Cocaine and Metabolites Urinary Excretion after Controlled Smoked Administration*

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Abstract

Understanding cocaine and metabolites urinary excretion following smoking is important for interpretation of urine test results in judicial, workplace and treatment settings. In National Institute on Drug Abuse approved studies on a secure research unit, six subjects smoked placebo, 10, 20, and 40 mg cocaine with a precise dose delivery device and six different subjects smoked 42 mg cocaine in a glass pipe. Urine specimens (n = 700) were collected for up to seven days and analyzed for cocaine (COC), benzoylecgonine (BE), ecgonine methylester (EME), m-hydroxybenzoylecgonine (mOHBE), p-hydroxybenzoylecgonine (pOHBE), norbenzoylecgonine (NBE), and ecgonine (EC) by gas chromatography–mass spectrometry. Results (mean ± SE) for the 40-mg precise delivery doses are as follows:

	COC	BE	EME	mOHBE	pOHBE	NBE	EC
Cutoff (ng/mL)	10	20	10	25	25	25	50
C_{max} (ng/mL)	4085 ± 2303	9196 ± 1569	4638 ± 1548	222 ± 69	540 ± 227	614 ± 347	852 ± 211
T_{max} (h)	2.2 ± 0.3	6.6 ± 0.9	5.6 ± 1.4	7.8 ± 0.5	4.4 ± 1.5	6.0 ± 2.0	9.3 ± 1.3
Last positive (h)	55	106	164	55	55	32	80

Mean C_{max} for all analytes linearly increased with increasing dose. T_{max} was not dose-dependent. All metabolites were detected in some subjects within 2 h. EC concentrations were significantly higher after smoked cocaine in a precise delivery coil compared to a glass "crack" pipe.

Introduction

Cocaine is the second most prevalent drug of abuse in the United States and is a required analyte for the U.S. Substance Abuse Mental Health Services Administration's (SAMHSA) federal workplace drug testing panel (1,2). Urine testing also is common for monitoring cocaine users in drug treatment and parole programs. Drug testing is expanding in Great Britain and Europe as cocaine consumption, a minor drug problem in the early 1990s, increases in frequency (3). Understanding cocaine metabolism and excretion is essential for the interpretation of cocaine urine test results.

The major metabolic routes for cocaine are well-documented (see Figure 1) (4). Three decades ago, Hamilton et al. (5) studied urinary excretion in six subjects who insufflated 1.5 mg/kg cocaine hydrochloride (105 mg for a 70 kg person).

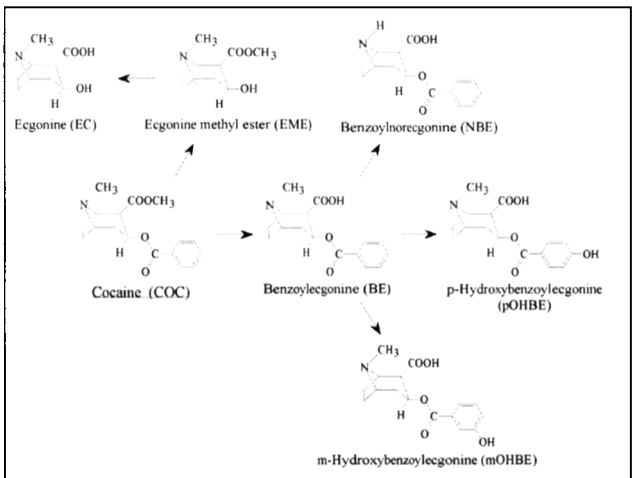

Figure 1. Metabolism of cocaine.

* The opinions in this article are those of the authors and do not necessarily reflect the views of the Department of Army, Department of Air Force, Department of Defense or the National Institute on Drug Abuse.

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Peak urine cocaine concentrations of 300 to 24,000 ng/mL were reached in less than 1 h. Peak urine benzoylecgonine (BE) concentrations ranged from 8100 to 70,800 ng/mL with a time to reach maximum concentrations (T_{max}) of 4 to 8 h. Ambre et al. (6) later examined urine from five subjects following 4 h of intravenous infusion of cocaine hydrochloride with total doses ranging from 253 to 700 mg. As expected, peak cocaine and BE concentrations were higher than those of lower single doses, and interestingly, peak ecgonine methyl ester (EME) concentrations often exceeded those of BE. Elimination half-lives of EME (2.3–4.1 h) and BE (2.8–6.5 h) were similar, but EME's was always shorter. In the last urine collections 24 h postdose, EME was always lower in concentration than BE.

