Clinical Chemistry 51:11
2085–2094 (2005)

Drug Monitoring and Toxicology

Disposition of Cocaine and Its Metabolites in Human Sweat after Controlled Cocaine Administration

Sherri L. Kacinko,[1] Allan J. Barnes,[1] Eugene W. Schwilke, [1] Edward J. Cone,[2] Eric T. Moolchan,[1] and Marilyn A. Huestis[1*]

Background: **Sweat testing is a noninvasive technique for monitoring drug exposure in treatment, criminal justice, and employment settings.**

Methods: **We evaluated cocaine excretion in 9 participants' sweat after they received 3 low doses (75 mg/70 kg) of cocaine HCl subcutaneously within 1 week and, 3 weeks later, 3 high doses (150 mg/70 kg). Six additional participants completed portions of the study. PharmChek® sweat patches (n = 1390) were collected throughout a 3-week washout period, reflecting previously self-administered drugs, and during and after controlled dosing.**

Results: **Cocaine was the primary analyte detected with 24% of patches positive at the gas chromatography–mass spectrometry limit of quantification of 2.5 ng/patch and 7% of patches at the proposed Substance Abuse and Mental Health Services Administration cutoff of 25 ng/patch. Ecgonine methyl ester (EME) was detected more often and at generally higher concentrations than benzoylecgonine. In patches containing both metabolites, there was no statistically significant difference in the benzoylecgonine/EME ratio based on length of patch wear. During washout, 2 participants' weekly patches tested positive (≥25 ng/patch) during the first week; one remained positive during week 2; and none were positive during week 3. Cocaine and EME were detectable within 2 h; benzoylecgonine was not detected until 4–8 h after low doses and slightly sooner after high doses. The majority of drug was excreted within 24 h. Over 70% of weekly patches worn during low doses were positive for cocaine (≥25 ng/patch), increasing to 100% during high doses.**

Conclusion: **Sweat testing is an effective and reliable method of monitoring cocaine exposure.**

Alternative biological matrices such as sweat provide a less invasive method of monitoring illicit drug use over a longer period of time in treatment, workplace, military, and criminal justice settings. Identification of the parent drug and its metabolites, analyte concentrations, time course of detection, and dose–concentration relationships are important aspects of evaluating sweat testing for cocaine (COC).[3] COC is included in federally mandated drug testing because of its abuse potential and performance-impairing effects. The Substance Abuse and Mental Health Services Administration (SAMHSA) has proposed screening cutoffs of 25 ng/patch for COC metabolite screening in sweat and a 25 ng/patch cutoff for confirmation of COC or benzoylecgonine (BE) *(1)*.

The mechanisms by which drugs are incorporated into sweat are not fully understood. The primary mechanism appears to be passive diffusion of nonionized drug from capillaries into sweat glands. At the lower pH of sweat, drugs may ionize and accumulate in sweat. In addition, drugs may diffuse through the dermal and epidermal layers of the skin *(2–4)*. Drugs excreted in sweat may be collected with the PharmChek® sweat patch, which con-

[1] Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD.
[2] Johns Hopkins School of Medicine, Department of Psychiatry and Behavioral Sciences, Baltimore, MD.
* Address correspondence to this author at: Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 5500 Nathan Shock Dr., Baltimore, MD 21224. Fax 410-550-2971; e-mail mhuestis@intra.nida.nih.gov.

Received May 13, 2005; accepted August 16, 2005.
Previously published online at DOI: 10.1373/clinchem.2005.054338

[3] Nonstandard abbreviations: COC, cocaine; SAMHSA, Substance Abuse and Mental Health Services Administration; BE, benzoylecgonine; NIDA, National Institute on Drug Abuse; EME, ecgonine methyl ester; BSTFA, *N,O*-bis(trimethyl)trifluoroacetamide; TMCS, trimethylchlorosilane; MTBSTFA, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide; TBDMCS, *tert*-butyldimethylchlorosilane; and LOQ, limit of quantification.

sists of an absorbent cellulose pad that is attached to cleansed skin with a Band-Aid®–like adhesive. The polyurethane backing protects the patch from environmental contamination while allowing water vapor and gases to escape. Patches are generally worn for 1 week, with drugs in sweat reflecting use as much as 24–48 h before patch application through the time of patch removal *(5)*. Advantages of sweat testing include ease of use, decreased opportunity for adulteration, and long detection windows *(6)*. There are limitations to the patch, however, including intra- and intersubject variability in sweat production, possible contamination from the environment, loss of drug as a result of degradation on the collection device or reabsorption through the skin, and occasional skin sensitivity *(6–9)*.

In this comprehensive study, we evaluated multiple aspects of COC and its metabolite excretion in sweat from prior illicit use and after controlled drug administration. The concentrations of COC and 10 metabolites in 3 weekly sweat patches applied before drug administration monitored COC excretion during washout from previously self-administered drug. After COC administration, the complete time course of COC excretion in sweat was examined with short-term sweat patches worn for up to 48 h after low and high COC doses and with weekly sweat patches. The relationship between administered dose and drug concentrations in sweat was examined at both COC doses. In addition, duplicate patches were applied to determine the reproducibility of sweat patch results, and the stability of COC and its metabolites during patch wear was evaluated by comparing the sum of short-term patch COC concentrations with those of weekly patches.

Materials and Methods

STUDY POPULATION

Participants (n = 15), who provided informed consent for this National Institute on Drug Abuse (NIDA) Intramural Research Program Institutional Review Board–approved study, resided on the closed research unit for 12 weeks and were compensated for their participation. Participants had a history of COC use and tested positive for COC metabolites in their urine before study recruitment.

DRUG ADMINISTRATION

There was a 3-week washout period to study excretion of previously self-administered drug. In the fourth week, participants received 3 subcutaneous injections of COC hydrochloride (75 mg/70 kg; Mallinkrodt) in saline approximately every 48 h. Three weeks later, 3 high doses (150 mg/70 kg) were given according to the timeline illustrated in Fig. 1. According to the March 2002 report by the Drug Enforcement Administration on COC abuse, it is "reasonable" to assume that 100 mg is the average dosage unit for crack or powder COC *(10)*.

