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Stability of Ecgonine Methyl Ester in Postmortem Urine Specimens

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ABSTRACT: In this study, 25 postmortem urine specimens testing positive, for cocaine and ecogonine methyl ester (EME) by full scan electron impact gas chromatography/mass spectrometry, were used to evaluate the stability of EME in refrigerated and frozen conditions. After an initial quantitation (t = 0), these specimens were split and stored at either 4°C or −20°C. At several intervals, over a six month period, the specimens were tested for cocaine and EME. Twenty-two of the frozen specimens were within 20% of their t = 0 EME concentration after 6 months; 19 of the 25 refrigerated specimens showed similar stability. At least 50% of the EME present was detected in all specimens under both storage conditions. In addition, there was no evidence to suggest that EME concentrations increased over time even though decreases in cocaine concentrations were observed over the same time period. This suggests that the presence of EME in urine specimens indicates in vivo conversion of cocaine and, therefore, use of cocaine.

KEYWORDS: forensic science, toxicology, cocaine, ecgonine methyl ester, urine, post-mortem stability

One premise in all forensic toxicological analyses is that analyte concentrations remain unchanged from specimen collection to specimen analysis. However, there are numerous examples in the scientific literature that demonstrate that this premise is not always appropriate. One such example is the analysis of cocaine. It is well known that cocaine in blood is rapidly converted by plasma cholinesterase to ecgonine methyl ester (EME). If an esterase inhibitor such as fluoride is added and the pH of the blood is lowered to 5, this transformation can be averted. Cocaine can also be converted chemically in aqueous solutions to benzoylecgonine (BE). BE has demonstrated greater stability in blood specimens than cocaine (1–3).

The widespread use of urine drug testing has shifted these same concerns to urine specimens. Baselt (4) showed that a 1 mg/L urine cocaine concentration was unchanged if the urine pH was 5, but urine cocaine concentrations decreased by 40–70% if the urine pH was 8. These observations were independent of the presence of fluoride in the urine specimens. Cody and Foltz (5) studied the stability of BE in both spiked and actual urine specimens. They found that BE showed great stability in some specimens, but other samples showed decreases of as much as 50% in a seven day period. Urine pH was one factor as greater stability was demonstrated at pH 5 than at pH 9. However, no single factor such as pH or temperature explained the observations. Romberg and Past (6) compared quantitative values for BE, obtained as a result of a retest request, with values originally obtained. The average decrease of BE concentration on 61 urine specimens was 19%, with a standard deviation of 28%. The distribution of percentage changes was bimodal, with one group centered around 10% and a second smaller distribution group centered around 80%. The stability of EME in spiked urine specimens has also been shown to be pH dependent (7); EME was stable in pH 3 and pH 5 urine specimens for up to 3 years when stored at 4–5°C. Conversely, EME was lost within 30 days when the pH was 9.

The purpose of this study was to investigate the stability of EME in actual urine specimens testing positive for cocaine, BE and EME. Since cocaine is converted in vitro to BE but not EME in urine specimens, the presence of EME in the specimen is indicative of cocaine metabolism and hence, of cocaine use. Therefore, the in vitro stability of EME in these specimens can be a critical issue.

Experimental

Specimen Acquisition

Specimens were obtained from autopsies performed at the Office of the Chief Medical Examiner, State of Maryland.

Study Design

Twenty-five urine specimens were identified as positive for cocaine and EME by full scan electron impact gas chromatography/mass spectrometry. After an initial quantitation they were divided into 2 portions; one was stored at 4°C and the other was stored at −20°C. At various intervals over a 6 month period, these specimens were analyzed for cocaine and EME.

Cocaine and EME Analysis

To 5 mL standard or urine specimen were added 2 mL 0.1 N sodium hydroxide, 100 uL 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

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vial for GC analysis. Quantitation was based on the area ratio of cocaine and 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.

Cocaine and EME analysis were performed on Hewlett Packard 5890 GC equipment 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.

Results and Discussion

Data from the stability studies of cocaine and ecgonine methyl ester are provided in Tables 1 and 2.

