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Note

Identification of Δ^9-tetrahydrocannabinol in human plasma by gas chromatography

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According to Agurell *et al.*[1], combined gas chromatography–mass spectrometry (GC–MS) allows the determination of Δ^9-tetrahydrocannabinol (Δ^9-THC) in human blood with the required high sensitivity and selectivity. The GC–MS method described by Rosenfeld *et al.*[2] gives much higher Δ^9-THC plasma levels than the method of Agurell *et al.*[1] using the same system. However, few laboratories possess such equipment. Therefore we found it useful to develop a method based on GC-alone.

Fenimore *et al.*[3] published a GC procedure which employs rather complicated equipment. The method of Garrett and Hunt[4] is sensitive but non-specific. Recently, these authors described a procedure involving a purification step based upon high pressure chromatography followed by GC analysis[5]. The sensitive and specific method of McCallum[6] published in 1973 has not yet been used for the analysis of human samples. The other possible approach includes high-pressure liquid chromatography (HPLC)[7–9] and radioimmunoassay[10] or the combination of these two methods. The recent method of Williams *et al.*[11] consists of an HPLC step followed by radioimmunoassay determination of THC and metabolites in the eluted fractions. The sensitivity is good: 0.1 ng THC can be detected in 1 ml plasma. The described method is not absolutely specific for Δ^9-THC as Δ^9- and Δ^8-THC isomers are not totally separated by HPLC. However, this separation is possible, but the analysis time is long and the required equipment expensive.

The purpose of our investigations was to improve first the purification and GC separation and then the detection sensitivity of Δ^9-THC. The procedure is applicable to pharmacokinetic, toxicological and psychopharmacological studies of this and related compounds.

MATERIALS AND METHODS

Two healthy volunteers participated in this study. Each smoked a cigarette loaded with 6 mg Δ^9-THC. Timing was started at the end of the inhalation of the drug.

Catheterization of the humeral vein during the first 25 min allowed collection of blood probes after 5, 10, 15 and 25 min. Further samples were collected after 1, 2 and 3 h. After centrifugation the sealed plasma was frozen at −20° and kept at this temperature in the dark until used.

Extraction and preliminary purification

A 5-ml volume of plasma was extracted successively with three portions of 20 ml dichloromethane containing 1.5% isoamyl alcohol in a Sorvall glass centrifuge-tube. The pooled extracts were concentrated to 10 ml, then washed successively with 10 ml Tris buffer pH 8.5[12] and 10 ml citrate buffer pH 3.5[12]. In both cases the buffers were discarded.

Derivatization and subsequent purification

The extract placed in a PTFE-lined screw-cap test-tube (Sovirel, France) was evaporated to dryness under a stream of nitrogen. The residue was then dissolved in 500 μl benzene (Mallinckrodt, St. Louis, Mo., U.S.A.), $2.851 \cdot 10^{-2}$ *M* in trimethylamine (Fluka, Buchs, Switzerland), and 25 μl heptafluorobutyric anhydride (HFB) were added.

The reaction mixture was left at room temperature for 15 min. Then 1 ml phosphate buffer (1 *M*, pH 6)[12] was added to neutralize excess of reactant and the mixture was shaken for 30 sec. After reduction of the organic layer in a nitrogen stream at 50°, the residue was dissolved in 200 μl cyclohexane (Mallinckrodt) and purified on a 38 × 55 mm silica gel 60 (Merck) microcolumn. The elution solvent contained 10% dichloromethane in cyclohexane. The first 3 ml of eluent containing Δ^9-THC-HFB were evaporated to dryness in a nitrogen stream and the residue dissolved in 100 μl cyclohexane.

Gas chromatography

A GI 450 gas chromatograph (Carlo Erba, Milan, Italy) was equipped with a splitless injector (Type Grob), a micro electron capture detector (tritiated scandium foil, 1 Ci; temperature regulator ±0.1°; Brechbuehler, Urdorf, Switzerland) and a glass capillary column (50 m × 0.37 mm I.D.) coated with SE-54 (Jaeggi, Trogen, Switzerland).

GC was carried out under the following conditions: carrier gas (helium) flow-rate, 1.5 ml/min; split 25 ml/min., electron capture detector, nitrogen flow 29 ml/min, constant voltage 2.1 V; injector temperature 260°, detector temperature 240°; oven temperature program 6 min isothermal at 190°, then increased by 4° per min up to 250°.

GC–MS

To control our GC identification we used an LKB 9000 mass spectrometer, equipped with a column (2.5 m × 3 mm I.D.), packed with 3% SE-30 GC-Grade on Gas-Chrom Q (100–120 mesh). Conditions: carrier gas (helium) flow-rate, 30 ml/min; oven, isothermal at 200°; acceleration voltage 2.5 kV; trap current 65μA; separator and ion source temperature 270°. The selective ion monitoring method was used, registering the molecular peak of Δ^9-THC-HFB: M^+ 510.

