The Determination of 11-Nor-Δ⁹-Tetrahydrocannabinol-9-Carboxylic Acid (THCCOOH) in Meconium

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Abstract

The determination of 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THCCOOH), the major urinary metabolite of Δ⁹-tetrahydrocannabinol (THC), in meconium is extremely difficult because of the high concentration of lipids in the specimen and the low concentration of drug present. A simple, robust, analytical procedure for the screening and confirmation of THCCOOH in meconium is presented. To our knowledge, the procedure is the first published method for the successful confirmation of THCCOOH in meconium and is used routinely in our laboratory for the purpose of detecting marijuana use during pregnancy.

Introduction

Adverse neonatal effects associated with drug exposure during pregnancy include low birth weight, intracranial bleeding, miscarriage, intrauterine growth retardation, and developmental problems.

The determination of drugs of abuse in meconium for the purpose of detecting use during pregnancy is becoming increasingly popular. Meconium analysis extends the window of detection of drug use to approximately the last 20 weeks of gestation as opposed to urinalysis, which only provides information about recent drug exposure (2-3 days before birth). Therefore, it is considered to be a superior sample to urine for the detection of drug exposure (1,2). The majority of the published procedures for drug analysis in meconium focus on the determination of cocaine and its metabolites. Confirmation methods for the analysis of the other NIDA-5 drugs are few.

A study released by the National Pregnancy and Health Survey showed that 118,700 (2.9%) women who gave birth between October 1992 and August 1993 used marijuana. Prevalence studies, which include the determination of marijuana metabolite, are predominantly screen-only methods (3). Of two confirmation procedures reported, one was unable to confirm screen positives (4) and the other did not give methodological details (5).

Experimental

Materials

Deuterated THCCOOH (THCCOOH-d₃) was obtained from Radian Corp. (Austin, TX). All solvents were high-performance liquid chromatographic grade, and chemicals were American Chemical Society reagent grade.

Methods

Screening. The meconium was mixed thoroughly, an aliquot (0.5–1 g) was homogenized in acetic acid (3 mL), and diphenylamine in acetone (1.67 mg/L; 6 mL) was added. The sample was centrifuged (5 min; 2500 rpm). The supernatant was then passed through a polypropylene filter (20–40 µm), collected, and evaporated to dryness. The residue was reconstituted in TDx buffer (pH 8) (Abbott Laboratories, Abbott Park, IL) for analysis by fluorescence polarization immunoassay. The cutoff was set at 25 ng/g meconium.

Confirmation. For confirmation, another aliquot of the meconium sample was homogenized in methanol (3 mL). Deuterated internal standard (100 ng/g) and 11.8M potassium hydroxide (0.5 mL) were added, and the sample was allowed to stand for 15 min. Following centrifugation (5 min), the supernatant was transferred to a clean tube, and deionized water (3 mL) was added. The specimen was then subjected to liquid–liquid extraction in hexane-ethyl acetate (9:1, v/v; 6 mL). The sample was allowed to stand for 15 min, and the organic layer was discarded. HCl (0.1N; 1 mL) was added, followed by hexane–ethyl acetate (9:1, v/v; 8 mL). After mixing and centrifugation, the supernatant was evaporated to dryness. The residue was reconstituted in ethanol, transferred to autosampler vials, and reevaporated to dryness. The sample was finally derivatized with N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (50 µL) to produce the tertiary butyldimethylsilyl derivative of THCCOOH; it was also analyzed by gas chromatography–mass spectrometry (GC–MS).

The monitored ions were as follows: THCCOOH-d₃, 575, 518, and 416; and THCCOOH, 572, 515, and 413.

GC–MS conditions

The GC–MS system used consisted of a 5890 gas chromato-
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Figure 1. Selected-ion monitoring trace of a meconium specimen containing 46 ng/g THCCOOH. Graph connected to a 5971A mass selective detector (Hewlett-Packard, Naperville, IL). The gas chromatograph was operated in the splitless mode using helium as the carrier gas. The column used was a DB-5 MS column (25 m x 0.2-mm i.d.; 0.53-μm film thickness) (J&W Scientific, Folsom, CA). The injector temperature was 270°C, and the detector temperature was 310°C. The oven was held at 100°C for 1 min, then programmed to a final temperature of 310°C at 30°C/min, where it was held for 6.6 min. The overall run time was 14.6 min, and the retention times of deuterated THCCOOH and unlabeled THCCOOH were 13.72 and 13.75 min, respectively. The limit of detection of the assay was 2 ng/g.

Results

The method was validated on 100 consecutive meconium samples that were received by our laboratory. The correlation between screening and confirmation results was 80%, using 25 ng/g as the cutoff screening value. Of the 100 samples, 80 were less than the screening cutoff; all 80 were confirmed as negative by GC–MS. Of the other 20 samples, all screened positive; 16 samples were confirmed as positive by GC–MS (Figure 1).

Discussion

A correlation rate of 80% between screening and confirmation samples gives an indication that other metabolites of THC that cross-react in the immunoassay system may be present in meconium. Recent data (6) have shown that in approximately 23% of cocaine-positive meconium samples, the only cocaine metabolite present is meta-hydroxy benzoylecgonine (a minor adult urinary metabolite), which is not routinely determined in drug-testing laboratories. The initial discovery of this metabolite in meconium (7) was made because of a discrepancy between screening and confirmatory data in cocaine metabolite analysis. Apparently, fetal metabolism is markedly different from adult drug metabolism, and unusual metabolites are produced.

Therefore, further work in our laboratory will focus on the determination of previously unidentified THC metabolites in meconium in an attempt to approach a 100% screen: confirmation correlation.

Conclusions

We believe this is the first published procedure for the determination of THCCOOH in meconium. The method is used routinely in our laboratory and is robust, rapid, simple, reproducible, and efficient.

The correlation of 80% suggests that other metabolites of marijuana may be present in meconium.

References


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