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Benzoylecgonine and Ecgonine Methyl Ester Concentrations in Urine Specimens

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ABSTRACT: The two major urinary metabolites of cocaine are benzoylecgonine (BE) and ecgonine methyl ester (EME). The major advantage of BE screening is that many commercial immunoassays are designed to detect BE. On the other hand, EME is more amenable to gas chromatographic screening. To ascertain the merits of screening BE versus EME for identifying cocaine use, 380 consecutive urine specimens presented to the Office of the Chief Medical Examiner-State of Maryland were tested for BE by EMIT (cutoff 0.3 mg/L) and for EME by gas chromatography-nitrogen-phosphorus detection (cutoff 0.05 mg/L). Each presumptive positive was confirmed by gas chromatography-mass spectrometry. One hundred four specimens tested positive for BE or EME. Ninety three specimens were positive for both BE and EME, seven were positive for BE (cutoff 0.05 mg/L) only and four were positive for EME only. BE concentrations ranged from 0.08–386 mg/L while EME concentrations ranged from 0.06–72 mg/L. The BE concentration was greater than or equal to the EME concentration in 73% of the cases. Using BE as a sole screen, 96% of the cases of cocaine use were identified while EME screening identified 93% of the cases.

KEYWORDS: toxicology, benzolyecgonine, ecgonine methyl ester, cocaine, urine

Cocaine is rapidly and extensively metabolized in man to two major metabolites, benzoylecgonine (BE) and ecgonine methyl ester (EME). The conversion to BE occurs by chemical hydrolysis at neutral and slightly alkaline pH. EME is formed by the action of plasma and liver esterases on cocaine. Work by Ambre [*1*] indicated that slightly more BE appears in the urine than BE; 46% of a dose of cocaine is excreted as BE while 41% of a dose is excreted as EME over time.

Some commercially available immunoassay systems are designed to identify BE in urine specimens [*2–5*]. Therefore, in high volume urine screening laboratories where these immunoassays are used, identifying cocaine usage by measuring BE is performed. However, in laboratories where immunoassays are not extensively used or gas chromatography (GC) is the predominant analytical technique employed, screening specimens for BE is more difficult. In addition to extraction difficulties, its poor gas chromatographic characteristics make derivatization an essential step in the analysis. Conversely, EME has better gas chromatographic characteristics and thus, does not require derivatization for detection by GC.

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The following is a study performed on consecutive urine specimens obtained from cases of the Office of the Chief Medical Examiner-State of Maryland (OCME). Specifically, each specimen was tested for BE by immunoassay and EME by GC.

Experimental

Specimen Acquisition

Random urine specimens were obtained from autopsies performed at OCME. Specimens were stored in the refrigerator at 4°C or in the freezer at −20°C until analyzed.

BE Analysis

The Syva ETS was used for screening urine specimens for BE. EMIT d.a.u. was used. The instrument was operated and the reagents were used in accordance with the manufacturer's instructions.

BE is confirmed and quantitated as its butylated derivative [*6*]. Briefly, to a standard or urine specimen is added d_3-BE (internal standard solution), pH 9.3 carbonate buffer and 20% ethanol in chloroform. After mechanical rotation and centrifugation, the organic layer is separated and evaporated to dryness. The residue is reconstituted in a TMAH/TMPAH/dimethyl sulfoxide solution (0.05/1/10) and derivatized with iodobutane at 60° for 15 min. The butylated product is acidified and washed with ethyl acetate. The acid layer is then alkalinized to pH 9.3 and extracted with chloroform. The organic layer is separated and evaporated to dryness. The residue is reconstituted in methanol and injected into the gas chromatograph/mass spectrometer (GC/MS).

GC/MS analysis was performed on a Hewlett-Packard 5890 gas chromatograph and a Hewlett-Packard 5970 mass selective detector. The oven temperature was 260°C, isothermal and the injector temperature was 270°C. The column was a HP-5 cross linked 5% phenyl methyl silicone fused silica capillary column (25 m × 0.32 mm ID × 0.17, μm film thickness) and helium was the carrier gas flowing at 1 mL/min. Three ions for BE were monitored: m/z = 82, 224 and 345; m/z = 348 for the internal standard was monitored. Quantitation was based on the ratio of 345/348 in comparison to urine standards. Qualitative identification was based on retention time and 345/82 and 345/224 ion ratios.

