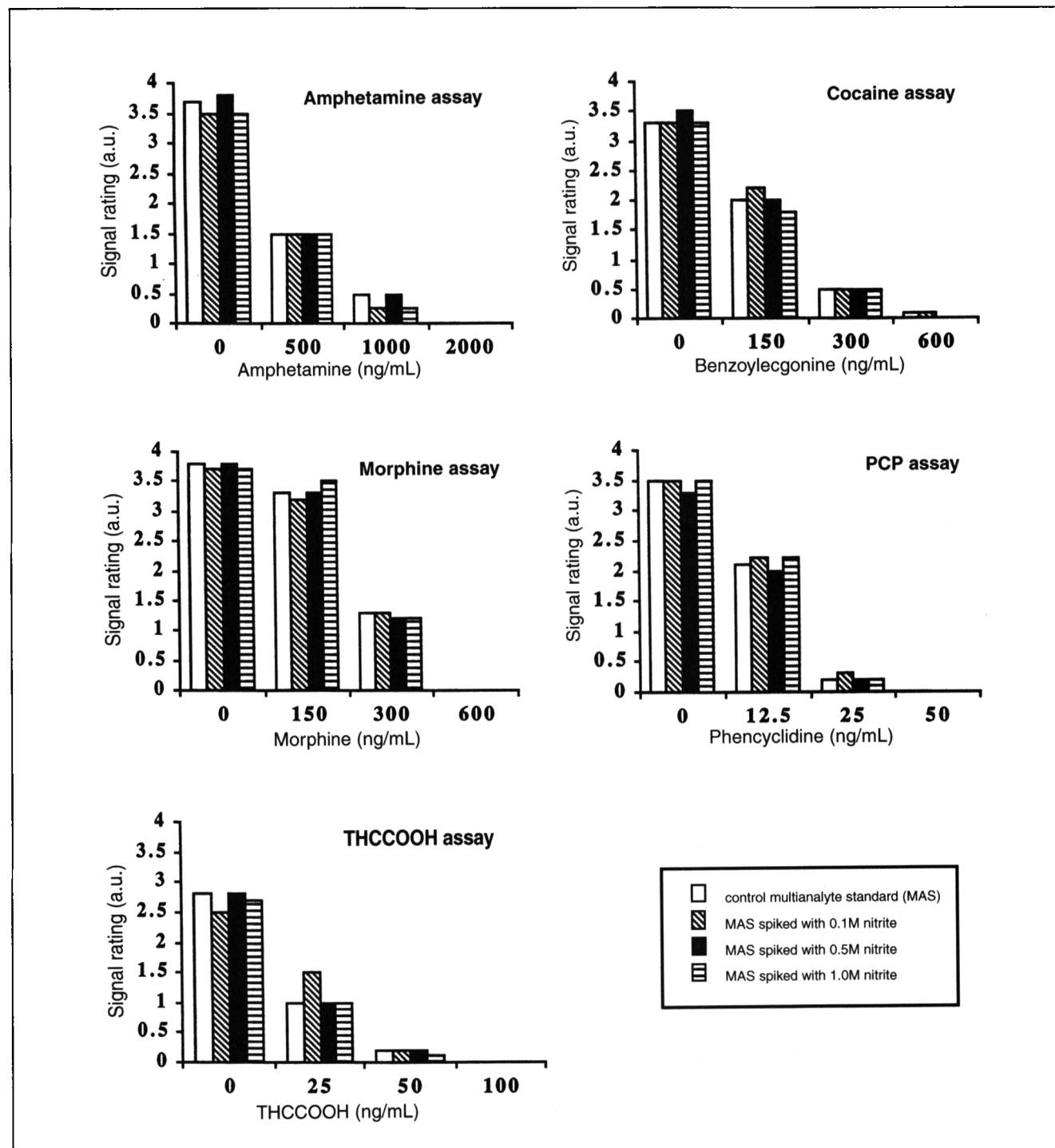
Figure 2. Evaluation of five abused drugs by the Abuscreen ONTRAK assays. The response of the ONTRAK assay was visually rated for the degree of latex agglutination based on an arbitrary unit (a.u.) of 0.0 (no agglutination) to 4.0 (total agglutination), with 0.5-unit increment. Results were expressed as the average agglutination score from three independent runs for each of the test conditions.

amount of amphetamines, respectively, in the ONLINE immunoassay. By contrast, urine standard containing 1.0M nitrite ion showed approximately 20% lower detection of THCCOOH than that of the nitrite-free controls in the ONLINE immunoassay. The dichromatic effects of nitrite on the ONLINE amphetamine and THCCOOH assays were also observed with standard containing lower drug concentrations. In the presence of 1.0M nitrite, the average ONLINE value for amphetamine was increased from 503 ng/mL in the control to 607 ng/mL (21% increase). In contrast, the average ONLINE value for THCCOOH was decreased from 25 ng/mL in control to 19 ng/mL (24% decrease). However, for all five drugs evaluated, the differences between the assays' response using the control standards and those containing 0.1 mol/L of potassium nitrite were within ± 10%.