SWEAT COLLECTION

Sweat was collected with PharmChek sweat patches provided by PharmChem, Inc. (Haltom City, TX). After thorough cleansing of the skin with alcohol swabs (saturated with 70% isopropyl alcohol), patches were applied to the back or abdomen and removed according to the schedule in Fig. 1. Patches were characterized as follows: weekly washout patches, patches worn for 4 h or less (short-term patches), patches worn for 15 h during the first and second days (15-h patches), weekly patches worn during COC dosing, and patches worn for consecutive

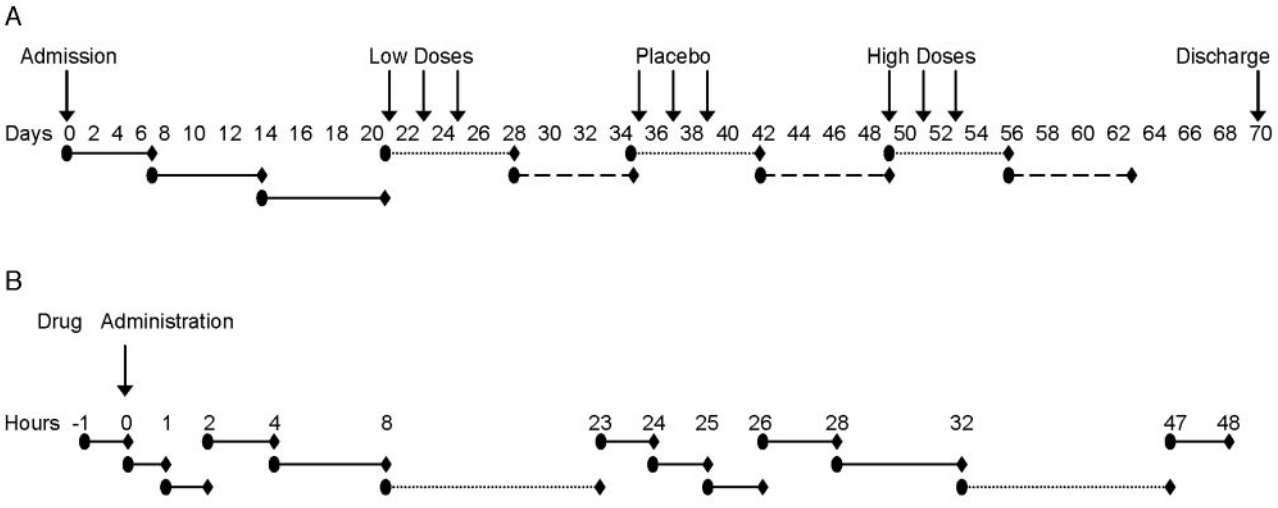

Fig. 1. Dosing schedule of patients (*A*), and times of application and removal of patches (*B*).

(*A*), dosing schedule, from participant admission to discharge, of 3 subcutaneous low (75 mg/70 kg), placebo, and high (150 mg/70 kg) doses of COC. Shown is the sweat patch application (●) and removal (♦) schedule for 3 weekly washout patches (*solid lines*), weekly patches worn during drug administration (*dotted lines*), and weekly patches worn the week after administration (*dashed lines*). (*B*), application (●) and removal (♦) schedule of patches worn for 1, 2, or 4 h (short-term patches; *solid lines*), and 15-h patches (*dotted lines*) worn for 48 h after each low, placebo, or high dose.

weeks after the completion of dosing. After removal, patches were stored in labeled plastic bags at −20 °C until analysis.

CHEMICALS, REAGENTS, AND MATERIALS

Chemicals were obtained from the following sources: COC hydrochloride (Mallinkrodt); BE, *m*- and *p*-hydroxycocaine · 0.5 H_2O, *m*-hydroxybenzoylecgonine · 0.5 H_2O, and *p*-hydroxybenzoylecgonine · 0.7 H_2O (Research Biochemicals International); ecgonine methyl ester (EME) hydrochloride, [$^2H_3$]-EME hydrochloride · H_2O, [$^2H_3$]-COC, [$^2H_3$]-cocaethylene, and [$^2H_3$]-BE · 4 H_2O (Sigma Chemicals and Cerilliant); cocaethylene, norcocaethylene · fumerate (Research Triangle Institute and Cerilliant); ecgonine ethyl ester (Intramural Research Program, NIDA, and Cerilliant); norcocaine hydrochloride (Research Biochemicals International and Cerriliant); and *N*,*O*-bis(trimethyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) containing 1% *tert*-butyldimethylchlorosilane (TBDMCS; Pierce Chemical and United Chemical Technologies). Solid-phase extraction columns (Clean Screen DAU; 200 mg, 10 mL) and 12-mL filtration columns were obtained from United Chemical Technologies. Methanol, methylene chloride, 2-propanol, and acetonitrile were HPLC grade; all other chemicals were reagent grade.

INSTRUMENTATION

Quantitative analyses were performed on an Agilent 6890 gas chromatograph interfaced with an Agilent 5973 mass-selective detector. A split–splitless capillary inlet system operated in splitless mode and an HP-1MS capillary column [30 m × 0.32 mm (i.d.); 0.25 μm film thickness] were used for analyses. The injection port and transfer line temperatures were 250 and 295 °C, respectively. Oven temperature was held at 70 °C for 1 min, ramped to 175 °C at 30 °C/min, to 250 °C at 23 °C/min, and finally to 310 °C at 18 °C /min and held for 5 min. The electron multiplier was set with a 400 EMV offset. Quantification was performed with the mass-selective detector in selected-ion monitoring mode with a dwell time of 10 ms for each compound. The ions monitored are listed in Table 1.

CALIBRATORS AND CONTROLS

Working solutions of COC and its metabolites were prepared at 0.25 and 5.0 mg/L. Aliquots of the appropriate working solutions were added to clean cellulose pads to provide calibrators at concentrations of 2.5, 5.0, 10, 25, 50, 100, 250, and 500 ng/patch. Control working solutions were prepared from a different stock solution at drug concentrations of 0.05, 0.25, 2.5, and 5.0 mg/L. Control working solutions were pipetted onto clean patches and air dried to produce final concentrations of 3.75, 12.5, 125, and 375 ng/patch.

Table 1. Target and qualifier ions for gas chromatographic–mass spectrometric analysis of COC, its metabolites, and internal standards in sweat.[a]

Analyte	Target ion	Qualifier ion 1	Qualifier ion 2
d_3-EME	185	259	
EME[b]	182	96	256
Ecgonine ethyl ester[b]	96	270	82
d_3-COC	185	306	
COC[c]	182	303	272
d_3-Cocaethylene	199	320	
Cocaethylene[d]	196	317	272
Norcocaine[c]	140	179	240
Norcocaethylene[d]	254	360	140
d_3-BE	285	349	
BE[e]	282	346	403
m-Hydroxycocaine[c]	182	433	82
p-Hydroxycocaine[c]	182	433	82
m-Hydroxybenzoylecgonine[e]	282	533	476
p-Hydroxybenzoylecgonine[e]	282	476	533

[a] Analytes are listed in elution order.
[b] d_3-EME as internal standard.
[c] d_3-COC as internal standard.
[d] d_3-Cocaethylene as internal standard.
[e] d_3-BE as internal standard.

