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Note

Marihuana metabolites in the urine of man

VIII. Identification and quantitation of Δ^9-tetrahydrocannabinol by thin-layer chromatography and high-pressure liquid chromatography

SAUL L. KANTER, LEO E. HOLLISTER and KAY O. LOEFFLER
Veterans Administration Hospital, Palo Alto, Calif. 94304 (U.S.A.)
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Some recent work has used high-pressure liquid chromatography (HPLC) for separating Δ^9-tetrahydrocannabinol (THC) from its metabolites as well as from other cannabinoids and endogenous materials in biological fluids[1,2]. Neither group used an ultraviolet (UV) wavelength sufficiently sensitive to quantify the amounts of THC in the effluent; one group used 254 nm, the other 274 nm, neither of which approaches the potential sensitivity of the procedure[3]. Maximum sensitivity is at the low wavelengths; absorbance at 215 nm is about 30 times greater than at 280 nm[4].

We have previously been able to detect unchanged THC in human urine using simple thin-layer chromatographic (TLC) methods, confirming its identity and quantifying it by gas chromatography–mass spectrometry (GC–MS)[5]. The possibility of combining TLC and HPLC for the detection and quantification of THC or other cannabis metabolites in urine appeared promising. The present study proved that this approach was feasible.

EXPERIMENTAL

Three young men were given single oral doses of 30 mg THC incorporated into a chocolate cookie. Urine specimens were collected during a 12–24-h period prior to administration of the drug and at intervals of 2, 6, 12 and 24 h after. Aliquots were removed for determination of creatinine; the remainder was stored frozen until analyzed for unchanged THC[6].

Volumes of urine containing from 50 to 100 mg of creatinine were hydrolyzed with β-glucuronidase–aryl sulfatase and concentrated in a rotary evaporator[7,8]. The concentrates were diluted to 10 ml with water, adjusted to pH 8 and extracted with hexane three times[7]. The combined hexane extracts were volatilized in a stream of nitrogen with heat (not over 50°). The residues were quantitatively transferred with microliter volumes of dichloromethane (DCM) to Analtech pre-coated silica gel G plates, 250 μm. A mixture of light petroleum (b.p. 30°–60°)–diethyl ether (85:15) was the mobile phase, which allowed the non-polar compounds, cannabidiol (CBD), THC and cannabinol (CBN) to separate from each other[9]. Simultaneously two reference chromatograms of THC, one at each edge of the TLC plate, were run. The

two reference chromatograms on the TLC plate were treated with freshly prepared, cold 0.1% Fast Blue Salt B in 1 *M* sodium hydroxide to stain the zones of THC or other cannabinoids. The zones corresponding to THC were scraped into test tubes and the silica eluted by extracting three times for 1 min each time on a vortex mixer with 3, 2 and 2 ml of ethanol[5]. The ethanol was volatilized and the residues were quantitatively transferred to 1-ml reacti-vials with microliter volumes of ethanol and stored in the freezer. Prior to HPLC, the ethanol was volatilized and the residue dissolved in 50 μl of hexane; 10-μl aliquots were used for analysis.

Liquid chromatography was performed with a Spectra-Physics Model 3500B liquid chromatograph, with two reciprocating piston type pumps. A Valco sampling valve permitted the injection of a 10-μl sample from a slightly larger volume. A Schoeffel variable wavelength detector was set at 220 nm to achieve maximum sensitivity. A 250 × 3 mm column of Spherisorb silica (5 μm) was used for separation of the urine extracts, and hexane–3% methanol in DCM (97:3) flowing at 1.6 ml/min, pressure *ca.* 1000 p.s.i., was used for elution. The small amount of methanol in the solvent mixture enhanced the sharpness of the peaks and effected base-line separation.