In the EME stability study, 22 of the 25 urine specimens stored at −20°C, had EME concentrations, after six months, that were within 20% of their initial quantitations. The other three specimens had ratios of final to initial EME concentrations of 0.69, 0.75, and 0.76 respectively. EME concentrations in refrigerated specimens demonstrated similar stability, with 19 of the 25 specimens being within 20% of their initial quantitations after six months. The final to initial concentration ratios of the remaining six specimens ranged from 0.49 to 0.78.

In the cocaine stability study, 17 of the 25 frozen urine specimens had a decrease in cocaine concentrations between 20 and 40% after six months. Only 6 of the 25 frozen specimens were within 20% of their original concentrations after six months. In the refrigerated specimens, only 9 of the 25 specimens had losses less than or equal to 30% after 6 months. Two urine specimens had losses greater than 80%.

TABLE 1—*Cocaine stability study.*

	Initial	6-Month Storage			
		Frozen		Refrigerated	
No.	Conc. (mg/L)	Final Conc. (mg/L)	Ratio	Final Conc. (mg/L)	Ratio
1	129	100	0.78	90	0.70
2	12	9.4	0.78	7.3	0.61
3	6.2	4.6	0.74	3.2	0.52
4	171	135	0.79	126	0.74
5	5.8	6.1	1.05	4.8	0.83
6	5.9	4.5	0.76	2.4	0.41
7	40	29	0.73	26	0.70
8	0.37	0.23	0.62	0.22	0.59
9	1.1	0.90	0.82	0.65	0.59
10	5.9	4.6	0.78	4.3	0.73
11	0.51	0.59	1.16	0.44	0.86
12	0.15	0.10	0.67	0.09	0.60
13	87	51	0.59	56	0.64
14	5.6	3.9	0.70	3.5	0.63
15	49	34	0.69	34	0.69
16	99	75	0.76	68	0.69
17	0.10	0.14	1.40	<0.05	—
18	9.1	7.3	0.80	5.5	0.60
19	27	20	0.74	18	0.67
20	102	76	0.75	64	0.63
21	11	7.8	0.71	1.7	0.15
22	44	37	0.84	20	0.45
23	16	15	0.94	14	0.88
24	24	16	0.67	15	0.68
25	1.5	1.0	0.67	0.74	0.49

TABLE 2—*EME stability study.*

	Initial	6-Month Storage			
		Frozen		Refrigerated	
No.	Conc. (mg/L)	Final Conc. (mg/L)	Ratio	Final Conc. (mg/L)	Ratio
1	78	72	0.98	67	0.86
2	20	18	0.90	17	0.85
3	16	13	0.81	7.8	0.49
4	43	42	0.98	43	1.00
5	21	18	0.86	17	0.81
6	24	27	1.12	24	1.00
7	58	51	0.88	54	0.93
8	12	11	0.92	11	0.92
9	26	24	0.92	22	0.85
10	4.6	4.5	0.98	4.3	0.93
11	10	10	1.00	8.9	0.89
12	3.8	4.0	1.05	3.7	0.97
13	32	22	0.69	27	0.84
14	68	60	0.88	59	0.87
15	83	95	1.14	92	1.11
16	38	29	0.76	26	0.68
17	1.2	1.2	1.00	1.0	0.83
18	12	11	0.92	9.4	0.78
19	5.8	5.0	0.86	5.1	0.88
20	126	115	0.91	111	0.88
21	76	82	1.08	39	0.51
22	173	177	1.02	128	0.74
23	114	97	0.85	96	0.84
24	8.4	6.7	0.80	6.5	0.77
25	1.2	0.9	0.75	1.4	1.17

This study indicated that EME is stable in urine stored in refrigerated and frozen condition for a six month period. This study also confirmed previously published data that indicate that cocaine concentrations decrease in urine specimens over time. One additional question posed in this study was whether cocaine broke down to any significant degree to EME in urine. This study showed that although there were some decreases in cocaine concentrations over time, this was not associated with corresponding increases in EME concentrations. Therefore, the presence of EME in urine specimens is evidence of in vivo and not in vitro conversion of cocaine and thus, use of cocaine. Previous work indicated that cocaine use can be associated with the presence of cocaine and/or BE without detectable amounts of EME in the urine (8). However the present work shows that this absence of EME is not due to an in vitro instability of EME in urine specimens.

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