TECHNICAL NOTE

Christine M. Moore,[1] *Ph.D.; Douglas E. Lewis,*[1] *B.A.; and Jerrold B. Leikin,*[1,2] *M.D.*

The Determination of Phencyclidine in Meconium Using Ion Trap Mass Spectrometry

REFERENCE: Moore CM, Lewis DE, Leikin JB. The determination of phencyclidine in meconium using ion trap mass spectrometry. J Forensic Sci 1996;41(6):1057–1059.

ABSTRACT: The use of meconium to determine the exposure of a fetus to drugs of abuse is becoming a widely accepted protocol. However, the quantity of sample available for testing varies greatly between newborns and the material itself is complex in nature. Because of the small amount often collected, the sensitivity of analytical assay is extremely important. A confirmatory procedure for the determination of phencyclidine (PCP) in meconium using the selected ion storage (SIS) functions of an ion trap mass spectrometer is reported. The method is reproducible and detector response is linear over the range 0 to 250 ng/g. The limit of detection is 5 ng/g which provides a significant improvement in sensitivity over selected ion monitoring (SIM) mode electron impact quadropole analysis. The method is currently applied in our laboratory where the confirmation of PCP in meconium is a required analytical procedure. It is particularly useful for the analysis of meconium in which sample size is limited and sensitivity is an important factor.

KEYWORDS: forensic science, phencyclidine, mass spectrometry, meconium drug testing

Reported adverse effects upon the fetus because of maternal phencyclidine use include a higher incidence of intrauterine growth retardation, precipitate labor, and meconium stained amniotic fluid (1), jitteriness, hypertonicity, vomiting, and similar symptoms to those of narcotic withdrawal (2). They are, however, less likely to be born prematurely than cocaine exposed neonates (1). In animal studies, PCP has been shown to cross rapidly the placenta where it reached concentrations 10 times higher in breast milk than in plasma (3). The placenta has also been shown to be an active site of biotransformation of PCP (4).

Meconium, the first fecal material passed by the neonate, is increasingly being analyzed as an alternative to neonatal or maternal urine for the determination of fetal drug exposure, because of its ability to give a longer historical record of drug use. But, it is a complex material consisting predominantly of epithelial cells, bile salts, water, blood group substances, squamous cells and sterol precursors, and is often only available in small amounts. Therefore, the development of sensitive analytical procedures and clean, efficient extraction methods are important for the determination of drugs in meconium.

Confirmatory procedures for cocaine and its metabolites (5,6), opiates (7), tetrahydrocannabinol metabolite (8), and amphetamines (9) have been reported, but only one method for the gas chromatography/mass spectrometry (GC/MS) confirmation of phencyclidine has been published with a detection limit of 20 ng/g (10).

We report a confirmatory procedure that lowers the limit of detection to 5 ng/g of meconium, using the selected ion storage-electron impact mode of an ion trap mass spectrometer.

Experimental Materials

Isolute HCX mixed mode solid phase extraction columns (200 mg/10 mL) were obtained from Jones Chromatography, Lakewood, CO. All reagents were of American Chemical Society grade or better and all solvents of high performance liquid chromatography grade. Deuterated phencyclidine and unlabeled phencyclidine were obtained from Radian Corporation, Austin, TX.

Methods

Extraction Procedure

Deuterated phencyclidine (250 ng) was added to the meconium (0.5 to 1.0 g). The specimens were homogenized in methanol (3 mL) and centrifuged (2500 rpm; 5 min). 0.1 M phosphate buffer (pH 3; 12 mL) was added to the supernatant and the specimen was filtered onto a mixed mode solid-phase extraction column previously conditioned with methanol (3 mL), deionized water (3 mL), and 0.1 M phosphate buffer (pH 3, 1 mL). During conditioning, the column bed was not allowed to dry. The sample was drawn slowly through, and the column was washed with deionized water (3 mL), 0.1 M hydrochloric acid (1 mL), and methanol (3 mL). The final eluent (methylene chloride—isopropanol—ammonium hydroxide (78:20:2 v,v; 3 mL) was collected and evaporated to dryness. The residue was reconstituted in butyronitrile (50 μL) and transferred to autosampler vials before injection into the GC/MS system.

GC/MS Analytical Conditions

Selected ion storage, electron impact (SIS–EI) GC/MS with a Varian Star 3400 bench top gas chromatograph connected to a

[1]US Drug Testing Laboratories, 2201 W. Campbell Park Drive, Chicago, IL 60612.

[2]Rush Presbyterian-St. Lukes Medical Center, 1653 Congress Parkway, Chicago, IL 60612.

