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Note

Simple, rapid method for extraction of urinary cannabinoids by liquid–solid chromatography

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The extraction of cannabinoids from biological fluids poses a problem the difficulty of which depends on the medium and on the complexity of the metabolism leading to the formation of many polar compounds mainly excreted in urine[1,2]. Liquid–liquid extraction at different pH values has been extensively described[3–5], usually in association with purification steps on Sephadex LH-20[6], since the use of Claisen alkali[7] permits elimination of lipophilic compounds. This paper describes a urine extraction method in which liquid–solid elution on a chromatographic column is used with appropriate solvents[8]. The elimination of unchanged Δ^9-tetrahydrocannabinol (Δ^9-THC) has also been investigated.

EXPERIMENTAL

^{14}C-Δ^9-THC (3 mCi/mmole) was synthesized by us at C.E.A. (Saclay, France). ^{3}H-8β-OH-Δ^9-THC (3 Ci/mmole) and ^{3}H-11-OH-Δ^9-THC (2.5 Ci/mmole) were a gift from the National Institute of Mental Health (Bethesda, MD, U.S.A.).

All solvents and other chemicals were standard reagent-grade materials.

Chromatography

Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates (Merck, Darmstadt, G.F.R.) and developed in three solvent systems: I (dioxane–*n*-hexane, 20:80); II (benzene–acetone, 90:10); and III (benzene–diethyl ether, 50:50). Impurities were preliminarly removed from the plates by one migration with the

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appropriate solvent. Cannabinoids were detected by spraying the plates with a freshly prepared, cold 0.1% solution of Fast Blue Salt B in 1 *M* sodium hydroxide.

Columns were prepared using 50-ml plastic syringes filled with 70 ml of granular diatomaceous earth (Extrelut®; Merck). The solvent flow-rate was regulated by steel needles connected to the syringe outlet.

Mass spectrometry

The silica gel zones corresponding to Δ^9-THC were eluted with ethanol and mass spectra were recorded with a Riber R 10-10 gas chromatograph–mass spectrometer controlled by a Digital Equipment Corp. PDP8/m data system. The electron ionization source temperature was 150°C, with a potential of 80 eV. Gas–liquid chromatographic (GLC) separations were performed with a Girdel gas chromatograph equipped with an SE-30 wall-coated open-tubular glass capillary column (25 m × 0.5 mm I.D.). The pressure and split for the carrier gas (helium) were 1.2 bar and 20 ml/min, respectively. The injection port and interface temperatures were 280°C and the column oven was operated isothermally at 240°C.

Radioactivity measurement

Radioactivity was measured by liquid scintillation counting and expressed as disintegrations per minute (dpm) after correction by the external standard method. The distribution of radioactivity on the TLC plates was determined with a Berthold LB-2723 scanner.

Procedure

A 10-ml volume of urine was acidified to pH 3 with 10 ml of 0.05 *N* hydrochloric acid, deposited on the column and chromatographed for 15 min, then 40 ml of diethyl ether or ethyl acetate were used to elute the 21st-min fraction. The eluate was evaporated to dryness in a Büchi rotary evaporator at 40–45°C and the residue was transferred quantitatively with microlitre volumes of diethyl ether or ethanol on to TLC plates or into scintillation vials.

Detection of non-metabolized Δ^9-THC

A volunteer male subject who was a frequent user of cannabis smoked a cigarette containing 15 mg of Δ^9-THC. Successive pooled 24-h urine specimens were collected prior to inhalation and for 24 h after smoking. Urine was stored frozen until used for detection of metabolites and unchanged THC.

RESULTS

Table I compares the cannabinoid extraction yields obtained for three different concentrations and the two elution solvents (diethyl ether and ethyl acetate). Extraction was quantitative for 8β- and 11-OH-Δ^9-THC with diethyl ether, but varied from 81.5 to 97% for Δ^9-THC. Urine pigments were eliminated from the final extract with diethyl ether only.

After spraying with Blue Salt B, TLC controls and radioactivity measurements showed the same R_F values as for the three standard cannabinoids. Fig. 1 shows the radiochromatograms of a normal urine extract loaded with 20 ng/ml of

TABLE I

EXTRACTION OF VARIOUS CANNABINOIDS AT THREE CONCENTRATIONS BY LIQUID–SOLID CHROMATOGRAPHY USING ETHYL ACETATE AND DIETHYL ETHER AS SOLVENTS

Results are means of duplicate extraction experiments.

Concentration (ng/ml)	*Ethyl acetate*			*Diethyl ether*		
	Δ^9-THC	*8β-OH-Δ^9-THC*	*11-OH-Δ^9-THC*	*Δ^9-THC*	*8β-OH-Δ^9-THC*	*11-OH-Δ^9-THC*
5	—	—	—	97	89	104
10	98.4	77.2	66.4	86.8	98.3	95.8
20	—	—	—	81.5	102	98