EME Analysis

To 5 mL standard or urine specimen were added 2 mL 0.1 N sodium hydroxide, 100 μL 100 mg/L mepivacaine (internal standard solution) and 21 mL n-butyl chloride. After mechanical rotation and centrifugation, the n-butyl chloride layer was separated

and extracted with 3 mL 1 N sulfuric acid. The acid layer was removed, alkalinized with 0.5 mL ammonium hydroxide and extracted with 5 mL methylene chloride. The methylene chloride was transferred to a conical centrifuge tube and 200 μL isopropanol was added. The methylene chloride was evaporated to the isopropanol layer at 40°C which was then transferred to an autosampler vial for GC analysis. Quantitation was based on the area ratio of EME to the internal standard in comparison to fortified standards. Appropriate dilution of specimens with distilled water was performed to ensure quantitation within the limits of the standard curve.

EME analysis was performed on a Hewlett Packard 5880 GC equipped with a nitrogen-phosphorus detector (GC-NPD) and a Hewlett Packard 7673A automatic sampler. The column used was an HP-5 cross linked 5% phenyl methyl silicone fused silica capillary column (25 m × 0.32 mm I.D. × 0.17 μm film thickness). Helium was the carrier gas flowing at 1 mL/min. The injector temperature was 250°C and the detector temperature was 310°C. The oven temperature began at 100°C for 1 min, increased at 30°C/min to 200°C, then increased at 10°C/min to 260°C and finally increased at 20°C/min to 300°C and held for 8 min. Splitless injection mode was utilized. Confirmation of EME was performed on the GC/MSD listed above; chromatographic conditions similar to the GC-NPD conditions were employed. A full scan electron impact mass spectrum in comparison to a library spectrum of EME provided qualitative confirmation.

Results and Discussion

A toxicology laboratory in a medical examiner's office is often required to perform comprehensive drug testing. This would include testing for therapeutic as well as abused drugs. To operate the laboratory as efficiently as possible, it is desirable to have a battery of tests which can identify a large number of substances in a variety of postmortem specimens. For instance, using an alkaline extraction, gas chromatography with nitrogen-phosphorus detection and temperature programming, classes of therapeutic drugs such as antiarrhythmics, antidepressants, antihistamines, local anesthetics, narcotic analgesics, phenothiazines, and sympathomimetic amines and abused drugs such as amphetamines, benzodiazepines, and phencyclidine can be detected in a single injection. This procedure is also capable of detecting cocaine and EME. One purpose of this study was to determine whether this GC procedure, which is performed on all OCME cases, is effective in identifying cocaine usage without necessitating BE screening.

A total of 380 urine specimens were tested for cocaine, BE and EME. The cut-off for BE screening by EMIT was 0.3 mg/L and the cut-off for EME screening by GC was 0.05 mg/L. GC/MS confirmation cut-offs were 0.05 mg/L for BE and EME. One hundred four specimens were positive for EME or BE; 93 specimens were positive for BE and EME, 4 specimens were positive for EME and negative for BE (<0.3 mg/L by EMIT) and seven specimens were positive for BE and negative for EME (<0.05 mg/L by GC). The four specimens with BE concentrations less than 0.3 mg/L by EMIT had responses between the negative and positive calibrators. Cocaethylene was detected, but not quantified in many of these specimens. The remaining 276 specimens were negative for both BE and EME.

Cocaine was also measured (limit of quantitation: 0.05 mg/L) in the 104 cases testing positive for BE or EME. Cocaine was detected in 69 of these specimens. The urine cocaine concentrations ranged from 0.07–78 mg/L. Cocaine was detected in 83% of the cases where BE concentrations exceeded 2.0 mg/L, but was detected in only 30% of the cases where the BE concentration was less than 2.0 mg/L.

The BE and EME concentrations in the 93 specimens positive for both BE and EME are given in Table 1. Since more of the cocaine dose appears in the urine as BE than EME [7], and since BE has a slightly longer half-life than EME [8], one would expect higher BE concentrations in these "randomly" collected urine specimens. Of these 93 specimens, 67 or 72% had BE concentrations exceeding EME concentrations. There were two cases with equal concentrations and the remaining 24 cases had EME concentrations exceeding BE concentrations. The concentration of BE ranged from 0.08 to 386 mg/L while the EME concentrations ranged from 0.06 to 72 mg/L. In addition, 46% of the cases where the BE concentrations equals or exceeds the EME concentrations had BE/EME ratios between 1.0 and 2.0.