Route of administration impacts the metabolic profile. Cone et al. (7) observed that cocaine was rapidly absorbed, metabolized, and excreted, and it was usually identified in the first urine void regardless of route of administration, including intravenous, intranasal, or smoking routes. However, BE concentrations were route-dependent and represented 39%, 30%, and 16% of administered dose, respectively. After oral administration of multiple doses of cocaine hydrochloride (n = 6, 375–2000 mg each), peak urine concentrations for cocaine, BE, and EME each exceeded 800,000 ng/mL for one subject (8). Elimination occurred in two phases for all subjects. Alpha half-lives were similar to those previously reported, but beta phases were much longer. All of the past studies found great interindividual variation in absorption of cocaine and elimination of metabolites.

When one interprets urine drug tests, pharmacokinetic complexity is not the only difficulty. Most workplace, treatment, and judicial programs identify cocaine use by identifying BE in urine. Testing for this metabolite is required for federally regulated urine drug testing programs (1). Cocaine can be converted to BE in urine when the pH is basic, allowing the possibility of a positive test due to external contamination (9). Some officials have suggested testing for EME to circumvent this problem, but Klette et al. (10) demonstrated that cocaine can be converted to EME when urine pH > 7. These authors recommended testing for m-hydroxybenzoylecgonine (mOHBE) and p-hydroxybenzoylecgonine (pOHBE) metabolites that are lower in concentration than BE or EME, but not produced in vitro. The hydroxyl metabolites appeared to be specific for cocaine ingestion, but their detection windows were not known. Another metabolite suggested to extend detection times was ecgonine (EC). Hornbeck et al. (11) developed a new analytical procedure for EC and examined random urine specimens with low BE concentration that were stored frozen more than one year. EC concentrations often exceeded those of BE. To date, no controlled clinical studies extended these original observations.

Cone et al. (12) reported the excretion patterns of cocaine and eight metabolites in six subjects after smoking 42 mg cocaine in a glass "crack" pipe. The metabolites studied were BE, EME, norcocaine, norbenzoylecgonine (NBE), mOHBE, pOHBE, m-hydroxycocaine, and p-hydroxycocaine. Norcocaine and hydroxycocaine metabolites were minimal following smoking. EC was not included because of the difficulty of analyzing for this polar metabolite. In the present study, urine was collected for up to seven days from subjects who used a precise delivery coil to smoke different doses of cocaine. Cocaine, BE, EME, mOHBE, pOHBE, NBE, and EC were quantified by a complex gas chromatography–mass spectrometry (GC–MS) assay of urine specimens collected after the precise delivery coil device and after cocaine smoking with a glass pipe. Peak concentrations and detection times were determined and compared.

Materials and Methods

Research protocols

Subjects were healthy adult males who provided written informed consent and were paid for participation. The protocol was approved by the Institutional Review Board for the National Institute on Drug Abuse and adhered to Federal guidelines for the protection of human subjects. All participants had a history of cocaine smoking. Participants resided on a secure research unit throughout the study and were monitored to prevent use of unauthorized drugs. Prior to controlled drug administration, subjects excreted self-administered cocaine until negative urine specimens were obtained at a cutoff concentration of 300 ng/mL by enzyme multiplied immunoassay technique (EMIT).

Study Group 1 consisted of six males (race 5 AA, 1 C; aged 27–39 years) who smoked three different doses of cocaine base (10, 20, and 40 mg) in a random ascending dose design (13). Additional doses were repeated to determine procedural reliability and to eliminate potential order effects. Cocaine base was delivered precisely to 3, 5, and 4 subjects for the 10-, 20-, and 40-mg doses, respectively. The smoking delivery system was a wire coil with cocaine deposited from a solution and evaporated prior to use. During smoking, the coil was placed inside a glass tube and heated to 200°C. The entire dose was inhaled in a single puff. Urine specimens were collected for up to seven days and stored at –20°C or lower until analyzed.

In Study Group 2, there were six males (race 2 AA, 4 C; aged 30–44 years) who smoked 42 mg of cocaine base in a glass pipe (12). Cocaine was administered in a randomized, crossover design with a separation of three days between doses. Urine specimens were collected for three days and stored at –20°C or lower until analysis.

Quality control samples were prepared in urine, contained all analytes except EC in concentrations covering the full analytical range of the assay, and were kept frozen with urine specimens collected from both clinical studies. Specimens and quality control samples were prepared at the National Institute on Drug Abuse, Baltimore, MD and submitted for blind analysis to the Armed Forces Institute of Pathology, Rockville, MD.

