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Note

Detection of marijuana metabolite 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in human urine by bonded-phase adsorption and thin-layer chromatography

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Thin-layer chromatography (TLC) is a widely used, cost-effective method for the qualitative assay of abused substances in urine. In recent years a new technique, bonded-phase adsorption chromatography, has been developed for selective extraction of drugs, pesticides, and vitamins from biologic and other materials [1]. We now report a qualitative method for the identification of the primary urinary metabolite of marijuana, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCA) [2]. The technique involves extraction of THCA onto a selective solid-phase material (Bond Elute-THC®) coupled with identification by TLC. The advantage of solid-phase extraction is that extracts obtained from hydrolyzed urine are concentrated in a small volume of solvent and are free of interfering substances.

EXPERIMENTAL

Materials

Fast Blue RR was obtained from Calbiochem-Behring (San Diego, CA, U.S.A.) and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid from Applied Science Labs. (State College, PA, U.S.A.). Bond Elute-THC columns (500 mg) were purchased from Analytichem International (Harbor City, CA, U.S.A.) and E. Merck silica gel 60 thin-layer plates (25 × 75 mm) from Applied Analytical (Wilmington, NC, U.S.A.). Thin-layer developing tank (No. 13265) is available from Fisher Scientific (Springfield, NJ, U.S.A.). A Baker 10 extraction system was purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.). All solvents and reagents were either HPLC or reagent grade and used without further purification.

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Method

Urine samples are hydrolyzed and extracted according to the following procedures:

A 10-ml aliquot of urine is transferred to a siliconized 15-ml screw-cap type centrifuge tube and 0.9 ml of 10 *M* sodium hydroxide is added. The tube is loosely capped and allowed to stand for 15 min in a boiling-water bath. After cooling the tube, the pH of the mixture is adjusted to a range of 1—3 by the addition of approximately 0.7 ml of concentrated hydrochloric acid. THCA is extracted from the urine using a Baker 10 extraction apparatus fitted with a Bond Elute-THC column (500 mg). The extraction column is activated by successive column full rinses of: methanol, water, methanol, water. Then 10—20 ml of hydrolyzed urine are drawn through the column followed by rinses with 10 ml of 0.1 *M* hydrochloric acid and 25 ml of 50 m*M* phosphoric acid (10% acetonitrile). THCA is eluted from the column into a siliconized 10 × 75 mm culture tube with exactly 1 ml of acetone. Methylene chloride (0.5 ml) is added to the eluate, the mixture is vortexed, then centrifuged briefly, and the upper phase removed by aspiration. To the remaining phase 0.5 ml of hexane is added, the mixture vortexed, and then centrifuged briefly. The upper organic phase is pipetted into a clean siliconized 10 × 75 mm tube leaving behind a small water droplet at the bottom of the tube. The solvent then is removed by evaporation at 60° C using a stream of nitrogen gas. The dry residue is dissolved in about 10 μl of acetone and spotted with a capillary tube onto a 25 × 75 mm thin-layer plate. The chromatogram is developed in 10 ml of ethyl acetate—methanol—water—concentrated ammonium hydroxide (12:5:0.5:1). THCA is visualized as a red spot at R_F 0.43—0.50 following spraying with freshly prepared 0.5% Fast Blue RR (50 mg/10 ml of 1:1 methanol—water).

A 10-ml pooled urine aliquot, which shows a positive EMIT® cannabinoid [3] reading at or above the medium calibrator level (equivalent to 75 ng/ml THCA), is run as a control in parallel with each assay.

RESULTS AND DISCUSSION

The method described here represents a significant modification of a THCA Bond Elute-THC extraction—HPLC assay [4]. The new method yields an eluate which is made free of water and thus suitable for TLC. Most of the water in the organic phase is separated by the addition of methylene chloride. The remaining water is then completely partitioned from the organic phase by the addition of hexane, a highly water-insoluble solvent. The final organic solvent volume is less than 2 ml and requires under 5 min for evaporation. Most liquid—liquid extraction procedures require the evaporation of a final solvent volume of 30—50 ml as well as repeated addition of other organics to remove remaining traces of water [5, 6].

The solid-phase adsorption residue left after evaporation of the solvent is dry, concentrated, and quite clean. The use of a 25 × 75 mm thin-layer plate reduces the development time to 7 min. The customary 20 × 20 cm plate requires 40 min development time. The TLC mini-chamber system is cost-effective since it uses much less solvent (10 ml) and a less expensive plate.

The solvent developing system places the THCA hydrolysis product midway in the chromatogram at R_F 0.43—0.50. The chromatogenic agent, Fast Blue RR, has been shown to be a sensitive and specific visualization reagent for marijuana [6, 7]. In addition, it is observed that the red color of a developed THCA spot sprayed with Fast Blue RR remains for many weeks when covered with a glass plate. In contrast to the marijuana chromatogenic spray reagent Fast Blue B, which has been linked with carcinogenicity [8], the question of safety has not been raised with the use of Fast Blue RR.

The Bond Elute-THC—TLC assay may serve as a confirmation method for the EMIT cannabinoid [3] drug screen procedure. It can detect 20 ng/ml of THCA in urine when the volume assayed is 10 ml. This sensitivity is sufficient to confirm the presence of THCA in a 10-ml clinical urine specimen which shows a positive EMIT cannabinoid response at the medium calibrator level [9]. However, a urine volume of 20 ml often is required to detect THCA when the positive EMIT cannabinoid response is at the low calibrator level [9].

We feel the combination of Bond Elute-THC extraction coupled with TLC assay represents a new and reliable technique for the qualitative detection of THCA in urine. It should prove particularly useful clinically when EMIT screening for cannabinoids is positive and a relatively simple confirmatory test is required.

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