CLIN. CHEM. 33/1, 118–119 (1987)

Detection and Confirmation of Cocaine Use by Chromatographic Analysis for Methylecgonine in Urine

Dennis R. Clark and Tarek M. Hajar

Methylecgonine is a common metabolite of cocaine in man. We prepared methylecgonine and developed thin-layer chromatographic and gas-chromatographic methods for its detection in urine. Seventy urine specimens from our drug screening laboratory were tested by our method and by EMIT. Both methods were positive for 26 urines, and both were negative for 42 urines. The other two urines were shown to contain cocaine by GC/MS, and no detectable metabolites. We thus demonstrated that detection of methylecgonine and cocaine is as sensitive a test for cocaine use as EMIT.

Additional Keyphrases: *preparation of methylecgonine*

Methylecgonine (ME), a major metabolite of cocaine in urine (*1, 2*), has been shown to be a sensitive indicator of cocaine use (*3*).[1] Currently, the most commonly used test for detecting cocaine use is EMIT® (Syva), which detects the presence of the cocaine metabolite, benzoylecgonine (BE), in urine. The immunochemical EMIT may be subject to interference from crossreacting substances, and BE, a carboxylic acid, is difficult to confirm by use of different techniques (*4, 5*). Methylecgonine, an ester, can be chromatographed conveniently by use of common thin-layer chromatographic (TLC) and gas-chromatographic (GC) systems.

We undertook this study to determine whether we could detect ME and (or) cocaine in urine by TLC and GC whenever EMIT was positive (i.e., whenever cocaine use was detected). We evaluated 70 patients' urine specimens submitted from our Emergency Department for drug screening.

Materials and Methods

pH 9.5 buffer: Saturated sodium bicarbonate solution was added to saturated potassium carbonate solution until the pH was 9.5.

Methylecgonine preparation: ME (currently not available commercially from Alltech–Applied Science, State College, PA) was prepared by the following procedure. Absolute methanol was dried with anhydrous sodium sulfate before use. We dissolved 20 mg of ecgonine (Alltech Associates, Applied Science Labs., Deerfield, IL) in an 8-mL culture tube with 1.0 mL of anhydrous methanol and 50 μL of concentrated sulfuric acid, and immersed the bottom of the tube in boiling water. The tube was loosely capped and reflux was maintained for 3 h. Methanol was added as needed to keep the total volume at approximately 1 mL. After refluxing, we cooled the solution to room temperature, added 1.5 mL of pH 9.5 buffer, 5 mL of dichloromethane ("Nanograde"; Mallinckrodt Inc., Paris, KY), vortex-mixed for 30 s, and centrifuged the tube at 1500 × *g* for 5 min. We separated the organic layer and evaporated it to dryness in a tared conical-bottom test tube. After further drying for 1 h at room temperature under reduced pressure, we weighed the tube, then added methanol to prepare a 1.0 g/L solution. We obtained a 72% average yield of ME from several preparations.

The product thus obtained was shown to be pure by TLC and GC (chromatographic conditions as described below). The product was indeed ME, as shown by the mass spectrum of the single capillary GC peak (conditions described below), which was virtually identical to that of Ambre et al. (*2*): molecular ion at *m/z* 199, base peak at *m/z* 82.

TLC procedure for urine: To 10 mL of urine in a 25-mL glass culture tube, we add 2 mL of pH 9.5 buffer, and 9 mL of chloroform/isopropanol (9/1 by vol). After capping the tube and rocking for 5 min we centrifuge the tube at 1500 × *g* for 5 min. We then aspirate the aqueous (upper) layer, pour the organic extract into a conical tube, and evaporate the extract to dryness under a stream of air at room temperature. We dissolve the dried extract with 50 μL of dichloromethane/methanol (1/1 by vol) and apply it to a silica gel TLC plate (250-μm Uniplate; Analtech, Newark, DE). We develop the plate in freshly prepared ethyl acetate/methanol/concd ammonium hydroxide (85/10/5 by vol) for 30 min. After air drying the plate for 5 min, we heat it in an oven at 110 °C for 5 min, then spray the plate, while hot, with ninhydrin (2 g/L solution in acetone). After heating in the oven for one additional minute, the plate is allowed to cool, then sprayed with iodoplatinate [1 g of chloroplatinic acid (J. T. Baker, Phillipsburg, NJ) and 10 g of potassium iodide in 500 mL of water]. The ME appears as a deep-blue spot with an R_f of 0.59. For comparison, cocaine has an R_f of 0.74, and ecgonine and BE do not migrate. We could detect 1 mg of ME per liter of urine with this procedure.

GC procedure for urine: To prepare a urine sample for GC analysis we pipet 0.25 mL of urine, 50 μL of pH 9.5 buffer, 50 μL of aqueous internal standard (30 mg of SKF-525A per liter), and 100 μL of chloroform into a 15-mL glass conical tube. After vortex-mixing for 30 s and centrifuging for 5 min, we withdraw 7 μL of the lower layer with a 10-μL syringe (Hamilton, Reno, NV) and inject it into the GC (HP-5711A; Hewlett-Packard Co., Avondale, PA). The glass GC column is 2 m by 2 mm (i.d.), packed with 3% OV 101 on 100/120 Gas Chrom Q (Alltech Associates, Applied Science Labs, Deerfield, IL). The nitrogen carrier gas flow-rate is 24 mL/min, and the oven is programmed from 125 to 250 °C at 8 °C per minute. Detection is by flame ionization detector. We can detect 1 mg of ME per liter of urine with this procedure. In this system, ME has a retention time of 4.5 min, the internal standard 14.5 min, and cocaine 13 min. BE and ecgonine do not give GC peaks.