GC–MS analysis

The GC–MS analytical method was previously reported (14). Briefly, 3 mL of urine was buffered to pH 5.5 and passed through solid-phase extraction columns (Clean-Thru® Clean-

Thru ZCDAU020L United Chemical Technologies, Bristol, PA). The aqueous eluent containing unabsorbed EC was collected. The remaining analytes (cocaine, BE, EME, pOHBE, mOHBE, and NBE) were eluted with methylene chloride/methanol/ concentrated ammonium hydroxide (9:1:0.2, v/v/v), divided into two parts (A and B), and evaporated to dryness. Part A was further divided into two portions (1 and 2). Sixty microliters of acetone was added to portion 1 of part A, which was analyzed for underivatized cocaine. Fifty microliters each of dimethylformamide and dimethylformamide-di-*n*-propylacetal (Aldrich Chemical, Milwaukee, WI) (2:1, v/v) were added to portion 2 of part A and injected into the GC–MS with injection port temperature of 280°C for on-column propylation BE, mOHBE, and pOHBE. Fifty microliters each of pentafluoropropanol (PFPOH) and pentafluoropropionic anhydride (PFPA) from Aldrich Chemical were added to dry extract Part B and heated at 50°C for 15 min. Excess reagent was evaporated to dryness under nitrogen at room temperature and dissolved in 50 µL of dry acetonitrile to yield pentafluoropropyl (PFP) derivatives of EME and NBE. The aqueous eluent containing EC was adjusted to pH 2–3, extracted using another set of SPE columns, and derivatized with 1-iodopentane. The solution was washed with 0.15M sulfuric acid and ethyl acetate, then made basic with 1.5M carbonate buffer (pH 9.5), and pentyl-EC was extracted with ethyl acetate, which was evaporated at room temperature under a minimal stream of nitrogen. The residue was reconstituted with 60 µL of acetone for GC–MS analysis. All solvents were purchased from Pierce (Rockford, IL). Additional detail is available in a previous publication (14).

Cocaine, Part A/Portion 1 Organic Phase, was analyzed by GC–MS in selected ion monitoring (SIM) mode. Selected ions, with ions used for quantification in parentheses, were *m/z* (182), 272, and 303 for cocaine and *m/z* (185) and 306 for cocaine-d_3. BE, mOHBE, pOHBE: Part A/Portion 2 Organic Phase was a separate injection with selected ions *m/z* (210), 272, and 331 for propyl-BE; *m/z* (213) and 331 for propyl BE-d_3; *m/z* (210), 330, and 389 for propyl mOHBE; *m/z* (213) and 392 for propyl mOHBE-d_3; *m/z* (210), 330, and 389 for propyl pOHBE; and *m/z* (213) and 392 for propyl pOHBE-d_3. EME, NBE: Part B Organic Phase was a separate injection with selected ions *m/z* (182), 314, and 345 for EME-PFP; *m/z* (185) and 348 for EME-d_3-PFP; *m/z* 214, 312, and (431) for NBE-PFP; *m/z* 303

Table I. Maximum Urine Concentrations (C_{max}) of Cocaine and Metabolites and Times to Reach these Maxima (T_{max}) for Subjects Smoking Cocaine Base

	Subject	COC	BE	EME	mOHBE	pOHBE	NBE	EC
Group 1 (*N* = 4): 40-mg dose, precise delivery coil								
A	C_{max} (ng/mL)	508	6881	1921	415	224	107	1425
	T_{max} (h)	1.7	6.8	2.4	6.8	2.4	9.9	6.8
B	C_{max} (ng/mL)	162	6295	3018	113	252	124	904
	T_{max} (h)	2.2	6.9	6.9	6.9	2.2	2.2	12.9
E	C_{max} (ng/mL)	5921	10,766	4645	129	489	640	600
	T_{max} (h)	3.0	8.7	8.7	8.7	8.7	3.0	8.7
F	C_{max} (ng/mL)	9748	12,844	8969	230	1197	1587	478
	T_{max} (h)	2.0	4.2	4.2	8.8	4.2	8.8	8.8
Mean ± SE	C_{max} (ng/mL)	4085 ± 2303	9196 ± 1569	4638 ± 1548	222 ± 69	540 ± 227	614 ± 347	852 ± 211
	T_{max} (h)	2.2 ± 0.3	6.6 ± 0.9	5.6 ± 1.4	7.8 ± 0.5	4.4 ± 1.5	6.0 ± 2.0	9.3 ± 1.3
Group 2 (*N* = 6): 42-mg dose, glass "crack" pipe								
G	C_{max} (ng/mL)	67	1021	410	36	ND*	ND	ND
	T_{max} (h)	3.2	3.2	3.2	3.2	NA	NA	NA
H	C_{max} (ng/mL)	521	5094	2052	81	53	ND	245
	T_{max} (h)	1.5	8.7	8.7	8.7	8.7	NA	27.5
I	Cmax (ng/mL)	94	2199	569	33	76	39	83
	T_{max} (h)	2.5	7.6	3.2	7.6	3.2	3.2	7.6
J	C_{max} (ng/mL)	1635	8871	4344	387	268	92	343
	T_{max} (h)	1.4	6.0	6.0	6.0	6.0	6.0	10.1
K	Cmax (ng/mL)	186	22,494	7676	37	564	3592	843
	T_{max} (h)	2.3	4.8	4.8	4.8	4.8	2.3	2.3
L	C_{max} (ng/mL)	178	3502	1751	47	46	29	149
	T_{max} (h)	3.4	3.4	3.4	8.2	3.4	13.5	8.2
Mean ± SE	C_{max} (ng/mL)	447 ± 247	7197 ± 3256	2800 ± 1133	104 ± 57	201 ± 99	938 ± 885	333 ± 135
	T_{max} (h)	2.4 ± 0.3	5.6 ± 0.9	4.9 ± 0.9	6.4 ± 0.9	5.2 ± 1.0	6.2 ± 2.5	11.1 ± 4.3