Received for publication 16 Jan 1996; revised manuscript received 4 March 1996; accepted for publication 6 March 1996.

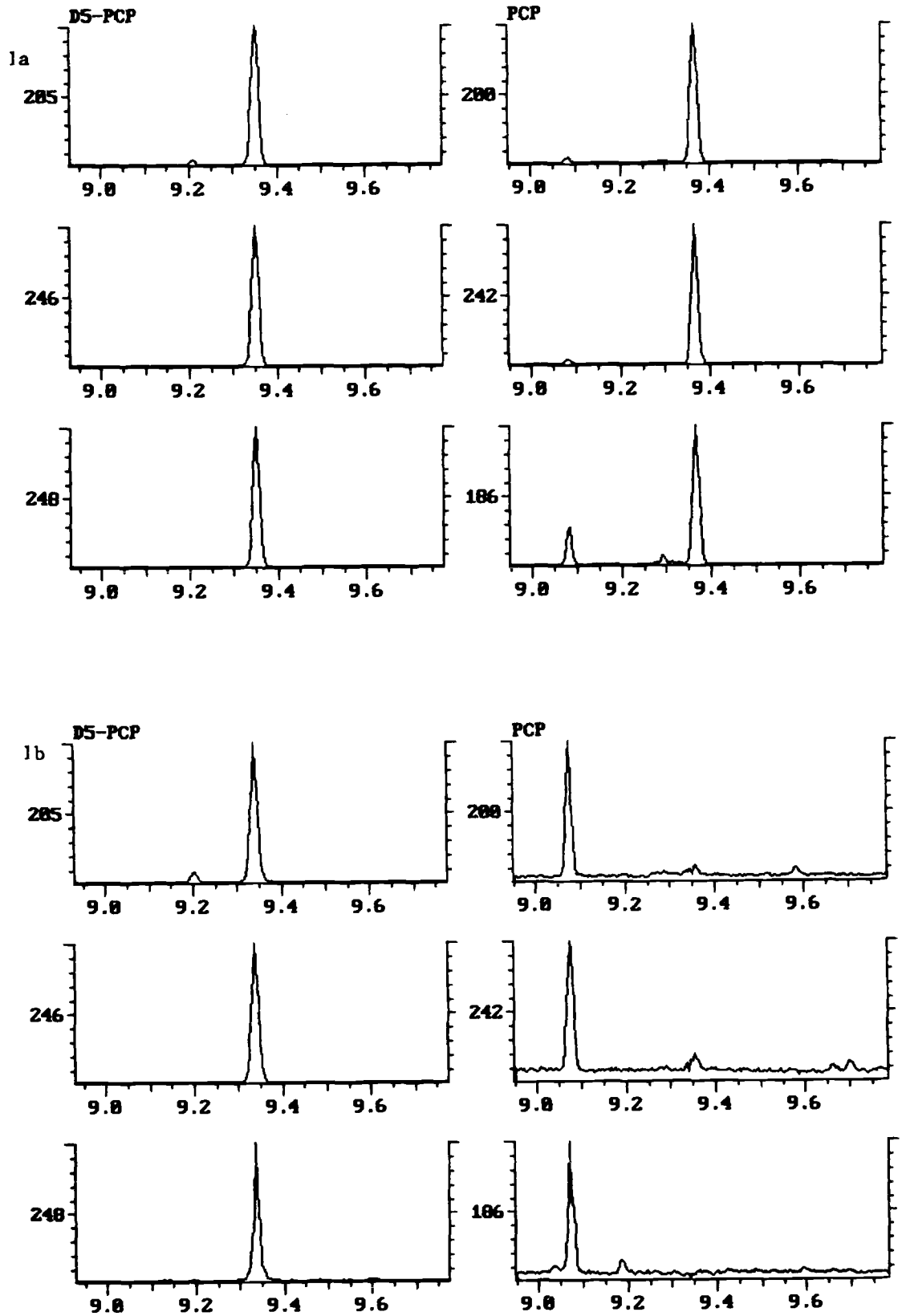

FIG. 1—(a) *Clinical meconium specimen containing phencyclidine,* (b) *negative meconium specimen.*

Saturn II ion trap mass spectrometer was used to analyze phencyclidine. The column was a DB5-MS (30-m length by 0.25-mm internal diameter by 0.25-micron film thickness; J & W Scientific, Folsom, CA). The injector was set at 160°C, the transfer line at 260°C, injection was carried out in splitless mode, and 2 μL of extracted meconium were injected into the system. The oven was held at 80°C for 1 min, then ramped at 22°C/min to 300°C. The manifold temperature was set to 250°C and the multiplier voltage was set at 2100 V. Helium was used as the carrier gas. The low mass monitored was 180 m/z and the high mass 250 m/z. The selected ions were 205, 246, and 248 for deuterated (D5); 200, 242, and 243 for PCP. The scan rate was 250 ms and the segment acquiring time was 11 min.