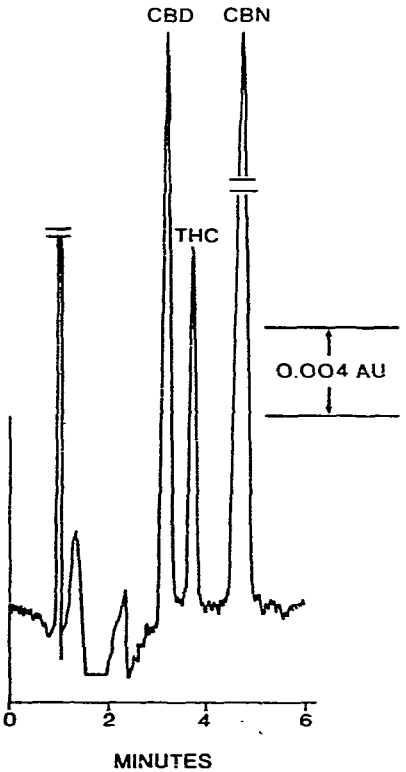

Fig. 1. Normal-phase HPLC separation of cannabinoids. Sample, 5 μl of a mixture of CBD, Δ^9-THC and CBN containing 25 ng/μl, respectively, in hexane. Column, 3 × 250 mm containing Spherisorb silica (5 μm). Solvent, hexane–3% methanol in dichloromethane (97:3) at a flow-rate of 1.6 ml/min. Detector, 220 nm, 0.04 a.u.f.s.

Separation of THC from selected natural cannabinoids and metabolites

Mixtures of hexane and DCM were tested for separation of CBD, Δ^8- and Δ^9-THC, CBN and several monohydroxy metabolites of Δ^9-THC (8α-, 8β-, and 11-hydroxy-Δ^9-THC). As the concentration of DCM in the mixture increased, the retention time (t_R) of Δ^9-THC decreased. At 25% DCM, the t_R was 5 min, a reasonable analysis time, but the peak was broad and affected by chemical tailing[10]. The addition of methanol to the DCM sharpened the peak, eliminated the tailing and effected base-line separation of peaks. With hexane–3% methanol in DCM (97:3) excellent separation of CBD, THC and CBN was obtained (Fig. 1). Under these conditions, Δ^8-THC had the same t_R as CBD, but this was of no consequence for analysis of THC, so further refinements of the HPLC system were not explored. The t_R value of 8β-hydroxy-Δ^9-THC, the earliest appearing monohydroxy metabolite, did not approximate that of THC until the concentration of DCM increased to 30%. This concentration was an order of magnitude greater than in the analytical eluent used. Thus a peak with t_R coincident with that of THC was considered to be more likely to represent that material rather than any other cannabinoids usually encountered in physiologic fluids.

RESULTS

Peaks coincident with the t_R of THC were detected in the extracts of the 0–2-h and 2–6-h post-drug urines. Equivalent peaks were not found in the pre-drug, the 6–12-h and 12–24-h post-drug urines (Fig. 2) confirming previous results[5]. By comparing peak heights of unknowns to peak heights of standard amounts of THC, a quantitative measure of the amount of THC in the residue was obtained. From this it was determined that approximately 0.003–0.008% of the THC dose was recovered (Table I). This compares favorably with recovery previously obtained by TLC and GC–MS[5]. The lowest recovery was from a frequent user of cannabis.

The CBD peaks of the spiked samples of Figs. 2B and C supported the identification of the THC peak in each chromatogram, respectively, by demonstrating that the relationship between peaks was the same as obtained for the mixture of standards.

DISCUSSION

The 6-h duration of excretion of THC, after its ingestion, provides a good marker for forensic purposes as this duration of excretion roughly parallels the duration of clinical effects of orally administered THC[11]. The identity of the HPLC peak of t_R coinciding with the standard THC is supported by previous work in which it was confirmed by GC–MS that the zone of the TLC plate that coincided with the retention time of THC did indeed contain THC[5].

Normal-phase chromatography using a polar adsorbent, silica, and a non-polar eluent was selected as the chromatographic mode as this system had already separated Δ^8- and Δ^9-THC on HPLC[12], and CBD, THC and CBN on TLC[9]. Reversed-phase HPLC will be tested in the future. This HPLC mode should enhance reliability of the identification of THC eluted from a thin-layer plate of silica gel as the latter would be processed through two different adsorbent conditions. Other polar compounds which make up the majority of the endogenous matrix would be eluted earlier