SPECIMEN ANALYSIS

Sweat patch specimens were analyzed for COC, BE, EME, ecgonine ethyl ester, cocaethylene, norcocaine, norcocaethylene, *m*- and *p*-hydroxycocaine, and *m*- and *p*-hydroxybenzoylecgonine by modifying the method published by Huestis et al. *(11)*. One hundred microliters of a deuterated internal standard solution containing 1 mg/L d_3-COC, d_3-BE, d_3-EME, and d_3-cocaethylene was added to previously prepared calibrator and quality-control samples and clinical specimens. Cellulose pads were placed in 12-mL filtration columns fitted with stopcocks, and 4-mL aliquots of 0.5 mol/L sodium acetate buffer (pH 4.0) were added to immerse the cellulose pads in solution for 30 min at room temperature. Buffer extracts were eluted into round-bottomed tubes. This step was repeated twice more with 2 mL of buffer.

Solid-phase extraction columns were conditioned sequentially with 1 mL of elution solvent (methylene chloride–2-propanol–ammonium hydroxide; 80:20:2 by volume), 3 mL of methanol, 3 mL of water, and 2 mL of buffer. Buffer eluates were decanted onto preconditioned columns, which were then washed with 2 mL of water, 1.5 mL of 0.2 mol/L hydrochloric acid, and twice with 1 mL of methanol. Columns were dried under full vacuum for 10 min and eluted into centrifuge tubes with five 1-mL volumes of elution solvent. MTBSTFA (20 μL containing 1% TBDMCS) was added, and the eluate was evaporated to dryness. Samples were reconstituted with 500 μL of acetonitrile, vortex-mixed, dried under nitrogen, reconstituted with 20 μL of acetonitrile, vortex-mixed, and transferred to labeled autosampler vials. BSTFA (20 μL containing 1% TMCS) was added, and the mixture heated for

15 min at 80 °C. After cooling for 10 min, MTBSTFA (20 μL containing 1% TBDMCS) was added, and vials were capped and heated at 80 °C for 45 min. An aliquot (2 μL) of the derivatized extract was analyzed by gas chromatography–mass spectrometry.

DATA ANALYSIS

Two calibration curves, 2.5–50 ng/patch and 50–100 ng/patch, were used to quantify quality-control samples and participant specimens. The method limits of quantification (LOQ) were 2.5 ng/patch for COC, BE, EME, ecgonine ethyl ester, and cocaethylene and 5.0 ng/patch for all other analytes.

Concentration ranges and mean values were calculated with only the positive patches in the group, whereas all values were used in the calculation of the median. Statistical analyses were performed with SPSS (Ver. 10.0) for Macintosh, release 10.0.7a (SPSS, Inc.). For comparisons between groups, the Student *t*-test for independent samples was used. Simple correlations were performed with Spearman correlation coefficients. A 2-tailed *P* value <0.05 was considered statistically significant.

Results

PARTICIPANT DATA

Eight males and 7 females [mean (SD) age, 35.3 (4.4) years; range, 26–43 years] participated in the study. Nine participants (6 males, 3 females) completed the entire study, receiving 3 low and 3 high doses. An additional 2 men and 3 women received all of the low doses, with 1 male participant also receiving a single high dose. One female participant received only 2 low doses. Participants who did not complete the study did so for a variety of reasons, including medical decisions not to administer high doses, behavioral compliance issues, and participant withdrawal of consent.

SWEAT PATCH RESULTS

Of 1390 sweat patches collected before, during, and after subcutaneous COC administration, 342 contained one or more COC analytes (Table 2). Overall, 24% of analyzed patches were positive for COC at the method LOQ of 2.5 ng/patch, and 7% were positive at the proposed SAMHSA cutoff of 25 ng/patch. BE concentrations were not available for 32 patches because of experimental error. Of the remaining patches (n = 1358), 5% were positive for BE at the LOQ with 0.5% positive patches at the proposed SAMHSA cutoff. EME was detected in more patches than BE for a total of 9% positive patches at the LOQ and 0.8% of patches with concentrations ≥25 ng/patch. In the positive patches, COC was the predominate analyte with 97% of patches testing positive at the LOQ and 29% positive at the proposed SAMHSA cutoff. Approximately two thirds of these patches contained only COC, 17% contained COC and EME, 17% contained all 3 analytes, and 4% contained COC and BE. Several minor metabolites were detected in patches from 2 individuals after the high doses. Participant K had 2 patches positive for ecgonine ethyl ester; 1 of these patches also contained *m*- and *p*-hydroxycocaine. One additional patch from participant M was positive for *p*-hydroxycocaine.

Duplicate results for the weekly washout patches for 12 participants are summarized in Table 3. The concentrations of COC and its metabolites reflected excretion of previously self-administered drug and were evaluated at 2 cutoffs: the LOQ of the method (2.5 ng/patch) and the proposed SAMHSA cutoff for COC or BE (25 ng/patch). Generally COC, BE, and EME concentrations decreased in consecutive weekly patches. However, patches from 2 participants were positive for COC in week 3, after the patches from week 2 had tested negative. One participant had higher BE and EME concentrations in a week 2 patch than in the week 1 patches, and an additional participant had an EME-positive week 2 patch when both week 1 patches were negative. At the SAMHSA cutoff, 2 participants had COC-positive patches during week 1, one of whom also had BE and EME concentrations >25 ng/patch. This individual was the only individual to remain positive for all 3 analytes at the proposed SAMHSA cutoff during washout week 2. There were no SAMHSA-positive patches during washout week 3.

Overall, COC was detected at the method LOQ during all short-term patch time periods (0–1, 1–2, 2–4, 4–8, and 23–24 h) in the first 24 h after low dose administration and

Table 2. Total positive sweat patches and detection rates for COC, BE, and EME at the LOQ of 2.5 ng/patch and the proposed SAMHSA cutoff for COC or BE of 25 ng/patch.

	Totals			Analytes detected in positive patches (n = 342)					
	COC	BE	EME	COC only	BE only	EME only	COC and BE	COC and EME	COC, BE, and EME
Total patches, n	1390	1358[a]	1390						
≥2.5 ng/patch									
No. of patches	332	73	125	202	2	8	13	59	58
% positive	23.9	5.4	9.0	59.0	<1	2.3	3.8	17.3	17.0
≥25 ng/patch									
No. of patches	99	7	11	84	0	0	4	8	3
% positive	7.1	0.5	0.8	24.6			1.2	2.3	<1

[a] 32 patches were not analyzed for BE because of experimental error.

