TECHNICAL NOTE

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Elimination of Fluconazole Interference in Gas Chromatography/ Mass Spectrometric Confirmation of Benzoylecgonine, the Major Metabolite of Cocaine Using Pentafluoropropionyl Derivative

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ABSTRACT: Cocaine is a widely abused drug and causes death from overdose. Benzoylecgonine, the major metabolite of cocaine in urine is usually confirmed after derivatization by gas chromatography/mass spectrometry to demonstrate cocaine abuse. Recently, Wu et al. demonstrated that fluconazole coelutes with benzoylecgonine after conversion to trimethylsilyl analogs and causes false-negative result in the confirmation test. However, fluconazole did not interfere with the screening assay using a enzyme multiplied immunoassay technique. We demonstrated that by converting benzoylecgonine to the corresponding pentafluoropropionyl derivative, the interference of fluconazole can be completely eliminated. The pentafluoropropionyl derivative of benzoylecgonine eluted at 14.7 min while the derivatized fluconazole eluted at 15.6 min. The mass spectral fragmentation pattern of derivatized benzoylecgonine was distinctively different from the mass spectral features of derivatized fluconazole in both electron ionization and chemical ionization mode of operation of mass spectrometers. The quantitation of benzoylecgonine in positive urine specimens has not affected when the specimens were supplemented with 50 μg/mL of fluconazole.

KEYWORDS: forensic science, fluconazole, benzoylecgonine, pentafluoropropionyl derivative

Cocaine is a naturally occurring stimulant found in the leaves of coca plants (*Erythroxylon coca*). Today, cocaine abuse is a significant problem in the United States. Cocaine is also lethal and in a limited review by the National Institute of Drugs of Abuse, over 111 deaths were documented related to cocaine (1). The medical examiners office of Dade County, Florida reported 68 deaths associated with recreational use of cocaine (2). Abuse of cocaine by a pregnant mother may cause severe damage to the fetus (3). Cocaine is considered the biggest producer of illicit income from street sales of all drugs (4). Cocaine is extensively metabolized by liver enzymes as well as plasma esterase to produce benzoylecgonine and ecgonine methyl ester (5,6). Cocaethylene has been detected in various amounts in patients abusing cocaine and ethyl alcohol simultaneously (7,8). Cocaethylene is nearly as potent as cocaine in its pharmacological action with an increased potential for toxicity.

The abuse of cocaine is usually demonstrated by confirming the presence of benzoylecgonine in urine, the major metabolite of cocaine. Usually immunoassays with a cut off benzoylecgonine concentration of 300 ng/mL are used to screen urine specimens. The specimens that test positive by immunoassay, are further analyzed for the presence of benzoylecgonine using gas chromatography/mass spectrometry (GC/MS). However, benzoylecgonine is polar and requires derivatization prior to analysis by GC/MS. Trimethylsilyl derivative of benzoylecgonine is widely used for GC/MS assay. Recently, Wu et al. reported that fluconazole, an antifungal medication interferes with the GC/MS confirmation of benzoylecgonine because the trimethylsilyl derivative of fluconazole co-elutes with trimethylsilyl derivative of benzoylecgonine. However, fluconazole does not interfere with the enzyme multiplied immunoassay technique (EMIT), the screening assay (9). Since a specimen positive by the screening assay and negative by the confirmatory assay is considered as negative for the presence of cocaine, fluconazole interferences in a urine specimen positive for benzoylecgonine is a serious problem. We would like to report our findings on eliminating this interferences by using a different derivative of benzoylecgonine, the pentafluoropropionyl derivative.