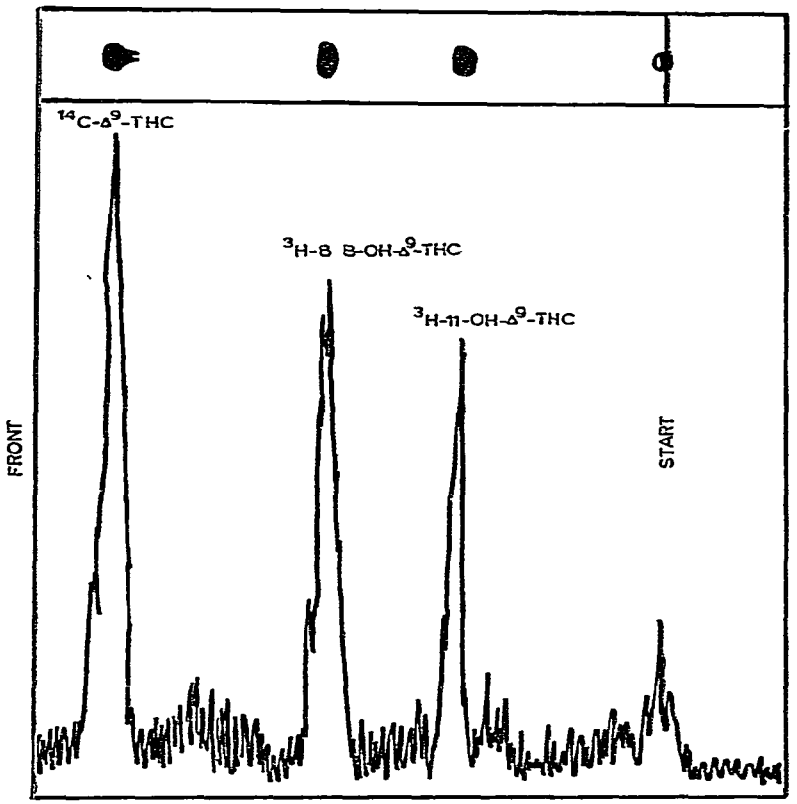

Fig. 1. Radiochromatogram showing TLC separation of a urine extract. Solvent: benzene–diethyl ether (50:50).

the three cannabinoids. The three spots are identical with those for the standard cannabinoids in the three TLC systems, which demonstrates the stability of the extracted compounds.

Extraction with diethyl ether was applied to the detection of unchanged Δ^9-THC in the urine of a cannabis smoker. The silica gel zones corresponding to the

Δ^9-THC eluted from the chromatograms prepared in solvent system I had the same mobility as the Δ^9-THC chromatographed sequentially in solvent systems II and III. Identification of Δ^9-THC was confirmed by GC–MS analysis, which led to typical fragmentation of Δ^9-THC with *m/e* 314, 299, 271, 258, 243 and 231 (ref. 9). The computer-reconstructed gas chromatogram is shown in Fig. 2. Verification of the urine sample collected prior to inhalation showed no specific Δ^9-THC fragments.

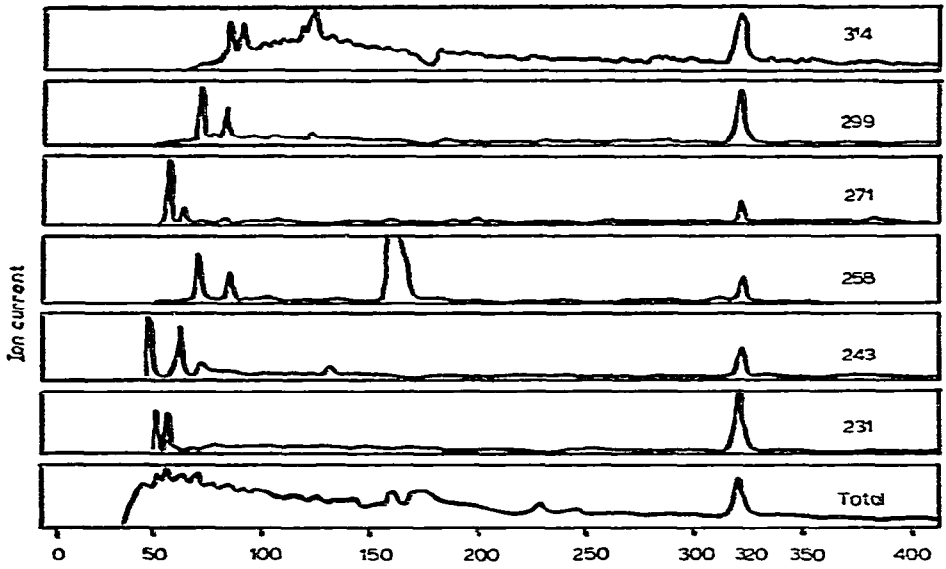

Fig. 2. Computer-reconstructed gas chromatogram of Δ^9-THC from the ethanol extract of the silica gel zone scraped off the TLC plate.

DISCUSSION

Liquid–solid chromatography applied to the extraction of cannabinoids from urine proved to be a simple and rapid procedure. Cannabinoids were quantitatively extracted with diethyl ether and characterized by TLC. Unchanged Δ^9-THC was found in a 24-h urine specimen after inhalation. Recently, Kanter *et al.*[10] combined high-performance liquid chromatography with TLC for the detection and quantitation of unchanged THC in urine during the first 6 h after cannabis absorption. Using dansylation, we previously found low concentrations of Δ^9-THC in urine ($\leqslant 15$ ng/ml) from habitual cannabis smokers[11]. These findings showed discrepancies with other studies demonstrating that unchanged Δ^9-THC was not eliminated in urine[1]. The present work confirms our previous findings and the results of Kanter *et al.* The use of the liquid–solid chromatography procedure described will permit further investigations and improvement of metabolic studies of Δ^9-THC.

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