ONTRAK assays

The effect of nitrites on the performance of ONTRAK immunoassays is shown in Figure 2. The degree of latex agglutination in the ONTRAK slide was assigned an arbitrary reading unit (a.u.) based on a 0 to 4.0 scale with increments of 0.5. Results from each of the ONTRAK slides in this study were individually compared to a set of photographs displaying readings corresponding to the appropriate degrees of latex agglutination. A reading of 0 showed complete inhibition (no agglutination), and a reading of 0.5 or 1.0 indicated only slight granulations. Readings from 1.5 to 4.0 were designated to gradually increasing degrees of latex agglutination with 4.0 indicating the strongest agglutination. For the field use, any agglutination (equivalent to ≥ 1.5 a.u. in this rating system) is considered a negative result. The normal noise between multiple replicates in this assay is ± 0.5 for samples showing agglutination. In this study, triplicate ONTRAK slides were run for each of the conditions tested and the average agglutination score was calculated and shown in Figure 2. No significant difference in the average agglutination scores was observed between the control standards and those containing 0.1, 0.5, or 1.0M of nitrite salts.

TESTCUP-5 assay

The effect of nitrites on the performance of the TESTCUP-5 assay is shown in Figure 3. For uniformity of on-site result interpretation, a signal-intensity rating system was developed. Each result was rated visually according to a 0 to 3.0 scale in a 0.5 increment, with 3.0 color unit (c.u.) representing the strongest color. Result readings using the rating system produced comparable standard curves to those obtained from colorimetric density measurements of the test strip result bands using a Minolta CR-241 Chroma Meter (26). Standard curves for each assay have been generated and photographed to serve as references for the designated rating system. The TESTCUP assays have been calibrated so that ratings of 0 (no color) or 0.5 (very faint color) indicate positive results, and ratings of 1.0 (a light blue band) and above denote negative results. As shown in Figure 3, the overall accuracy of the TESTCUP-5 assay to distinguish a positive from a negative result was not affected by the presence of nitrite ion. However, the signal intensity of the negative result for THC and phencyclidine assays was slightly suppressed by the presence of high levels of nitrites.

GC–MS analysis

Table III summarizes the GC–MS analysis of the control standards, the urine MAS spiked with 1.0M nitrite, and nitrite-spiked urine specimens that were treated with sodium bisulfite as described in the Materials and Methods section. For the five assays analyzed, the most significant interference was only observed with the THCCOOH analysis. There was no detectable THCCOOH or its internal standard in the nitrite-containing urine. However, the bisulfite-treatment step alleviated this problem and resulted in 89% recovery of THCCOOH. The spiking of nitrite ion increased the recovery of amphetamine. It is interesting because the presence of nitrite significantly increased the immuno-reactive response for amphetamine (Table II). Further studies are warranted to provide possible explanations for this observation.

Sample storage

In this study, the nitrites were left in contact with the drugs or drug metabolites for 2 days before the assays were performed to simulate the time lag between specimen collection and laboratory analysis. It should be noted that the duration between initial sample collection and the GC–MS confirmation test may be longer than a few days, and therefore, the stability of THCCOOH following storage of urine in the presence of nitrite may influence the drug-test results. In addition, the possible degradation of THCCOOH metabolite by the nitrite adulterant over time can be problematic for the analysis of bottle B in regulated testings.

In a preliminary study, urine standards (pH 7) that had been spiked with 1.0M nitrite and refrigerated for 2 weeks showed substantial recovery of THCCOOH following bisulfite treatment (47.3 ng/mL after 2 days of storage versus 43.4 ng/mL after 2 weeks of storage). However, subsequent study demonstrated that the degradation of THCCOOH in the presence of Klear was pH dependent. After a 3-week exposure to 0.15M nitrite in refrigerated storage, the THCCOOH was not recoverable (0% recovery) even with bisulfite treatment if the urine standard was adjusted to pH 5 or pH 6. By contrast, >90% of THCCOOH could be recovered following bisulfite treatment if the urine standard was adjusted to pH 8.