Materials and Methods

We obtained drug standards, benzoylecgonine, ecgonine methyl ester, cocaethylene and the corresponding deuterated standards from Radian Corporation (Austin, TX). The derivatizing reagents, 2,2,3,3,3, pentafluoropropanol was purchased from Aldrich Chemical company (Milwaukee, WI), while pentafluoropropionic anhydride was obtained from Pierce (Rockford, IL). We supplemented negative urines with those drug standards to study the separation and mass spectral features. We also used specimens screened positive for benzoylecgonine for further analysis. Preliminary screening of clinical urine samples was performed with a Syva EMIT-II assay for benzoylecgonine (Syva, San Jose, CA) on a Monarch

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2000 analyzer (Instrumentation Laboratory, Lexington, MA). Derivatized samples were analyzed by either a Hewlett Packard 5890 gas chromatograph coupled to a 5970 mass selective detector with electron ionization capability or a 5890 series II gas chromatograph coupled with a 5972 series mass selective detector with chemical ionization capability (Hewlett Packard, Palo Alto, CA). We used methane as a ionizing gas for chemical ionization mass spectrometry.

We supplemented negative urine with a fluconazole concentration of 50 μg/mL, and benzoylecgonine, ecgonine methyl ester and cocaethylene concentrations between 150 and 3000 ng/mL. We also supplemented clinical urine specimens positive for benzoylecgonine with 50 μg/mL of fluconazole. Then a 5 mL aliquot of the specimen was supplemented with deuterated benzoylecgonine, ecgonine methyl ester and cocaethylene (internal standards), and 1 mL of sodium borate buffer (pH 8.5) was added to it. We extracted the drugs along with internal standards using dichloromethane/isopropyl alcohol (2:1 BY vol) as described by Ambre et al. (10). We discarded the aqueous phase and evaporated the organic phase to dryness. Then, we added 50 μL of pentafluoropropionic anhydride and 25 μL of 2,2,3,3,3 pentafluoropropanol to the dried extract and incubated the reaction mixture for 20 min at 85°C. After derivatization, the excess derivatizing reagent was evaporated to dryness and the dry residue was reconstituted with 50 μL of ethyl acetate. We injected 2 μL of the reconstituted mixture into GC/MS. We operated GC/MS either in the selected ion mode or the scan mode for electron ionization mode and in the scan mode only for chemical ionization. The initial oven temperature of the gas chromatograph was 140°C. After maintaining that temperature for 2 min, the oven temperature was raised at a rate of 10°C/min to reach a final oven temperature of 290°C. For the chemical ionization mode, we operated the mass spectrometer in the scan mode with a scanning range of 40–700 m/z. We operated our mass spectrometer either in scan mode (scanning range m/z 40–450) or in the selected ion mode for obtaining electron ionization mass spectra.

Results and Discussion

The free carboxyl group of benzoylecgonine was esterified by 2,2,3,3,3 pentafluoropropanol while the free hydroxyl group of fluconazole was converted to the corresponding pentafluoropropionyl derivative by pentafluoropropionic anhydride. Mule and Casella also described derivatization of benzoylecgonine and ecgonine methyl ester with pentafluropropanol and pentafluoropropionic anhydride (11). We observed excellent separation between derivatized benzoylecgonine (retention time: 14.7 min) and derivatized fluconazole (retention time: 15.6 min). Cocaethylene eluted after derivatized fluconazole (retention time: 16.4 min), while ecgonine methyl ester was eluted at 6.3 min. Therefore, our chromatographic separation condition not only separated derivatized fluconazole from derivatized benzoylecgonine, but also separated other important components like ecgonine methyl ester and cocaethylene.

The mass spectral fragmentation patterns of derivatized benzoylecgonine and derivatized fluconazole were distinctly different, both in electron ionization and chemical ionization modes. In the electron ionization mass spectrum of derivatized benzoylecgonine, a distinct molecular ion peak was observed at m/z 421 (Relative abundance: 48.7%), while the base peak was observed at m/z 300. We also observed a characteristic peak at m/z 272 (Relative abundance: 10.1%) and another strong peak at m/z 82 (Relative