*ND, not detected; NA, not applicable.

and (424) for BE-d_3-PFP, IS to NBE. EC was a separate injection with selected ions *m/z* (168), 238, and 255 for pentyl-EC and *m/z* (171) and 258 for pentyl-EC-d_3. mOHBE and pOHBE reference materials and internal standards were purchased from ElSohly Laboratories (Oxford, MS). All other calibrators and internal standards were purchased from Cerilliant (Austin, TX).

Quantifications were accomplished with a single-point calibration and four intra-assay controls. Limits of quantification (LOQ) for cocaine, BE, EME, mOHBE, pOHBE, NBE, and EC were 1, 4, 1, 1, 1, 2, and 16 ng/mL, with cutoff concentrations of 10, 20, 10, 25, 25, 25, and 50 ng/mL, respectively. Cutoff concentrations for all analytes except EC were selected based on performance of quality control samples stored with specimens. More than 90% of quality control samples stored with specimens and having concentrations at or above these cutoffs had analytical results with acceptable chromatography, quantification, and two ion ratios within ± 20% of expected values. The EC cutoff was selected based on calibrator and quality control performance over time.

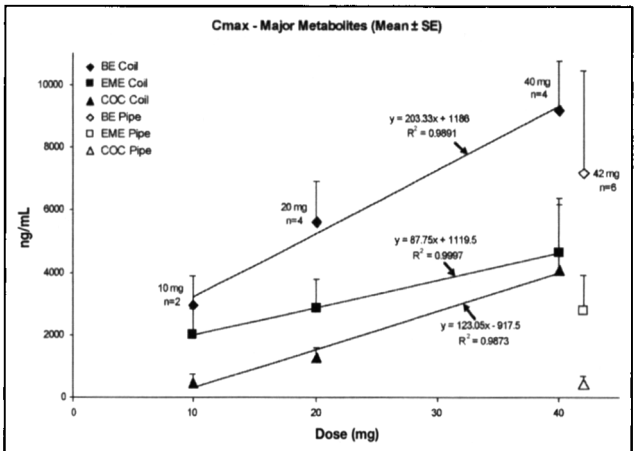

Figure 2. Correlation of mean peak urine concentrations (C_{max}) ± SE of cocaine, benzoylecgonine (BE), and ecgonine methyl ester (EME) with the dose of smoked cocaine administered with the precise delivery coil device and a glass pipe.

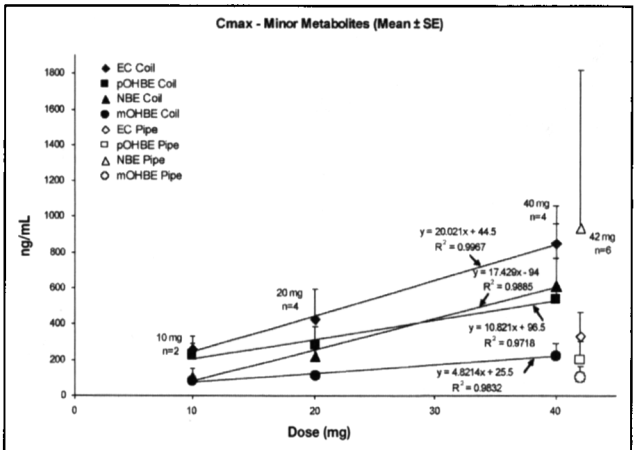

Figure 3. Correlation of mean peak urine concentrations (C_{max}) ± SE of meta-hydroxybenzoylecgonine (mOHBE), para-hydroxybenzoylecgonine (pOHBE), norbenzoylecgonine (NBE), and ecgonine (EC) with the dose of smoked cocaine administered with the precise delivery coil device and a glass pipe.

Statistical comparisons

Between group comparisons employed the Student *t* test if distributions were normal; if skewed, the Mann-Whitney Rank Sum test was used. Pearson product moment correlations also were used to compare analyte concentration with dose of cocaine. (SigmaStat version 3.5, Systat Software, Inc., Point Richmond, CA).