The time and concentration limits alleviated by the bisulfite could be different from sample to sample because of the variation between samples in pH, ionic strength, and storage conditions. However, at the time of analysis in this study, a sufficient quantity of the bisulfite was used to decompose all nitrite ions in the

Table IV. GC–MS Analysis of THCCOOH

| Concentration of nitrite ion in urine	Percent of control THCCOOH value*
0.294 mM (25 µg KNO$_2$/mL)	96.9%
0.588 mM (50 µg KNO$_2$/mL)	95.3%
2.941 mM (100 µg KNO$_2$/mL)	98.9%
5.882 mM (500 µg KNO$_2$/mL)	96.4%
11.765 mM (1 mg KNO$_2$/mL)	97.8%

* Percent of control = 100% × (ng/mL THCCOOH in nitrite-containing urine)/(ng/mL THCCOOH in control standard)

sample to recover any undegraded THCCOOH. Nevertheless, the net recovery of THCCOOH would vary from sample to sample depending on urine property and storage time and conditions. Further studies are currently being conducted to investigate the stability of THCCOOH in the presence of nitrite in various urine matrices and in different temperature conditions. Moreover, patient specimens (rather than spiked specimens) contain predominantly the acid glucuronide in combination with the THCCOOH. The effect of nitrites on the acid glu-

curonide under different conditions will also be the subject of a future report.

Nitrite from natural sources

Although nitrite is not a constituent of normal urine, nitrites can be present in urines from individuals suffering from urinary tract infections with nitrate-reducing microorganisms or in specimens from patients on medications that may metabolize to nitrite (13,22). It has been reported that there is a substantial
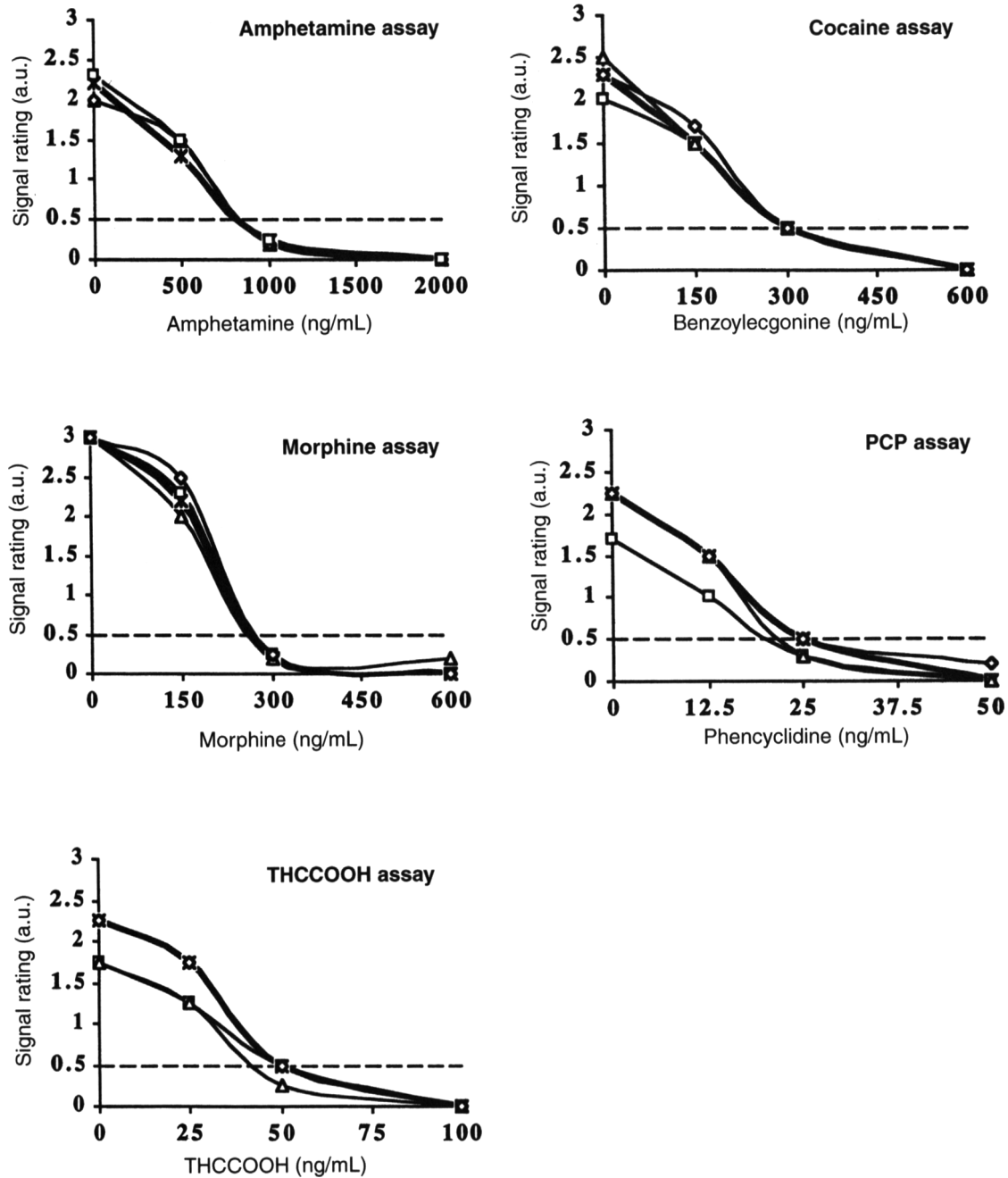
Figure 3. Evaluation of 5 abused drugs by the ONTRAK TESTCUP-5 assay. The response of the TESTCUP-5 assay was visually rated for the degree of color formation (color units: c.u.) from 0.0 (no color) to 3.0 (strongest color) with 0.5-unit increment. Results were expressed as the Average Signal Color Rating (c.u.) from two independent runs for each of the test conditions. — X —, MAS urine control; — ◇ —, MAS spiked with 0.1M nitrite; — △ —, MAS spiked with 0.5M nitrite; — □ —, MAS spiked with 1.0M nitrite. The dotted line indicates the upper limit of a positive result interpretation.