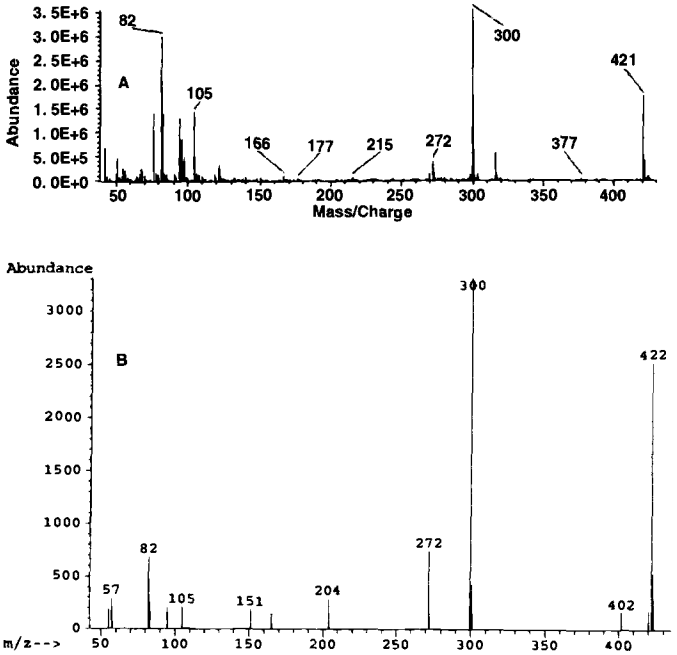

FIG. 1—*Mass spectrum of pentafluoropropionyl derivative of benzoylecgonine* (A) *electron impact,* (B) *chemical ionization.*

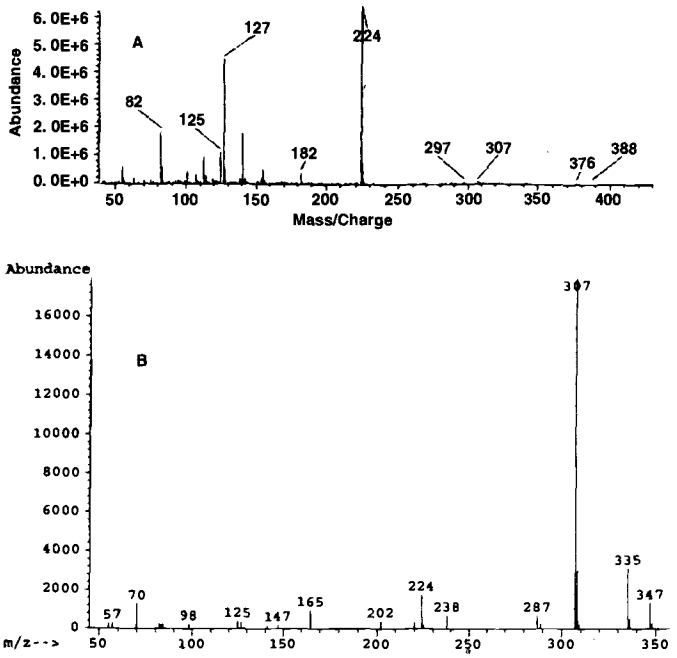

FIG. 2—*Mass spectrum of pentafluoropropionyl derivative of fluconazole* (A) *electron impact,* (B) *chemical ionization.*

abundance: 83.6). In the chemical ionization, the derivatized benzoylecgonine showed a strong protonated molecular ion at m/z 422 (Relative abundance: 69.2%) and a base peak at m/z 300. We also observed a stronger m/z 272 peak (Relative abundance: 20.3%), but the m/z 82 peak was relatively weak (Fig. 1).

The derivatized fluconazole showed a base peak at m/z 224 in the electron ionization mode. We observed strong peaks at m/z 127 (Relative abundance: 70.5%) and at m/z 82 (Relative abundance: 29.3%). In the chemical ionization mode, we observed a base peak at m/z 307. Other peaks at m/z 335 (Relative abundance 11.8%)

TABLE 1—*Quantitation of benzoylecgonine in the original EMIT positive specimens and after supplementing them with fluconazole.*

Specimen	EMIT Screening Δ Absorption		GC/MS Confirmation of BE ng/mL	
	Original	Fluconazole	Original	Fluconazole
1.	0.429	0.427	460	415
2.	0.347	0.348	393	403

and m/z 224 (Relative abundance 7.3%) were observed as well (Fig. 2). Therefore, the mass spectrum of derivatized fluconazole was very different from the mass spectrum of benzoylecgonine in both electron ionization and chemical ionization mode.