Results

For Group 1, concentrations of the major urinary analytes in the first voided specimen were in the order BE > EME > cocaine. Minor metabolites, mOHBE and pOHBE, were in the first void of all subjects, after the high (40 mg) precise delivery dose. NBE was found in 75% of first voided specimens after this dose. Interestingly, EC that is formed later in cocaine's metabolism also could be observed in the first urine void, but only after the high precise dose. For ease of reference, EC will be included in the minor metabolite group, although its concentrations may equal or exceed those of the major urinary cocaine analytes late in the timecourse. EC is rarely measured because of analytical challenges.

Mean maximum concentrations (C_{max}) for Group 1 decreased in the order BE > EME > cocaine > EC > NBE ≈ pOHBE > mOHBE (Table I). C_{max} increased linearly by increasing dose for the major ($r^2 > 0.98$, $p < 0.05$) and minor metabolites ($r^2 > 0.97$, $p < 0.05$) (Figures 2 and 3) when cocaine was delivered by the precise coil device.

The T_{max} for cocaine was the shortest for all analytes, occurring in the first urine void for all but one subject after the 10-mg smoked dose. BE, EME, pOHBE, and NBE had mean T_{max} (± standard error of the mean, SE) of 6.6 ± 0.9, 5.6 ± 1.4, 4.4 ± 1.5, and 6.0 ± 2.0 h, respectively, for subjects administered the 40-mg dose and in the range of 2.2–9.5 h for all subjects and all doses. Mean T_{max} at the highest dose for mOHBE and EC were 7.8 ± 0.5 and 9.3 ± 1.3 h, respectively, with all T_{max} within 4.6–12.9 h for all doses. Mean T_{max} were not significantly different between doses for any analyte using an unpaired *t*-test (all $p > 0.05$). Detection times for Groups 1 and 2 are shown in Table II. EME (cutoff 10 ng/mL) had the longest mean detection time for all subjects and doses, except for Subject E after 40 mg who had detectable EC (cutoff 50 ng/mL) in the urine for 77 h. Cocaine was generally absent after about 24 h. In one exception, Subject D after 20 mg, had cocaine detectable for 55 h. This subject also had long detection times for most cocaine metabolites. The hydroxylated BE analytes could be detected for approximately 1–3 days. Detection times after 10 mg cocaine were shorter than after smoking either of the two higher doses.

C_{max} and T_{max} for all analytes are shown in Table I for subjects in Group 2 who smoked a 42-mg dose of cocaine in a glass pipe. Comparing results for this group to those for Group 1 after coil delivery of 40 mg smoked cocaine, C_{max} was lower for all analytes except NBE. With the large interindividual variability in analyte concentrations after cocaine smoking, the only significant difference between group concentrations was

for EC (unpaired $t = 2.5$, $p < 0.05$). Mean T_{max} were not statistically different between groups.

Discussion

Cocaine was measurable in all first urine voids after cocaine delivery by the precise coil device, regardless of dose. T_{max} was independent of dose and generally occurred in the first void (i.e., within 1–4 h). Only one subject administered a 20-mg dose had cocaine above the 10 ng/mL cutoff concentration after 26 h. This detection time is relatively short but longer than one might expect for a single moderate dose, given the one-hour elimination half-life of cocaine. However, this longer excretion time is consistent with previous reports of chronic cocaine users with lengthy urinary terminal elimination half-lives, some with detectable cocaine for 162 h (15). A controlled study with high oral cocaine doses demonstrated a two-phase elimination with cocaine present at or above 10 ng/mL for as long as 133 h (8). In the present study, cocaine C_{max} was lower than that of BE or EME as previously reported (5,6,12).

The major metabolites, BE and EME, were present in each first void for Group 1. T_{max} was independent of dose and generally occurred within 2–10 h for both major metabolites. The detection times for BE ranged from 31 to 106 h. They were similar for the 20- and 40-mg doses and lower following the 10-mg dose. Applying the 100 ng/mL cutoff proposed by SAMHSA about half the specimens were negative at 48 h. Some remained above this concentration at three days. EME could be detected for a longer time, that is, 31 to 164 h. For all dosing sessions, the detection time for EME was either the same or

Table II. Detection Times for Cocaine and Metabolites in Urine from Subjects Smoking Cocaine Base*