Table 3. Sweat patch concentration ranges and detection rates for COC, BE, and EME in duplicate weekly patches collected the first 3 weeks from 12 participants residing on the clinical research unit.

	Week 1 (n = 24)[a]			Week 2 (n = 22)[b]			Week 3 (n = 20)[c]		
	ng/patch	≥2.5 ng/patch, %	≥25 ng/patch, %	ng/patch	≥2.5 ng/patch, %	≥25 ng/patch, %	ng/patch	≥2.5 ng/patch, %	≥25 ng/patch, %
COC	3.7–197.1	62.5	16.7	2.6–59.7	36.4	9.1	5.0–13.8	25	0
BE	2.6–62.3	33.3	8.3	4.3–162.2	18.2	9.1	ND[d]	0	0
EME	3.6–29.2	41.7	4.2	3.9–41.9	27.3	4.5	5.5–6.1	10	0

[a] Twelve participants with duplicate weekly patches.
[b] Eleven participants with duplicate weekly patches.
[c] Nine participants with duplicate weekly patches, and 2 participants with single weekly patches.
[d] ND, no drug detected above the method LOQ.

in patches worn at least 2 h the day after administration. Short-term patches were rarely positive for COC at the proposed SAMHSA cutoff (8 of 92 patches; 8.7%) during the first 24 h with no patches COC positive during the second 24 h. BE was detected at the LOQ in patches applied and removed 2–4 h (3.6%), 4–8 h (10.3%), and 23–24 h (3.3%) after dosing but was never detected at concentrations greater than the proposed SAMHSA cutoff after a low dose. EME was detected sooner, more often, and at generally higher concentrations than BE. EME detection rates in 1–2, 2–4, 4–8, and 23–24 h patches were 5.6%, 10.7%, 10.3%, and 3.3%, respectively.

More than 50% of short-term patches had concentrations greater than the method LOQ during the first 24 h after high dose administration. The day after drug exposure, 15% of short-term patches were positive for COC at the method LOQ. Each short-term time period in the first 24 h had at least one SAMHSA positive patch; no patches had COC concentrations >25 ng/patch the day after high dose administration. The maximum COC concentration was 375.4 ng/patch detected 2–4 h after the first high dose. The 2–4 h time period also had the highest percentage of SAMHSA-positive patches (55%). BE was present above the LOQ in 9%–15% of patches worn for 1 to 4 h, with none positive for BE at the proposed SAMHSA cutoff. EME was detected during the same time periods and more frequently than BE at the method LOQ (11%–45% positive). A single patch, worn 2–4 h after the first high dose, had an EME concentration >25 ng/patch.

More than 75% of patches worn for 15 h during the first day of low dose administration were positive for COC at the LOQ, with 22% having concentrations ≥25 ng/patch (range, 3.8–84.7 ng/patch). The percentage of positive patches decreased to 31% during the second day (32–47 h after dose); no patches were positive for COC at the proposed SAMHSA cutoff more than 24 h after dosing. Consistent with other time points, EME was detected more often and at similar or higher concentrations than BE during both the first and second 24-h periods. After low doses, EME detection rates were 27% and 3% during the first and second 24 h, respectively, with 19% and 3% positive for BE at the LOQ. BE concentrations in the 8–23 h patches ranged from 2.6 to 12.8 ng/patch with a mean (SD) concentration of 6.0 (3.5) ng/patch; the mean (SD) EME concentration was 5.8 (2.6) ng/patch (range, 2.5–9.6 ng/patch). Two 15-h patches (3%) from 2 different participants were positive for EME and BE 32–47 h after dose administration. No 15-h patches were positive for BE or EME at the 25 ng/patch cutoff after low doses.

After the high dose of 150 mg/70 kg, more than 90% of 15-h patches in the first 24 h were positive for COC at the method LOQ [range, 3.5–149.0 ng/patch; mean (SD), 33.6 (51.5) ng/patch]. The rate of BE- and EME-positive samples also increased to 39% and 46%, respectively. The mean BE concentration was 7.9 (3.3) ng/patch with EME concentrations almost twice as high at 11.1 (13.5) ng/patch. More than one third of 15-h patches collected on the day of dosing were positive for COC at the proposed SAMHSA cutoff, whereas only a single patch was positive for EME at a concentration >25 ng/patch. The number of positive samples also increased during the 32–47 h time period after dosing for all 3 analytes when the high dose was administered. COC was detected above the LOQ in almost 50% of patches with a mean concentration of 15.3 (19.5) ng/patch. BE and EME were detected in 14% of these patches with concentration ranges of 2.7–13.9 and 2.9–6.6 ng/patch, respectively. COC was the only analyte detected above 25 ng/patch in 15-h patches worn the day after dose administration; ~10% were positive.

The time courses of COC, BE, and EME excretion in sweat after low and high doses are shown in panels A and B, respectively, of Fig. 2. To account for the length of patch wear, the mean analyte concentration of each patch was divided by the time the patch was worn. COC was detected within the first hour after dosing and was detectable during all time periods in the first 24 h. During the second 24-h period, COC generally was detected only in patches worn for 15 h. EME usually was detected at higher concentrations than BE. In most cases, analytes were detected earlier and at higher concentrations after high doses.

All weekly patches (n = 18) worn during dosing were positive for COC above the LOQ [range, 5.1–191.3 ng/patch; mean (SD), 63.1 (53.4) ng/patch; median, 45.4 ng/patch)], and 72% were positive at the proposed SAMHSA cutoff during low dose administrations. Less than one half