Linearity

Unextracted PCP standards at concentrations of 5, 10, 50, 100, and 250 ng/mL were prepared and injected into the system (internal standard concentration 50 ng/mL). The system was linear (r = 0.998) over the analytical range tested. Seven replicate injections of concentration 50 ng/mL were made giving a relative standard deviation of less than 10%.

Extraction Efficiency and Limit of Detection

The extraction efficiency of PCP from meconium was 84.4% (C.V. 1.0%) at the 50 ng/g level (n = 10). To determine the limit of detection, deuterated D5-PCP (250 ng) and various concentrations of PCP (5 − 250 ng/g) were added to negative meconium samples (0.5 − 1 g) and the specimens were extracted according to the procedure outlined. The limit of detection (LOD) was 5 ng/g meconium.

Results and Discussion

The outlined method is routinely used in our laboratory and is particularly useful when sample size is limited and sensitivity is an important issue. A chromatogram of a clinical specimen containing phencyclidine and a negative meconium specimen are shown in Fig. 1. Positivity of clinical samples is based on retention time (9.35 min) and the "goodness of fit" value incorporated into the software. Because of the complexity of the meconium matrix, a "goodness of fit" value of 800 is used (1000 = perfect match). The SIS program effectively performs a limited scan library search on a specific peak representing a small range of ions as opposed to the determination of abundance ion ratios as is used in SIM. The ion trap procedure lowers the limit of detection four fold over other reported GC/MS electron impact methods.

Relative to other abused drugs (cocaine, opiates etc.,) the frequency of phencyclidine detection in meconium is low. This may be because PCP is commonly used in conjunction with other drugs, and its overall use is not as common as cocaine, for example. However, it is possible that phencyclidine itself is not the predominant compound found in meconium, in a similar way that benzoylecgonine is not the predominant cocaine metabolite found in meconium (11). Fetal metabolism is not well understood particularly regarding route of administration, frequency, and amount of maternal drug use.

Ongoing work in our laboratory includes the investigation of possible PCP metabolites in meconium, their degree of conjugation (if any), and their extraction and analysis.

References

(1) Tabor BL, Snith-Wallace T, Yonekura ML. Perinatal outcome associated with PCP versus cocaine use. Am J Drug Alcohol Abuse 1990;16(3 & 4):337–48.
(2) Strauss AA, Modanlou HD, Bosu SK. Neonatal manifestations of maternal phencyclidine (PCP) abuse. Pediatrics 1981;68:550–2.
(3) Nicholas JM, Lipshitz J, Schreiber EC. Phencyclidine: Its transfer across the placenta as well as into breast milk. Am J Obstet Gynecol 1982;143:143–6.
(4) Rayburn WF, Holsztynska EF, Domino EF. Phencyclidine: Biotransformation by the human placenta. Am J Obstet Gynecol 1984;148(1):111–2.
(5) Browne SP, Moore CM, Negrusz A, Tebbett IR, Covert R, Dusick A. Detection of cocaine, norcocaine and cocaethylene in the meconium of premature neonates. J Forensic Sci 1994;39:1515–9.
(6) Clark GD, Rosensweig IB, Raisys VA. The analysis of cocaine and benzoylecgonine in meconium. J Anal Toxicol 1992;16:261–3.
(7) Moore C, Deitermann D, Lewis D, Leikin J. The detection of hydrocodone in meconium: Two case studies. J Anal Toxicol 1994;19(6):514–8.
(8) Moore C, Lewis D, Becker J, Leikin J. The determination of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) in meconium. J Anal Toxicol 1996;20:50–1.
(9) Franssen RME, Stolk LML, Van den Brand W, Smit BJ. Analysis of morphine and amphetamine in meconium with immunoassay and HPLC-diode array detection. J Anal Toxicol 1994;18:294–5.
(10) Moriya F, Chan KM, Noguchi TT, Wu PYK. Testing for drugs of abuse in meconium of newborn infants. J Anal Toxicol 1994;18:41–5.
(11) Lewis DE, Moore CM, Leikin JB. Incorrect diagnosis of cocaine-exposed babies: A report. Neonatal Intensive Care 1994;7(5):24–7.

Address requests for reprints or additional information to
Christine M. Moore, Ph.D.
Associate Scientific Director,
US Drug Testing Laboratories
2201 W. Campbell Park Drive
Chicago, IL 60612
TEL: 1-312-421-7333
FAX: 1-312-421-7249