Mass spectrometer operating conditions: Our GC/MS system consists of an HP-5970A Mass Selective Detector, an

Core Clinical Laboratories, Stanford University Hospital, Stanford, CA 94305.

[1] Nonstandard abbreviations: ME, methylecgonine; BE, benzoylecgonine; TLC, thin-layer chromatography(-ic); GC, gas chromatography(-ic); GC/MS, gas chromatography/mass spectrometry.

Received August 18, 1986; accepted September 22, 1986.

HP-5790A GC, and a 12-m fused-silica capillary column coated with cross-linked methyl silicone (all from Hewlett-Packard, Palo Alto, CA). We program the GC oven in three temperature ramps from 100 to 280 °C. Under these conditions, the retention time for ME is 3.6 min, for the internal standard 11 min, and for cocaine 9.8 min.

EMIT *Cocaine Assay procedure:* We used EMIT Cocaine Assay kits (Syva Co., Palo Alto, CA) according to the manufacturer's directions. These assays were performed in a Cobas-Bio automated centrifugal analyzer (Roche Analytical Instruments, Nutley, NJ), following the procedure for Syva's Cobas-Bio application.

Urine specimens: We tested 70 urine specimens by both methods (EMIT and TLC/GC). We selected specimens from among those submitted over a two-month period according to the following criteria: *Group 1* (32 specimens) consisted of specimens that either were positive for cocaine by our usual TLC/GC procedure, had the presumed (before we had identified it) ME spot on TLC, or were specifically requested to be tested for cocaine. *Group 2* (38 specimens) consisted of randomly selected urines submitted for drug testing to which none of the Group 1 criteria applied.

Results

Specimens were considered positive by TLC/GC if they had cocaine or ME, or both, identified and confirmed by chromatography. Specimens were considered positive by EMIT if they produced an increase in absorbance greater than or equal to that of the 0.3 mg/L BE standard. The results are shown in Table 1. There are eight possible combinations of positive and negative results for the three analytes. Six of these eight possibilities should be positive by chromatography, and four should be positive by EMIT. For 68 of our 70 specimens, results for the two methods were either both positive or both negative. There are four possible cases where TLC/GC and EMIT can disagree; only two of our 70 specimens fell into this category. Both of these specimens were positive by chromatography and negative by EMIT. They were analyzed by GC/MS, and the presence of cocaine was confirmed in both. The incidence of positives and negatives is summarized in Table 2.

Table 1. Possible Combinations of the Presence and Absence of Cocaine and Metabolites, and Those Found in the Two Groups of Specimens

Specimens		Constituents found			Test results	
Group 1	Group 2	Cocaine	ME	BE	TLC/GC	EMIT
15	1	+	+	+	+	+
0	0	+	−	+	+	+
0	0	+	+	−	+	−
1	1	+	−	−	+	−
8	2	−	+	+	+	+
0	0	−	−	+	−	+
0	0	−	+	−	+	−
8	34	−	−	−	−	−

Table 2. Summary of Positive and Negative Results by the Two Methods

	TLC/GC +	TLC/GC −
EMIT +	26	0
EMIT −	2	42

Discussion

We show ME to be as sensitive and specific an indicator of cocaine use as BE. With detection limits of 1 mg/L for ME, and 0.3 mg/L for BE, every one of our 70 urine specimens was either positive for both metabolites or for neither. These results indicate that the chromatographic procedure described is at least as sensitive and specific as EMIT in detecting cocaine use by analysis of urine.

We were surprised to find two specimens that contained cocaine and neither metabolite. Many of our specimens were stored frozen for several months before being analyzed, including these two. Perhaps the metabolites were originally present but became non-detectable during storage. We would not expect to find cocaine in the absence of its metabolites in fresh urine specimens, and in this study, we did not. We did note, however, that if this situation does occur, our method would give a positive result, while the EMIT would be negative.

Our findings are important for laboratories performing drug screening by TLC and GC. Owing to the poor chromatographic qualities of BE (*4*), as well as potential urine specimens which contain only metabolites and no cocaine, it has not been possible to incorporate cocaine use screening into TLC and GC procedures. With our technique, cocaine use may now be screened for and confirmed chromatographically, or screened for by a rapid technique such as EMIT, then confirmed by TLC and (or) GC. We believe this will improve the availability and reliability of detection of cocaine use.

We thank Mr. Don Guidoux (Syva Co.) for providing the EMIT reagents. We are also indebted to Mr. Scott Casseday (Stanford Drug Assay Lab.) for initially bringing ME to our attention and getting us interested in the project.

References

1. Inaba T, Stewart DJ, Kalow W. Metabolism of cocaine in man. Clin Pharmacol Ther 1978;23:547–52.

2. Ambre JJ, Ruo TI, Smith GL, Backes D, Smith CM. Ecgonine methyl ester, a major metabolite of cocaine. J Anal Toxicol 1982;6:26–9.

3. Ambre J, Fischman M, Ruo T. Urinary excretion of ecgonine methyl ester, a major metabolite of cocaine in humans. J Anal Toxicol 1984;8:23–5.

4. Wallace JE, Hamilton HE, Christenson JG, Shimek EL, Land P, Harris SC. An evaluation of selected methods for determining cocaine and benzoylecgonine in urine. J Anal Toxicol 1977;1:20–6.

5. Jindal SP, Vestergaard P. Quantitation of cocaine and its principle metabolite, benzoylecgonine, by GLC–mass spectrometry using stable isotope labeled analogs as internal standards. J Pharm Sci 1978;67:811–4.