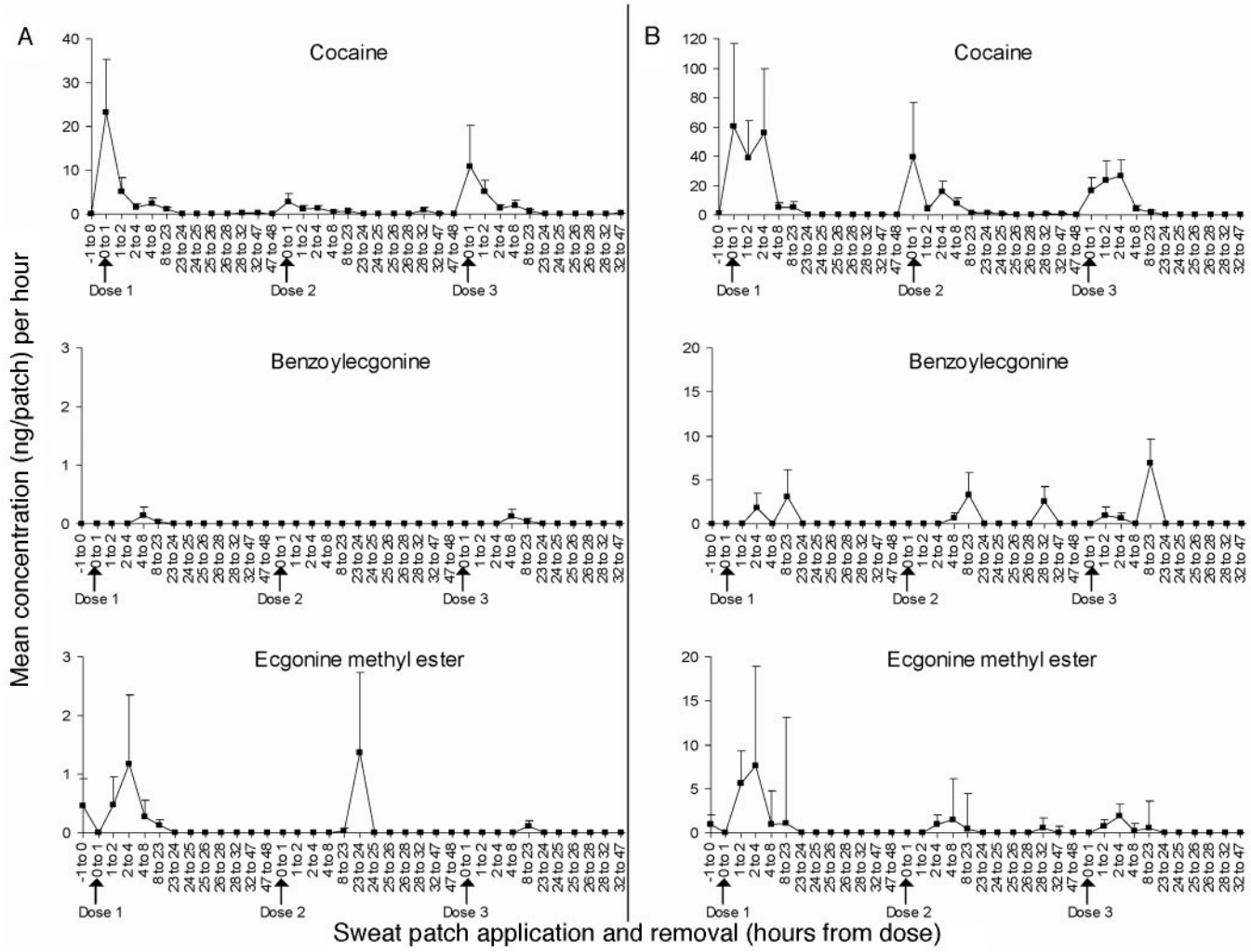

Fig. 2. Mean COC, BE, and EME concentrations excreted per hour in sweat after (*A*) three 75-mg/70 kg low-dose and (*B*) three 150-mg/70 kg high-dose subcutaneous COC hydrochloride administrations.

of these patches were positive for BE at the LOQ, with only a single positive patch at the higher cutoff. A higher percentage of patches (67%) were positive for EME at the LOQ, with a single patch exceeding 25 ng/patch.

All of the weekly patches worn during high dose administrations (n = 10) were positive for COC at the LOQ, with concentrations ranging from 8.2 to 128.4 ng/patch. A single participant did not have a COC-positive patch at the proposed SAMHSA cutoff. BE was detected at the LOQ in 70% of these patches (range, 3.7–47.0 ng/patch); however, only a single weekly patch was positive at the proposed SAMHSA cutoff. Eighty percent of weekly patches were positive for EME with concentrations ranging from 6.1 to 35.9 ng/patch; 4 had EME concentrations >25 ng/patch.

We examined the relationship between dose administered and concentrations of COC metabolites in sweat, using 15-h patches collected during the first 24 h after dose administration and weekly patches. The mean COC concentration in 15-h patches after low doses was 16.2 (20.0) ng/patch and increased to 31.6 (1.1) ng/patch after high doses; the mean BE concentrations were 1.8 (3.4) ng/patch after low doses and 3.1 (4.4) ng/patch after high doses. Similarly, the mean EME concentration increased from 2.1 (3.4) ng/patch to 5.2 (10.8) ng/patch after the low and high doses, respectively. There were no significant differences in any of these means ($\alpha = 0.05$), and comparison of individual participants' concentrations indicated that 64%, 36%, and 44% had higher 15-h sweat patch COC, BE, and EME concentrations, respectively, after high dosing compared with patches collected during low dose administrations.

Mean differences between weekly high- and low-dose COC, BE, and EME concentrations are depicted in Fig. 3. If a participant had duplicate weekly patches, concentrations were averaged for comparison purposes. Comparison of concentrations of all 3 analytes during low and high doses could be performed for 5 participants: 2 participants' weekly patches demonstrated an increase in analyte concentrations during the high dosing week com-

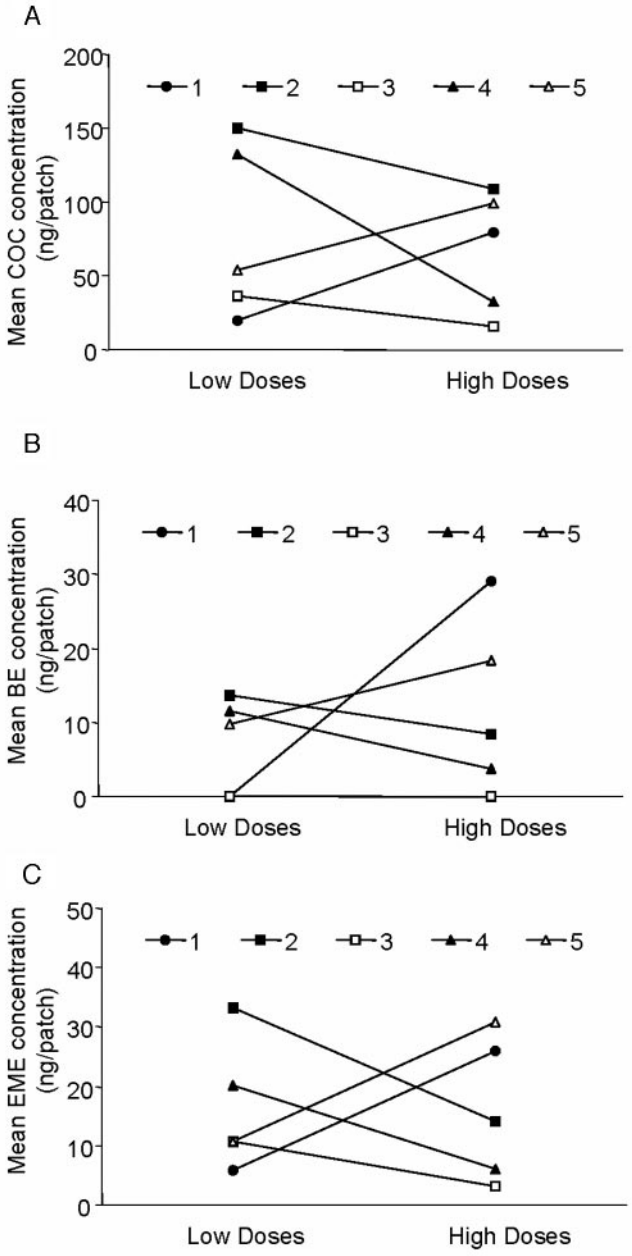

Fig. 3. Mean COC (*A*), BE (*B*), and EME (*C*) concentrations in weekly sweat patches collected from 5 participants after 3 low (75 mg/70 kg) and 3 high (150 mg/70 kg) doses.