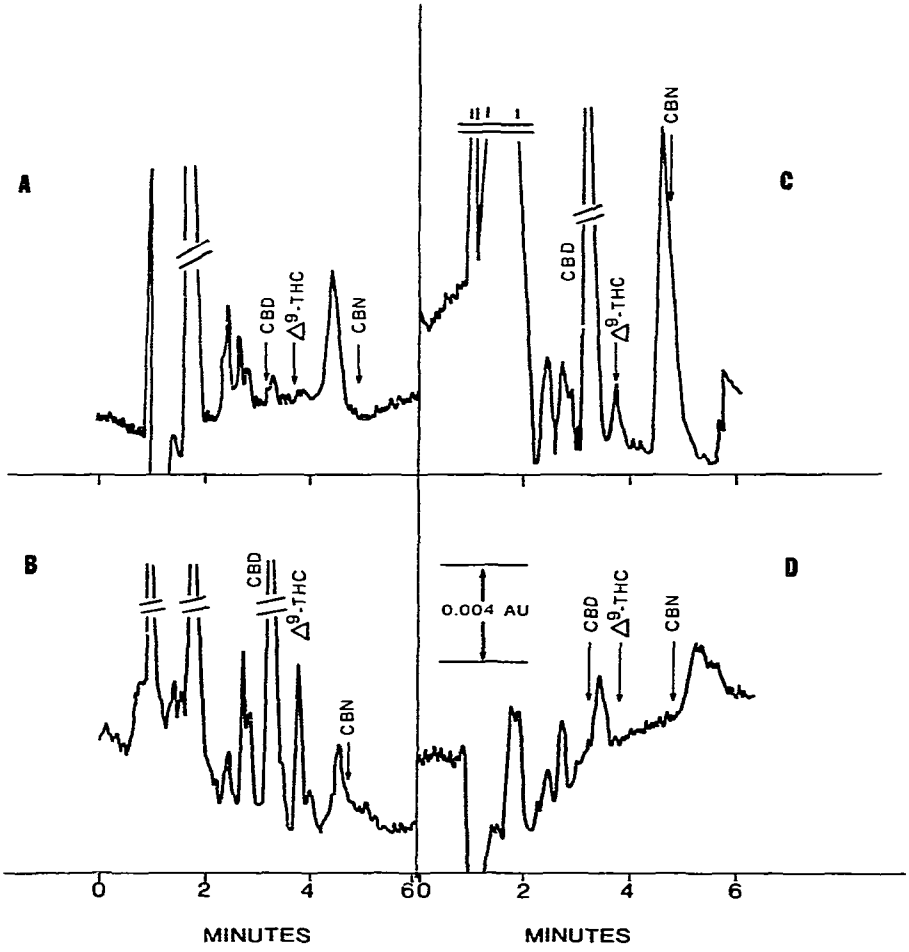

Fig. 2. Normal-phase HPLC chromatograms of the urines of an infrequent user of cannabis who ingested 30 mg of Δ^9-THC orally. HPLC conditions, see legend Fig. 1. A, pre-drug urine, injected equivalent to 20 mg creatinine. B, C, and D, post-drug urines, 0–2 h, 2–6 h and 6–12 h, respectively; injected equivalent to 10, 10 and 15 mg creatinine, respectively. Arrows indicate retention times of designated standards. A peak for unchanged Δ^9-THC appeared only up to 6 h after drug ingestion. CBD peaks in B and C are due to spiking of the samples.

than the cannabinoids, thus reducing both their potential interference with identification of THC and their contamination of the adsorbent column[4].

We have confirmed greater sensitivity of the HPLC detector to cannabinoids using a low wavelength of 220 nm. While using fixed-wavelength UV detectors, such as 254 nm for the quantitative estimation of THC by HPLC is a relatively insensitive procedure, using two UV detectors in series (215 and 280 nm) not only increases the sensitivity of detection but also enhances the reliability of the identification by providing absorbance ratios characteristic of cannabinoids[4]. A fluorescence detector combined with dansylated derivatives increases sensitivity 100-fold compared to that at 215 nm[4]. The potential of HPLC for the reliable and sensitive detection of minute amounts of THC in urine seems only to have been slightly realized as yet.

TABLE I

RECOVERY OF THC MEASURED BY HPLC

Subject	*Time after THC administration*	*Urine*			*THC recovered (ng)*		
		Total volume (ml)	*Per analysis*		*Per analysis***	*Total*	*Percent of drug ingested*
			ml	*mg**			
A	0–2 h	400	114	50	357	1254	0.0042
	2–6 h	700	119	50	138	813	0.0027
	0–6 h	1100				2067	0.0069
B	0–2 h	800	417	100	214	410	0.0014
	2–6 h	300	72	83.5	140	588	0.0020
	0–6 h	1100				998	0.0034
C	0–2 h	180	55	50	375	1227	0.0041
	2–6 h	400	120	100	354	1180	0.0039
	0–6 h	580				2407	0.0080

* Creatinine.

** Actual measurement, 1/5 these amounts.

A procedure that successfully combines HPLC with TLC for the detection and quantitation of unchanged THC in urine after oral doses of 30 mg has been described.

Future work in the development of HPLC combined with TLC will be directed towards the detection of THC after social use.

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