	COC Detection time		BE Detection time		EME Detection time		mOHBE Detection time		pOHBE Detection time		NBE Detection time		EC Detection time	
Subject	Initial (h)	Last (h)	Initial (h)	Last (h)	Initial (h)	Last (h)	Initial (h)	Last (h)	Initial (h)	Last (h)	Initial (h)	Last (h)	Initial (h)	Last (h)
Group 1: 40-mg dose, precise delivery coil (N = 4)														
A	1.7	11.9	1.7	37.3	1.7	164.4	1.7	33.3	1.7	13.4	2.4	13.4	1.7	33.3
B	2.2	6.9	2.2	44.4	2.2	68.7	2.2	16.9	2.2	6.9	2.2	12.9	2.2	44.4
E	3.0	25.3	3.0	46.3	3.0	46.3	3.0	16.8	3.0	16.8	3.0	25.3	3.0	77.4
F	2.0	17.8	2.0	69.8	2.0	94.1	2.0	37.0	2.0	37.0	2.0	32.4	2.0	56.7
Mean	2.2	15.5	2.2	49.4	2.2	93.4	2.2	26.0	2.2	18.5	2.4	21.0	2.2	53.0
± SE	± 0.3	± 4.0	± 0.3	± 7.0	± 0.3	± 25.6	± 0.3	± 5.3	± 0.3	± 6.5	± 0.2	± 4.8	± 0.3	± 9.4
Group 1: 20-mg dose, precise delivery coil (N = 5)														
C	3.5	3.5	3.5	40.5	3.5	40.5	3.5	25.4	3.5	15.5	3.5	9.5	3.5	25.4
D	4.1	55.3	4.1	106.3	4.1	112.7	4.1	55.3	4.1	55.3	7.4	11	4.1	80.3
E	2.2	9.5	2.2	35.1	2.2	35.1	9.5	10.8	2.2	10.8	2.2	10.8	2.2	35.1
E#†	1.8	31.3	1.8	57.8	1.8	88.6	4.6	14.2	1.8	14.2	1.8	31.3	1.8	79.0
F	2.3	25.2	2.3	66.2	2.3	66.2	2.3	25.2	2.3	29.0	2.3	29.0	2.3	47.5
Mean	2.8	25.0	2.8	61.2	2.8	68.6	4.8	26.2	2.8	25.0	3.4	18.3	2.8	53.5
± SE	± 0.4	± 9.1	± 0.4	± 12.6	± 0.4	± 14.6	± 1.2	± 7.8	± 0.4	± 8.2	± 1.0	± 4.8	± 0.4	± 11.2
Group 1: 10-mg dose, precise delivery coil (N = 3)														
E	2.2	2.2	2.2	44.8	2.2	44.8	ND‡	NA	2.2	10.9	2.2	2.2	ND	NA
F	1.2	15.8	1.2	31.3	1.2	31.3	4.3	11.9	4.3	15.8	1.2	11.9	4.3	31.3
F#†	0.8	23.5	0.8	40.5	0.8	40.5	2.1	31.0	0.8	31.0	0.8	31.0	2.1	31.0
Mean	1.4	13.8	1.4	38.9	1.4	38.9	3.2	21.4	2.4	19.2	1.4	15.0	3.2	31.2
± SE	± 0.4	± 6.2	± 0.4	± 4.0	± 0.4	± 4.0	± 1.1	± 9.6	± 1.0	± 6.0	± 0.4	± 8.5	± 1.1	± 0.1
Group 2: 42-mg dose, glass "crack" pipe (N = 6)														
G	3.2	5.2	1.4	31.0	1.4	12.3	3.2	3.9	ND	NA	ND	NA	ND	NA
H	1.5	27.5	1.5	57.0	1.5	57.0	8.7	27.5	8.7	8.7	ND	NA	8.7	57.0
I	2.5	7.6	2.5	47.0	2.5	34.5	7.6	7.6	3.2	7.6	3.2	3.2	4.2	15.0
J	1.4	28.2	1.4	62.0	1.4	53.8	6.0	28.2	1.4	10.1	1.4	24.0	6.0	28.2
K	2.3	11.4	2.3	73.4	2.3	38.8	4.8	9.2	2.3	12.8	2.3	31.9	2.3	50.2
L	3.4	21.7	3.4	55.2	3.4	68.8	3.4	13.5	3.4	8.2	13.5	13.5	3.4	33.5
Mean	2.4	16.3	2.1	54.3	2.1	44.2	5.6	15.0	3.8	9.5	5.1	18.2	4.9	36.8
± SE	± 0.3	± 4.2	± 0.3	± 5.8	± 0.3	± 8.2	± 0.9	± 4.2	± 1.3	± 0.9	± 2.8	± 0.2	± 1.1	± 7.6

* Cut-off concentrations for cocaine (COC), benzoylecgonine (BE), ecgonine methyl ester (EME), meta-hydroxybenzoylecgonine (mOHBE), para-hydroxybenzoylecgonine (pOHBE), norbenzoylecgonine (NBE), and ecgonine (EC) were 10, 20, 10, 25, 25, 25, and 50 ng/mL, respectively.
† Randomly repeated dose.
‡ ND, not detected and NA, not applicable.

greater than for BE. Cone et al. (12) reported mean T_{max} for BE and EME of 4 h, slightly less than the 5–6 h found for Group 1 but not statistically different. They reported elimination half-lives for each of these metabolites of about 5–6 h. One might expect BE to have the longer detection time since its peak concentration is higher and elimination rate similar to EME. However, Jufer et al. (8) found that, following high oral doses, cocaine, BE, and EME each had a biphasic elimination (8). The beta elimination half-life varied between individuals (15–52 h) but was always greater for EME than BE. This might explain why BE concentrations were often higher than EME in the first 48 h during alpha elimination but were lower during beta elimination.