pared with those collected during low dose administrations. Three participants had a decrease in weekly patch concentrations of COC and EME, 2 of whom also had a decrease in BE concentrations. One participant had negative weekly patches for BE after low and high doses.

We compared the sum of analyte concentrations in short-term patches worn during dosing with cumulative analyte concentrations in weekly patches. There were 9 complete sets of short-term patches with at least 1 weekly patch for comparison. Listed in Table 4 are the weekly COC, BE and EME concentrations; the mean concentrations of duplicate patches; and the sum of concentrations in short-term patches worn throughout the dosing week. The sum of COC concentrations in short-term patches was higher than mean weekly patch concentrations in all but one case. More variable results were found for BE and EME concentrations. The correlation between the sum of short-term and the weekly COC concentrations was significant; however, there were no such correlations for BE and EME.

In addition to the weekly patch worn during the dosing week, 9 participants had patches applied, in duplicate, a few days after the last low dose. Eleven of 18 patches (61%) were positive for COC at the LOQ, with concentrations ranging from 3.6 to 30.7 ng/patch. BE was detected above the LOQ in only a single patch, whereas 3 patches were positive for EME. A single patch was positive at the proposed SAMHSA cutoff with COC and BE concentrations of 30.7 and 26.3 ng/patch, respectively. Four participants wore duplicate patches for the week after the last high dose; one half of these patches had measurable COC, but no patches were positive for BE or EME. No patches were positive for COC or either metabolite at the proposed SAMHSA cutoff.

Duplicate weekly patches, applied and removed at the same time, were compared to determine the reproducibility of excretion of COC and its metabolite in sweat. There were 6 possible outcomes for duplicate patches: (*a*) both patches negative at the method LOQ; (*b*) one patch negative and one patch positive at the method LOQ; (*c*) both patches positive at the LOQ; (*d*) one patch negative at the method LOQ and one patch positive at the proposed SAMHSA cutoff; (*e*) one patch positive at the LOQ and one patch positive at the proposed SAMHSA cutoff; and (*f*) both patches positive at the proposed SAMHSA cutoff. The total number of duplicate patch sets (n = 68 for COC and EME and 65 for BE) and the number of sets for each possible outcome are shown in Table 5. When both patches were positive for COC at either the LOQ or SAMHSA cutoff (n = 24; 35%), the difference between the 2 values was calculated. This difference ranged from 0.2 to 82.4 ng/patch with a mean difference of 12.4 (19.9) ng/patch. In addition, we calculated the percentage differences between mean concentrations and those of individual patches. Fifteen (63%) were within ±20% of the mean. BE was detected above the method LOQ in duplicate patches in 7 cases (10%). Differences between the patches ranged from 0.8 to 127.5 ng/patch [mean (SD), 26.9 (46.2) ng/patch]. Less than one half of the sets (4 of 9) were within 20% of the mean value for the pair. EME was positive in 13 sets (19%) of duplicate patches. The mean difference between paired patches was 7.4 (8.3) ng/patch (range, 0.1–23.1 ng/patch). Almost 70% of these sets quantified within ±20% of the pair mean.

Discussion

This comprehensive controlled COC administration study addressed the disposition of COC and its metabolites in

Table 4. Individual and mean concentrations of COC, BE, and EME in duplicate weekly sweat patches and cumulative amounts of COC and metabolite concentrations in short-term patches worn for 1 to 15 h.

COC, ng/patch				BE, ng/patch				EME, ng/patch			
Patch				Patch				Patch			
1	2	Mean	Sum	1	2	Mean	Sum	1	2	Mean	Sum
28.4	11.3	19.9	57.7	0.0	0.0	0.0	0.0	8.4	3.4	5.9	14.6
191.3	108.8	150.0	573.3	0.0	23.4	11.7	55.5	41.7	18.7	30.2	38.4
88.1	–[a]		17.1	0.0	–		0.0	12.6	–		0.0
36.5	–		61.6	0.0	–		0.0	10.7	–		6.9
139.2	125.1	132.1	287.7	12.8	10.3	11.6	0.0	22.6	17.9	20.3	13.9
52.1	54.9	53.5	123.6	10.3	9.3	9.8	18.3	9.5	11.7	10.6	16.2
92.2	96.9	94.6	157.1	14.1	13.3	13.7	0.0	18.0	17.9	17.9	0.0
51.2	107.8	79.5	155.8	11.1	47.0	29.1	13.9	16.2	35.9	26.0	58.8
128.4	88.9	108.7	1249.8	0.0	16.8	8.4	34.2	27.9	0.0	14.0	39.6

[a] Missing data.

human sweat. The study was conducted on a closed research unit and included a 3-week washout period to ensure that COC detected during and after controlled drug administration was not a result of self-administered drug. Sweat patches (n = 1390) were analyzed for COC and 10 metabolites. In the 342 positive patches (concentration ≥2.5 ng/patch), COC was the primary (97.0%) and frequently the only (59.0%) analyte detected in sweat. Although BE and EME were prevalent in positive patches—21.3% and 36.5%, respectively—they were detected in only 2 and 8 patches, respectively, in the absence of COC. Only 3 minor metabolites (ecgonine ethyl ester and *m*- and *p*-hydroxycocaine) were detected in <1% of patches. Huestis et al. *(11)* reported detecting *m*- and *p*-hydroxycocaine along with norcocaine, *m*- and *p*-hydroxybenzoylecgonine, and benzoylnorecgonine in sweat of 3 of 4 individuals after 3 low (75 mg/70 kg) and 3 high (150 mg/70 kg) subcutaneous COC administrations. Results of these studies indicated that no additional information would be gained by the inclusion of minor COC metabolites in the routine analysis of COC in sweat.