Cocaethylene produced a protonated molecular ion at m/z 318 as the base peak and another strong peak at m/z 196 (Relative abundance: 98.6%) in the chemical ionization mode, while the molecular ion at m/z 317 in the electron ionization mode had a relative abundance of only 20.3%. In the electron ionization mode, cocaethylene showed a base peak at m/z 82 and another strong peak at m/z 196 (Relative abundance 67.5%). Therefore, cocaethylene produced characteristic much stronger peaks in the higher mass range in the chemical ionization mode than the electron impact mode. Although a limited study reported chemical ionization mass spectra of silyl derivatives of benzoylecgonine, chemical ionization mass spectrum of cocaethylene has not been reported before.

We supplemented negative urine with benzoylecgonine concentrations between 150 ng/mL and 3000 ng/mL. After adding fluconazole to achieve a final concentration of 50 μg/mL, we extracted the urine specimens, derivatized the drugs and analyzed them by GC/MS. In all cases we were able to distinguish derivatized benzoylecgonine from derivatized fluconazole. We were able to obtain good quality mass spectrum of derivatized benzoylecgonine at a concentration of 150 ng/mL in electron impact using the scan mode. However, we obtained good quality mass spectrum of derivatized benzoylecgonine only at a concentration of 300 ng/mL (NIDA recommended cut off for screening of benzoylecgonine) in chemical ionization using the scan mode.

We also supplemented two clinical urine specimens positive for benzoylecgonine with fluconazole to achieve a final concentration of 50 μg/mL. We observed comparable values in EMIT screening assay confirming the report of Wu et al. that fluconazole does not interfere with the screening assay of benzoylecgonine. We observed comparable quantitation of benzoylecgonine in the original specimen and after adding fluconazole (Table 1) indicating that our protocol completely eliminates interference of fluconazole in the GC/MS confirmation of benzoylecgonine.

References

(1) Petersen RC, Stillman RE (editors). Cocaine, 1977 NIDA Research Monograph 13, National Institute of Drug of Abuse, US Government Printing Office, Washington DC: 1977.
(2) Wetti CV, Wright RK. Death caused by recreational use of cocaine. JAMA, 1977;241:2519–22.
(3) Hadeed AJ, Siegel ST. Maternal cocaine use during pregnancy: Effects on the fetus and newborn infant. Pediatrics 1989;84:205–10.
(4) Haddad LM. "Cocaine" in clinical management of poisoning and drug overdose (Haddad LM and Winchester JF editors). W. B. Saunders Company, Philadelphia, 1983;443–47.
(5) Ambre J. The urinary excretion of cocaine and metabolites in human: A kinetic analysis of published data. J Anal Toxicol 1985;9:241–45.
(6) Ambre J, Connelly TJ, Rou TI. A kinetic model of benzoylecgonine disposition after cocaine administration in human. J Anal Toxicol 1991;15:17–20.
(7) Jatlow P, Elsworth JD, Bradberry CW, Winger G, Taylor JR, Russell R, Roth RH. Cocaethylene: a neuropharmacologically active metabolite associated with concurrent cocaine-ethanol ingestion. Life Sciences 1991;48:1787–94.
(8) Bailey DN. Plasma cocaethylene concentrations in patients treated in the emergency room or trauma unit. Am J Clin Pathol 1993;99: 123–27.
(9) Wu A, Ostheimer D, Cremese M, Forte E, Hill D. Characterization of drug interferences in gas chromatography/mass spectrometry confirmation of targeted drugs in full scan and selected ion monitoring. Clin Chem 1994;40:216–20.
(10) Ambre J, Ruo TI. Ecgonine methyl ester, a major metabolite of cocaine. J Anal Toxicol 1982;6:26–29.
(11) Mule SJ, Casella GA. Confirmation and quantitation of cocaine, benzoylecgonine, ecgonine methyl ester in human urine by GC/MS. J Anal Toxicol 1988;12:153–55.

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