BE had the highest C_{max} of any metabolite, ranging from 6295 to 12,844 ng/mL after the highest dose. An earlier study reported higher EME C_{max} following continuous intravenous infusion and after multiple oral doses, but BE was the highest concentration metabolite following single dose intravenous, intranasal, and smoking administration (6,8,12). As expected, our concentrations are lower than those reported by Jufer et al. (8), who administered multiple oral doses up to 2000 mg/day. One purpose of this dosing regimen was to provide a better comparison to actual cocaine users who often administer multiple doses throughout a day. C_{max} concentrations for BE in the Jufer et al. (8) study were 115,000 to 890,000 ng/mL. Because BE C_{max} following smoking tends to be higher than for the oral or intranasal route, one might expect multiple smoked doses to produce even higher urine concentrations. This may be one explanation for the occasional specimen in urine testing programs with BE concentration above 1,000,000 ng/mL. In the present study, C_{max} for EME was lower than that for BE for all subjects. C_{max} decreased for the major metabolites with dose. For Group 1, this decrease was linear for cocaine and all metabolites by the precise dosing method. The coil device provided superior cocaine delivery efficiency and precision for pharmacokinetic analysis; however, rarely would it be expected that individuals could self-administer crack cocaine via the smoked route in so precise a manner.

Of the minor metabolites, only pOHBE was present in every first urine specimen. The T_{max} for this metabolite and NBE were in the range of 2–10 h except for one subject administered a 10-mg dose whose NBE T_{max} was 20 h. mOHBE and EC were not present in every first urine void and tended to reach maximum concentrations later, in the range of 4–12 h. EC had the latest T_{max} of all metabolites tested, mean ± SE of 9.3 ± 1.3 h for the highest dose. Detection times for pOHBE and mOHBE were up to 55 h with a median for most subjects of about one day following the two highest doses. These two metabolites were recommended as markers of cocaine ingestion by Klette et al. (10) after they noted that both BE and EME could be produced from cocaine when added to urine with a pH > 7. This finding demonstrated that both BE, the most common analyte in urine testing programs for identifying cocaine use, and EME, a recommended substitute analyte, could be produced from contamination of urine with cocaine. If programs choose to analyze urine for the hydroxyl metabolites as added proof of ingestion, they can expect to find them for less than two to three days after individuals smoke cocaine.

EC reached its peak concentration later than the other metabolites. Detection times were always longer than two days and extended to as long as 80 h. Even though EC C_{max} was generally one-tenth that of BE, it appears that EC could be an acceptable analyte in some specimens for detecting cocaine use. It also is more stable in urine than some of the other metabolites and is the end product of any BE or EME hydrolysis during storage. Two previous studies demonstrated that specimens collected in a random urinalysis program with low concentrations of BE often have high concentrations of EC (11,14). The principal problem observed in our study was that precision and accuracy of quantifying EC below 50 ng/mL were less reliable. It is possible that a lower cutoff concentration could be achieved for verifying cocaine use in urine specimens that could extend detection times. For the assay used in these analyses, EME appeared to be the best metabolite for detecting cocaine use in specimens collected days after suspected ingestion.

Group 2 urine specimens collected after smoking 42 mg of cocaine in a glass pipe were compared to Group 1 urine specimens collected after 40 mg cocaine delivered with the precise coil device. The mean C_{max} for cocaine and all metabolites were higher for Group 1, although no differences were statistically significant except for EC. Part of the reason the EC comparison reached significance was the smaller relative variability in analyte concentrations between subjects. This was not true for the other analytes. For example, NBE concentrations for Group 2 ranged from 0 to 3592 ng/mL with a median value of 197 ng/mL. The most likely reason for the higher urine concentrations is that the coil avoided loss of cocaine during volatilization and increased the actual delivered dose. Although the glass pipe better mimics real smoking situations, the coil minimized variability of dose delivery that is greatly affected by an individual's smoking topography. Smoking topography refers to the manner in which an individual smokes the drug, that is, the number and lengths of puffs, the hold time in the lung, the time between puffs, and other variables. The result was less variation between individuals. With a more controlled dose delivery, we observed that mean C_{max} for all metabolites decreased linearly with dose.

We examined the possibility that metabolite concentrations were lower due to deterioration during storage (16). Urine specimens were stored frozen for an extended timeframe at –20°C before analysis in this study. However, quality control samples were prepared at the time of collection of Groups 1 and 2 urine specimens and were stored under identical conditions. Ninety percent of these quality control samples quantified within ± 20% of target, documenting stability of cocaine analytes during extended storage.