Table 5. Reproducibility of qualitative COC, BE, and EME sweat test results in duplicate weekly patches at the method LOQ of 2.5 ng/patch and the proposed SAMHSA cutoff for COC and BE of 25 ng/patch.

		Patch 1		
	Patch 2	Negative	≥2.5 ng/patch	≥25 ng/patch
COC (n = 68)	Negative	31	12	1
	≥2.5 ng/patch		13	1
	≥25 ng/patch			10
BE (n = 65)	Negative	51	5	2
	≥2.5 ng/patch		4	1
	≥25 ng/patch			2
EME (n = 68)	Negative	49	5	1
	≥2.5 ng/patch		9	4
	≥25 ng/patch			0

The relative frequencies of EME and BE in our sweat patches were similar to those reported by Cone et al. *(12)* after administration of 1, 2.5, 5, 10, and 25 mg of intravenous, 32 mg of intranasal, and 42 mg of smoked COC to 4 participants *(12)*. In contrast, 3 published field studies *(13–15)* reported that BE is detectable more often and at higher concentrations than EME. Liberty et al. *(15)* analyzed sweat patches from 22 active drug users at a cutoff of 5 ng/patch and reported BE to be more prevalent than EME in patches worn from 0.5 h to 2 weeks. Similarly, Preston et al. *(13)* found that BE (cutoff, 5 ng/patch) was present in more patches and at higher concentrations than EME in weekly patches collected during a study evaluating the effectiveness of sweat testing for monitoring COC use in 44 methadone-maintained patients. Finally, Moody et al. *(14)* reported more patches positive for BE than EME (cutoff, 4 ng/patch) and at slightly higher concentrations in patches worn for 2–7 days. Those participants were enrolled in a clinical trial testing the effectiveness of methylphenidate treatment of COC users diagnosed with attention deficiency disorder. In our study, 17% of patches contained all 3 analytes, whereas Moody et al. *(14)* and Preston et al. *(13)* reported that the majority of patches worn for 1 to 7 days—68.1% and 72.5%, respectively—contained all 3 analytes. More than 20% of patches contained only COC, compared with nearly 60% in our study. When the 4 and 5 ng/patch cutoffs were applied to weekly patches in our study, the percentage of positive patches that contained all 3 analytes increased to 25.7% and 28.3%, respectively, and COC was detected alone in less than one half.

In our study, patches containing both BE and EME had mean BE concentrations of 14.2 (23.4) ng/patch and mean EME concentrations of 13.4 (13.3) ng/patch. However, at the method LOQ, EME concentrations exceeded those of BE in 53% of patches containing both analytes. In sweat patches containing both analytes, the mean BE/EME ratio was 1.1 (median, 0.9) because of increased BE concentrations in some patches. There was no statistically signifi-

cant difference in mean ratios in patches worn for <24 h [1.0 (0.7)], 1–6 days [1.3 (0.6)], or weekly [1.2 (0.7); α = 0.05]. Moody et al. *(14)* evaluated the ratio of BE to EME based on duration of patch wear and found that mean BE/EME ratios increased from 0.76 in patches worn for 1 day (n = 36) to 1.15 in patches worn for 7 days (n = 96). However, no statistical analyses were provided to determine whether these were significantly different. Application of a 4 ng/patch cutoff to our data yielded no change in the mean or median BE/EME ratios or the percentage of patches that had greater concentrations of EME than BE. Overall, our data indicate that although EME is detected at higher concentrations than BE when both analytes are present, the difference is not statistically significant and the length of time the patch is worn does not influence the ratio of analytes.

This study was the first to evaluate excretion of COC and its metabolites from drug exposure before patch application. Participants had no access to COC while residing on the closed research unit. During the first week of washout, 9 of 12 participants had measurable COC concentrations; 5 had a positive patch during washout week 2, and 4 had a positive patch during week 3. The possibility of residual COC excretion from a previous COC administration should be taken into consideration when interpreting sweat results. During the first few weeks of abstinence, sweat may be positive for COC even if the individual is no longer using the drug. Preston et al. *(13)* used a 10 μg/L ELISA cutoff to analyze sweat patches applied on a Tuesday and removed the following Tuesday. Urine samples collected on Wednesday, Friday, and Monday were analyzed by an ELISA with a cutoff of 300 μg/L. More than 21% of patches were positive for COC when the corresponding urine specimens were negative. Although this could reflect COC use before patch application, or after the Monday urine sample but before patch removal on Tuesday, it also suggests that sweat testing may be more sensitive than urine testing at these cutoff concentrations.

COC was first detectable in sweat within 1 h of drug administration, in agreement with previous studies *(11, 12, 16)*. EME was detectable within 2 h and BE within 8 h. Burns and Baselt *(16)* compared the effectiveness of sweat and urine testing for detecting COC use up to 7 days after a single low (50 mg) or single high (126 mg) intranasal COC dose. In participants who had negative predose patches, COC first appeared 2 h after the low and 1 h after the high dose. When collecting sweat using Torso and Hand-Held Fast Patches, which use heat to stimulate sweat production, Huestis et al. *(11)* reported detectable COC within 30 min of the first COC administration. Our study design did not allow determination of peak excretion because patches were worn for various lengths of time; however, the majority of COC excretion occurred within the first 24 h after dosing. When analyzing patches applied at the time of dosing and removed 24, 48, or 72 h later, Cone et al. *(12)* reported only small increases in COC concentrations in patches worn for longer periods of time, indicating that the majority of COC was excreted within 1 day of drug administration.

We used 15-h patches collected during the first 24 h after dosing and weekly patches to evaluate the relationship between the administered COC dose and the excretion of COC and its metabolites in sweat. Although mean analyte concentrations were higher in 15-h sweat patches collected after high doses, they were not significantly different from concentrations after low doses. Statistical analysis could not be performed on the weekly patches because of the low number of participants who had weekly patches available after both low and high doses. The available data indicated that at least 50% of the participants had lower concentrations of COC, BE, and EME in weekly patches after high doses compared with low-dose patches. Cone et al. *(12)* also examined the relationship between dose and COC excretion in sweat after administration of 4 different doses of intravenous COC hydrochloride. COC was detected after all 4 doses, and concentrations increased with increasing dose, although no statistical analyses were reported. Another study reported a statistically significant increase in COC concentrations after intranasal administration of 126 mg compared with 50 mg of COC in 15 pairs of patches *(16)*. Our data do not provide sufficient evidence to establish a clear relationship between the COC dose administered and the concentrations of COC analytes detected in sweat.