TABLE 1—*BE and EME concentrations in 93 specimens positive for each.*

No.	EME (mg/L)	BE (mg/L)	No.	EME (mg/L)	BE (mg/L)
1	16.1	19.5	48	12.9	24.0
2	24.6	30.0	49	2.2	1.4
3	0.21	0.50	50	7.3	10.2
4	0.56	0.47	51	28.2	118.5
5	1.5	1.6	52	0.20	5.0
6	34.3	43.2	53	29.3	386.5
7	14.0	16.5	54	1.2	1.1
8	4.2	1.3	55	0.50	0.50
9	1.7	11.7	56	18.1	38.5
10	32.2	67.5	57	12.0	7.9
11	1.7	2.0	58	2.0	0.9
12	8.3	2.5	59	2.5	0.5
13	1.6	0.80	60	47.8	165.0
14	0.12	1.6	61	14.6	79.0
15	70.5	98.6	62	0.45	6.0
16	32.9	22.5	63	41.1	173.0
17	72.0	79.0	64	42.1	81.0
18	0.39	1.4	65	0.44	0.70
19	57.0	59.5	66	0.65	0.60
20	4.1	3.6	67	7.6	8.7
21	2.2	8.1	68	49.0	94.5
22	0.6	0.4	69	9.4	6.1
23	10.2	52.5	70	3.0	4.5
24	4.0	6.4	71	1.2	2.4
25	6.4	6.8	72	1.4	2.4
26	13.1	39.5	73	4.2	20.0
27	10.1	11.0	74	3.6	0.40
28	12.7	23.0	75	4.0	96.0
29	0.80	2.8	76	0.40	0.55
30	8.8	14.5	77	18.2	46.0
31	12.4	43.3	78	15.2	27.6
32	0.18	0.17	79	7.6	20.0
33	0.17	0.50	80	1.5	8.7
34	7.4	4.0	81	25.2	85.5
35	12.9	51.5	82	27.3	89.0
36	2.0	7.5	83	3.7	4.5
37	20.2	72.0	84	1.6	1.1
38	0.53	1.0	85	5.5	11.0
39	5.0	177.5	86	39.8	37.1
40	37.7	145.0	87	20.0	76.0
41	8.5	8.5	88	35.8	8.0
42	0.43	0.50	89	21.7	33.0
43	8.3	4.5	90	2.4	9.7
44	11.2	2.8	91	15.9	19.0
45	16.0	72.0	92	0.19	0.10
46	0.45	3.6	93	0.41	0.10
47	0.83	1.4			

There were 11 specimens positive for BE or EME, but not both. The urine concentrations of BE and EME in these cases is provided in Table 2. Specimens 6, 8, and 9 were also positive for cocaine at concentrations of 0.17, 1.1, and 0.10 mg/L respectively.

The qualitative findings in this study are similar to a study previously reported [9]. In that study, 70 specimens were tested for BE by EMIT and for EME and cocaine by thin layer chromatography and GC. Twenty-six specimens were positive for both BE and EME and 42 specimens were negative for both BE and EME. Interestingly, the other 2 specimens were positive for cocaine and negative for BE and EME.

Numerous defenses that have been raised in forensic urine drug testing cases. One such defense is that due to its extensive metabolism, cocaine is not normally present in urine specimens of individuals using cocaine. Instead, its presence in the urine means that the specimen had been "spiked" with cocaine. The formation of BE then occurs by the hydrolysis of cocaine. However, 66% of the cases that were positive for BE or EME in this study were also positive for cocaine. Contamination of the specimens is not an issue in these cases since they were collected from the bladder by a pathologist at autopsy.

Another defense argument is that EME should be identified along with BE to properly document metabolism and thus use of cocaine. Ambre [1] indicated that BE remains in the urine for a longer period of time than does EME. Therefore, it is possible that only BE being present is related to the length of time from the last dose of cocaine. In this study, there were seven cases where EME was not identified in the urine. From this observation, one point that arises is what is the BE concentration in urine above which EME would be detected in all of the urine specimens. Table 3 demonstrates that at BE concentrations less than or equal to 0.5 mg/L, EME was detected in 67% of the samples. Once the BE concentration exceeded 2.0 mg/L, all specimens were positive for EME.

TABLE 2—*BE and EME concentrations of urine specimens positive for BE only or EME only.*

No.	EME (mg/L)	BE (mg/L)
1	neg	0.5
2	neg	0.13
3	1.0	neg
4	neg	0.08
5	0.09	neg
6	0.06	neg
7	neg	0.55
8	0.39	neg
9	neg	1.9
10	neg	0.15
11	neg	0.30

TABLE 3—*Qualitative EME results versus range of BE concentrations.*

BE concentration range (mg/L)	Total	Total positive for EME
0.05–0.15	3	1
0.16–0.50	12	9
0.51–2.0	15	13
>2.0	70	70

Based on the data collected in this study, the following conclusions can be drawn: 1) screening for BE alone by immunoassay at the HHS cut-off of 0.3 mg/L identified 96% of the cases where cocaine was used; 2) screening for EME alone at a cut-off of 0.05 mg/L identified 93% of the cases where cocaine was used; and, 3) the BE concentration exceeded the EME concentration in 72% of the cases.

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