Conclusions

This study confirmed previous published findings that BE, EME, and cocaine are the major analytes excreted in human urine after smoked cocaine. Other analytes in descending concentration order were EC > pOHBE ≈ NBE > mOHBE. C_{max} for

all metabolites decreased linearly with dose when cocaine was delivered by the precise coil device. EC, not previously reported in a controlled administration study, appears later than the other metabolites and has detection times up to 80 h when the smoked dose is in the 20 to 40 mg range. With an analytical cutoff concentration of 10 ng/mL, EME appears to have the longest detection time extending to 164 h after a 40-mg dose. The detection time of EME for each subject and each dose was always equal to or longer than that of BE. Part of the reason for the longer EME detection time was the longer terminal elimination half-life and part was due to the lower cutoff concentration, 10 ng/mL compared to 20 ng/mL for BE. The metabolites mOHBE and pOHBE, recommended as indicators of ingestion in urinalysis cases where there is a potential for contamination of urine with cocaine, have detection times as long as 55 h but typically are not detectable past one day. The concentration of cocaine excreted into urine is significantly higher and more uniform for subjects using a precise delivery coil than a glass "crack" pipe.

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References

1. Department of Health and Human Services. Mandatory guidelines and proposed revisions to mandatory guidelines for federal workplace drug testing programs. *Fed. Regist.* **69(71):** 19644–19673 (2004).
2. Crack and cocaine. Department of Health and Human Services, *NIDA InfoFacts* **May:** 1–4 (2006).
3. World Drug Report, Vol. 2, United Nations, 2004, pp 422–427.
4. D.S. Isenschmid. Cocaine. *In Principles of Forensic Toxicology,* B.S. Levine, Ed. AACC Press, Washington, D.C., 2003, pp 207–228.
5. H.E. Hamilton, J.E. Wallace, E.L. Shimek, Jr., P. Land, S.C. Harris, and J.G. Christenson. Cocaine and benzoylecgonine excretion in humans. *J. Forensic Sci.* **22:** 697–707 (1977).
6. J. Ambre, T.I. Ruo, J. Nelson, and S. Belknap. Urinary excretion of cocaine, benzoylecgonine, and ecgonine methyl ester in humans. *J. Anal. Toxicol.* **12:** 301–306 (1988).
7. E.J. Cone, A. Tsadik, J. Oyler, and W.D. Darwin. Cocaine metabolism and urinary excretion after different routes of administration. *Ther. Drug Monit.* **20:** 556–560 (1998).
8. R.A. Jufer, A. Wstadik, S.L. Walsh, B.S. Levine, and E.J. Cone. Elimination of cocaine and metabolites in plasma, saliva, and urine following repeated oral administration to human volunteers. *J. Anal. Toxicol.* **24:** 467–477 (2000).
9. D.S. Isenschmid, B.S. Levine, and Y.H. Caplan. A comprehensive study of the stability of cocaine and its metabolites. *J. Anal. Toxicol.* **13:** 250–256 (1989).
10. K.L. Klette, G.K. Poch, R. Czarny, and C.O. Lau. Simultaneous GC–MS analysis of meta- and para-hydroxybenzoylecgonine and norbenzoylecgonine: a secondary method to corroborate cocaine ingestion using nonhydrolytic metabolites. *J. Anal. Toxicol.* **24:** 482–488 (2000).
11. C.L. Hornbeck, K.M. Barton, and R.J. Czarny. Urine concentrations of ecgonine from specimens with low benzoylecgonine levels using a new ecgonine assay. *J. Anal. Toxicol.* **19:** 133–138 (1995).
12. E.J. Cone, A.H. Sampson-Cone, W.D. Darwin, M.A. Huestis, and J.M. Oyler. Urine testing for cocaine abuse: metabolic and excretion patterns following different routes of administration and methods for detection of false-negative results. *J. Anal. Toxicol.* **27:** 386–401 (2003).
13. A.J. Jenkins, R.M. Keenan, J.E. Henningfield, and E.J. Cone. Correlation between pharmacological effects and plasma cocaine concentrations after smoked administration. *J. Anal. Toxicol.* **26:** 382–392 (2002).
14. B.D. Paul, S. Lalani, T. Bosy, A.J. Jacobs, and M.A. Huestis. Concentration profiles of cocaine, pyrolytic methyl ecgonidine and thirteen metabolites in human blood and urine: determination by gas chromatography–mass spectrometry. *Biomed. Chromatogr.* **19:** 677–688 (2005).
15. K.L. Preston, D.H. Epstein, E.J. Cone, A.T. Wtsadik, M.A. Huestis, and E.T. Moolchan. Urinary elimination of cocaine metabolites in chronic cocaine users during cessation. *J. Anal. Toxicol.* **26:** 393–400 (2002).
16. D.E. Moody, K.M. Monti, and A.C. Spanbauer. Long-term stability of abused drugs and antiabuse chemotherapeutical agents stored at –20°C. *J. Anal. Toxicol.* **23:** 535–540 (1999).