Winhusen et al. *(7)* evaluated the correlation between COC, BE, and EME concentrations in weekly sweat patches and cumulative drug concentrations in short-term patches worn in the same week, using sweat patches collected from 27 patients diagnosed with comorbid COC dependence and attention deficit disorder. Pearson correlation coefficients indicated a strong correlation (α = 0.001) between weekly and cumulative short-term patch COC results. In our study, the Spearman correlation coefficient was used because normality in the data could not be assumed. We also found a significant correlation between weekly and cumulative short-term COC results. In contrast, BE and EME concentrations were not significantly correlated. Loss of COC and its metabolites from sweat patches by various means was demonstrated by Uemura et al. *(8)*, who placed 6 patches containing 100 ng of deuterated COC on the bodies of 8 individuals. Patches were removed after 1, 3, 8, 24, 48, or 72 h. COC concentrations decreased significantly (P <0.001) in patches worn for 8 h or longer. Minimal conversion to BE (<2%) was reported. In addition, a drug-free patch was placed over a COC-containing patch to measure the transfer of drug through the patch membrane. One half of drug-free patches were positive for deuterated COC (3.5–5.9 ng/patch) at the LOQ of 2 ng/patch, demonstrating the passage of COC through the adhesive patch membrane.

Reproducibility of results is an important aspect of the effectiveness of sweat testing to monitor COC use. Discrepant COC results (results greater than ±20% of the

mean) occurred in >21% of duplicate patches, but only 2 of 68 pairs (3%) had one negative and one positive at the SAMHSA cutoff. Preston et al. *(13)* reported a 6-fold or greater difference in COC concentrations in 4% of duplicate pairs (n = 287). Cone et al. *(12)* also reported a relatively low intrasubject variability in duplicate pairs of patches from participants after acute COC administration. In our study, the maximum variation between duplicate patches was ~3-fold. These data support the conclusion that sweat patch tests are reproducible and appropriate for qualitative monitoring of COC use.

Current proposed SAMHSA guidelines include a screening cutoff of 25 ng/patch for COC metabolites and a confirmation cutoff of 25 ng/patch for COC or BE. In this study, application of these guidelines yielded fewer than 8% confirmed positive patch results. No patches had BE or EME concentrations >25 ng/patch without concurrent positive COC results. Because the proposed guidelines suggest that the patch be worn for 7 days, weekly patch results offer the best information regarding the effectiveness of sweat testing to monitor COC use in real-life settings *(1)*.

Our study design was the first to permit an investigation of the duration of detection of self-administered illicit COC. Comparison of weekly washout patch results with the proposed SAMHSA cutoff showed that 2 participants were positive during the first washout week and that 1 participant remained positive during washout week 2. By washout week 3, all sweat patches were below the proposed cutoff. These data demonstrate that residual COC excretion from previously self-administered drug may contribute to positive patch results for several weeks after monitoring begins. Eight of 10 participants had sufficient COC excretion in sweat to produce positive weekly sweat tests at a 25 ng/patch cutoff during low dosing, and 5 of 6 participants had at least one positive patch during high dose administration. Weekly sweat patches applied after the last low and high doses provided additional information on the duration of COC excretion. COC, BE, and EME were occasionally detected at the LOQ of the method for 3 weeks after drug administration; a single patch had COC and BE concentrations above the proposed SAMHSA cutoff the week after drug administration.

This research was supported by the Intramural Research Program of the NIH, National Institute on Drug Abuse. PharmChem Inc. kindly provided the sweat patches. We also thank David Darwin and Deborah Price for invaluable technical assistance.

References

1. US Department of Health and Human Services. Proposed revisions to mandatory guidelines for federal workplace drug testing programs. Fed Regist 2004;69:19673–732.
2. Skopp G, Potsch L. Perspiration versus saliva—basic aspects concerning their use in roadside drug testing. Int J Legal Med 1999;112:213–21.
3. Huestis MA, Cone EJ. Alternative testing matrices. In: Karch SB, ed. Drug abuse handbook. Boca Raton, FL: CRC Press, 1998: 799–857.
4. Kidwell DA, Holland JC, Athanaselis S. Testing for drugs of abuse in saliva and sweat. J Chromatogr 1998;713:111–35.
5. Stimmel B, Kreek MJ. Neurobiology of addictive behaviors and its relationship to methadone maintenance. Mt Sinai J Med 2000; 67:375–80.
6. Kidwell DA, Smith FP. Susceptibility of Pharmchek drugs of abuse patch to environmental contamination. Forensic Sci Int 2001;116: 89–106.
7. Winhusen TM, Somoza EC, Singal B, Kim S, Horn PS, Rotrosen J. Measuring outcome in cocaine clinical trials: a comparison of sweat patches with urine toxicology and participant self-report. Addiction 2003;98:117–324.
8. Uemura N, Nath RP, Harkey MR, Henderson GL, Mendelson J, Jones RT. Cocaine levels in sweat collection patches vary by location of patch placement and decline over time. J Anal Toxicol 2004;28:253–9.
9. Saito T, Wtsadik A, Scheidweiler KB, Fortner N, Takeichi S, Huestis MA. Validated gas chromatographic-negative ion chemical ionization mass spectrometric method for δ(9)-tetrahydrocannabinol in sweat patches. Clin Chem 2004;50:2083–90.
10. US Department of Justice. Federal cocaine offenses: an analysis of crack and powder penalties. Washington, DC: US Department of Justice, 2002:42pp.
11. Huestis MA, Oyler JM, Cone EJ, Wstadik AT, Schoendorfer D, Joseph RE Jr. Sweat testing for cocaine, codeine and metabolites by gas chromatography-mass spectrometry. J Chromatogr 1999; 733:247–64.
12. Cone EJ, Hillsgrove MJ, Jenkins AJ, Keenan RM, Darwin WD. Sweat testing for heroin, cocaine, and metabolites. J Anal Toxicol 1994;18:298–305.
13. Preston KL, Huestis MA, Wong CJ, Umbricht A, Goldberger BA, Cone EJ. Monitoring cocaine use in substance abuse treatment patients by sweat and urine testing. J Anal Toxicol 1999;23:313–22.
14. Moody DE, Spanbauer AC, Taccogno JL, Smith EK. Comparative analysis of sweat patches for cocaine (and metabolites) by radioimmunoassay and gas chromatography-positive ion chemical ionization-mass spectrometry. J Anal Toxicol 2004;28:86–93.
15. Liberty HJ, Johnson BD, Fortner N. Detecting cocaine use through sweat testing: multilevel modeling of sweat patch length-of-wear data. J Anal Toxicol 2004;28:667–73.
16. Burns M, Baselt RC. Monitoring drug use with a sweat patch: an experiment with cocaine. J Anal Toxicol 1995;19:41–8.