difference between nitrite concentrations from natural sources and adulteration; however, it is important to ensure that the nitrite levels in non-adulterated samples are not sufficient to produce false-negative results for the THCCOOH confirmation testing. Additional experiments were performed using a urine standard that contained THCCOOH at its cutoff level to determine the effect of low concentrations of nitrite on the GC–MS analysis of THCCOOH. Although the presence of 30mM or more of nitrite ion considerably reduced the recovery of THCCOOH and its internal standard, a negligible difference in the THC-COOH recovery was observed when the THCCOOH single-analyte standard was spiked with a series of nitrite concentration ranging from 0.3 to 12mM (Table IV). The comprehensive study by Urry et al. (13) indicated that the highest nitrite concentration in urine from natural sources in their study was 129 μg/mL (2.8mM nitrite ion). They further estimated that the maximum nitrite concentration in urine from all sources other than purposeful addition was 300 mg/mL (6.5 mM nitrite ion). Therefore, urine nitrite derived from natural sources should not produce false-negative results for THCCOOH confirmation testing.

References

1. M. DeLancey and E.S. Kinkennon. *Does Drug Testing Work?* The Institute for a Drug-Free Workplace, Washington D.C., 1994.
2. T. Mieczkowski and K. Lersch. Drug testing in criminal justice. *J. Natl. Inst. Justice* **234**: 9–15 (1997).
3. G. Hemphill. Drugs of abuse testing. *Adv. Med. Lab. Professionals* **Oct**: 10–14 (1995).
4. J.M. Walsh. Drug testing, a standard business practice. *Empl. Assist.* **10**: 26–29 (1995).
5. American Management Association. *Workplace Drug Testing and Drug Abuse Policies.* New York, NY, 1995.
6. D.B. Fraser. Labs scramble to keep pace with schemes for defeating tests. *Forensic Urine Drug Test.* **Aug**: 1–6 (1997).
7. L.A. Broussard. Sample adulteration and other challenges. *Forensic Urine Drug Test.* **Dec**: 1–6 (1993).
8. S.D. Pearson, K.O. Ash, and F.M. Urry. Mechanism of false-negative urine cannabinoid immunoassay screens by Visine eye drops. *Clin. Chem.* **35**: 636–638 (1989).
9. A. Warner. Interference of common household chemicals in immunoassay methods for drugs of abuse. *Clin. Chem.* **35**: 648–651 (1989).
10. E. O'Connor, D. Ostheimer, and A.H.B. Wu. Limitations of forensic urine drug testing in methodology and by adulteration. *AACC TDM/Tox.* **14**: 277–288 (1993).
11. S. George and R.A. Braithwaite. The effect of glutaraldehyde adulteration of urine specimens on Syva EMIT II drug-of-abuse assays. *J. Anal. Toxicol.* **20**: 195–196 (1996).
12. M.A. ElSohly, S. Feng, W.J. Kopycki, T.P. Murphy, A.B. Jones, and D. Carr. A procedure to overcome interferences caused by the adulterant "Klear" in the GC-MS analysis of 11-nor-Δ^9-THC-9-COOH. *J. Anal. Toxicol.* **21**: 240–242 (1997).
13. F.M. Urry, G. Komaromy-Hiller, B. Staley, D.K. Crockett, M. Kushnir, G. Nelson, and R.E. Struempler. Nitrite adulteration of workplace urine drug testing specimens I. Sources and associated concentrations of nitrite in urine and distinction between natural sources and adulteration. *J. Anal. Toxicol.* **22**: 89–95 (1998).
14. A.H.B. Wu and K. Rymut. The adulteration of urine drug testing samples with "Klear". *AACC TDM Clin. Tox. Newsletter* **12**: 1–2 (1997).
15. F.M. Esposito. Problems associated with the confirmation of marijuana metabolites in urine specimens suspected of being adulterated with KLEAR (potassium nitrite). Proc. Am. Acad. *Forensic Sci.* **4**: 249–250 (1998).
16. S.D. Ferrara, L. Tedeschi, G. Frison, G. Brusini, F. Castagna, B. Bernardelli, and D. Soregaroli. Drugs-of-abuse testing in urine: statistical approach and experimental comparison of immunochemical and chromatographic techniques. *J. Anal. Toxicol.* **18**: 278–291 (1994).
17. D.A. Armbruster and J.M. Krolak. Screening for drugs of abuse with the Roche ONTRAK assays. *J. Anal. Toxicol.* **16**: 172–175 (1992).
18. J. Towt, S.-C.J. Tsai, M.R. Hernandez, A.D. Klimov, C.V. Kravec, S.L. Rouse, H.S. Subuhi, B. Twarowska, and S.J. Salamone. ONTRAK TESTCUP: a novel, on-site, multi-analyte screen for the detection of abused drugs. *J. Anal. Toxicol.* **19**: 504–510 (1995).
19. D.J. Crouch, M.L. Cheever, D.M. Andrenyak, D.J. Kuntz, and D.L. Loughmiller. A comparison of ONTRAK TESTCUP, Abuscreen ONTRAK, Abuscreen ONLINE and GC-MS urinalysis test results. *J. Forensic Sci.* **43**: 35–40 (1998).
20. D.A. Armbruster. On-site assays bring drug testing to workplace-literally. *Adv. Med. Lab. Professionals* **Oct**: 6–8 (1996).
21. D.A. Armbruster. On-site on the rise. *Med. Lab. Observer* **Apr**: 40–44 (1997).
22. S.K. Strasinger. *Urinalysis and Body Fluids,* 3rd ed. F.A. Davis, Philadelphia, PA, 1994.
23. Abuscreen ONLINE for Cocaine; ONLINE for Opiate; ONLINE for THC (50 ng/mL); ONLINE for Amphetamine; and ONLINE for Phencyclidine: Package insert Rev. 3/96. Abuscreen ONLINE Cannabinoids Calibration Pack; Abuscreen ONLINE Calibration Pack: Package inserts, Rev. 4/95. Roche Diagnostic Systems, Inc., Somerville, NJ.
24. Abuscreen ONTRAK for Cocaine, ONTRAK for Morphine, ONTRAK for THC (50 ng/mL), ONTRAK for Amphetamine and ONTRAK for Phencyclidine, package inserts, Rev. 9/94; ONTRAK TESTCUP-5, package insert 3/97, Roche Diagnostic Systems, Inc., Somerville, NJ.
25. M.A. ElSohly, T.L. Little, Jr., and D.F. Stanford. Hexadeutero-11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid: a superior internal standard for the GC/MS analysis of Δ^9-THC acid metabolite in biological specimens. *J. Anal. Toxicol.* **16**: 188–191 (1992).
26. A.D. Klimov and S.-C.J. Tsai. U.S. Patent application serial no. 08/385,300, 1995.