RESULTS AND DISCUSSION

Figs. 1 and 2 show gas chromatograms obtained from plasmas taken respectively before and 5 min after inhalation of smoke containing Δ^9-THC.

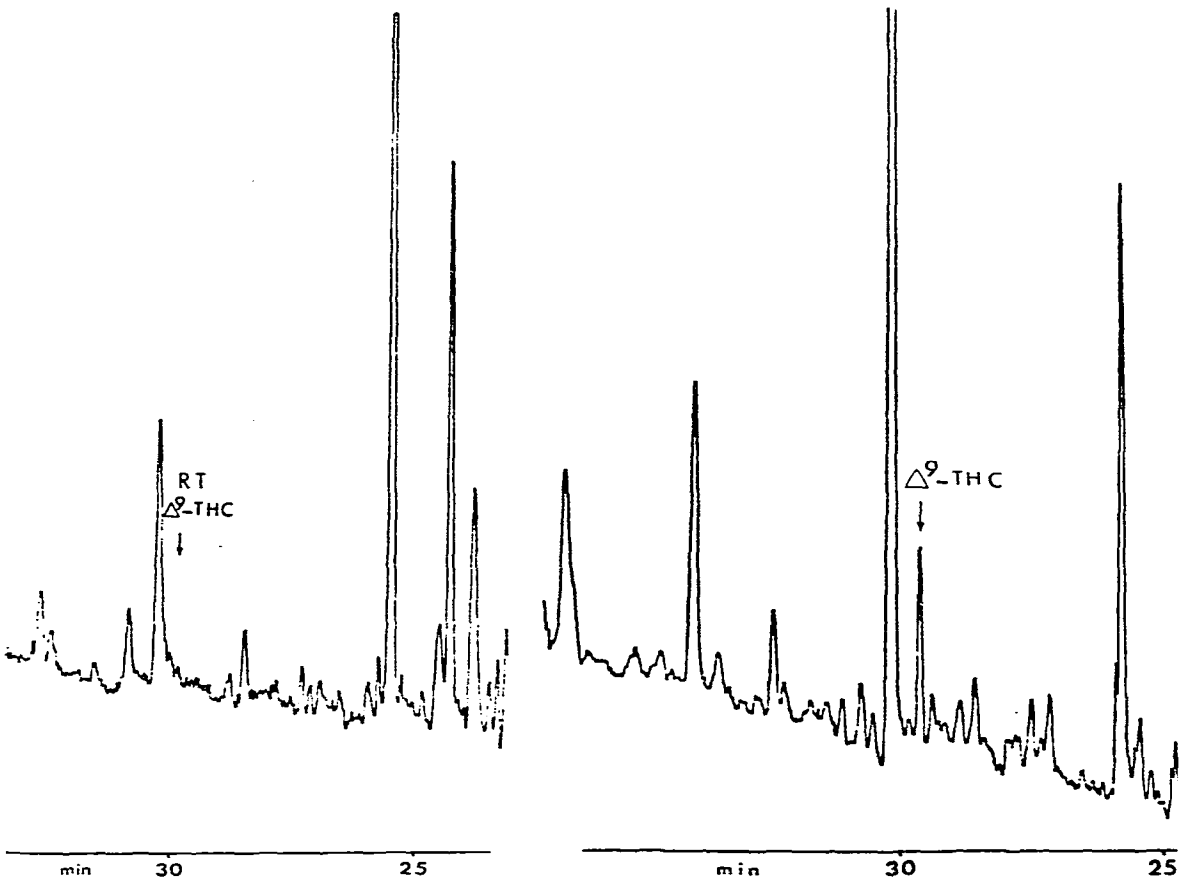

Fig. 1. Gas chromatogram of human plasma extract before Δ^9-THC inhalation. Arrow indicates Δ^9-THC-HFB retention time.

Fig. 2. Gas chromatogram of human plasma extract 5 min after Δ^9-THC inhalation.

The total recovery of the procedure, including extraction, partition and derivatization, is 80%, whereas the yield of purification on a silica gel microcolumn is only 30%. Despite this poor recovery, 2.5 ml plasma are sufficient to identify Δ^9-THC during 2–3 h after inhalation of the compound. The 50 m capillary column coated with SE-54 suppresses any interference from plasma components. Δ^8-THC and Δ^9-THC are totally separated from one another. In a reference solution, 8 pg of synthetic Δ^9-THC-HFB can be detected. In plasma the Δ^9-THC level must be higher than 1 ng/ml.

The GC–MS verification based on the selective determination of the molecular peak of Δ^9-THC-HFB (M^+ 510) yields the fragmentograms shown in Figs. 3 and 4.

This procedure is not more time-consuming than those using GC–MS, where a column purification is also necessary to obtain the required selectivity.

Improvement of the recovery could probably be achieved by using a Sephadex LH-20 column or a reversed-phase RP-8 microcolumn in the subsequent purification step.

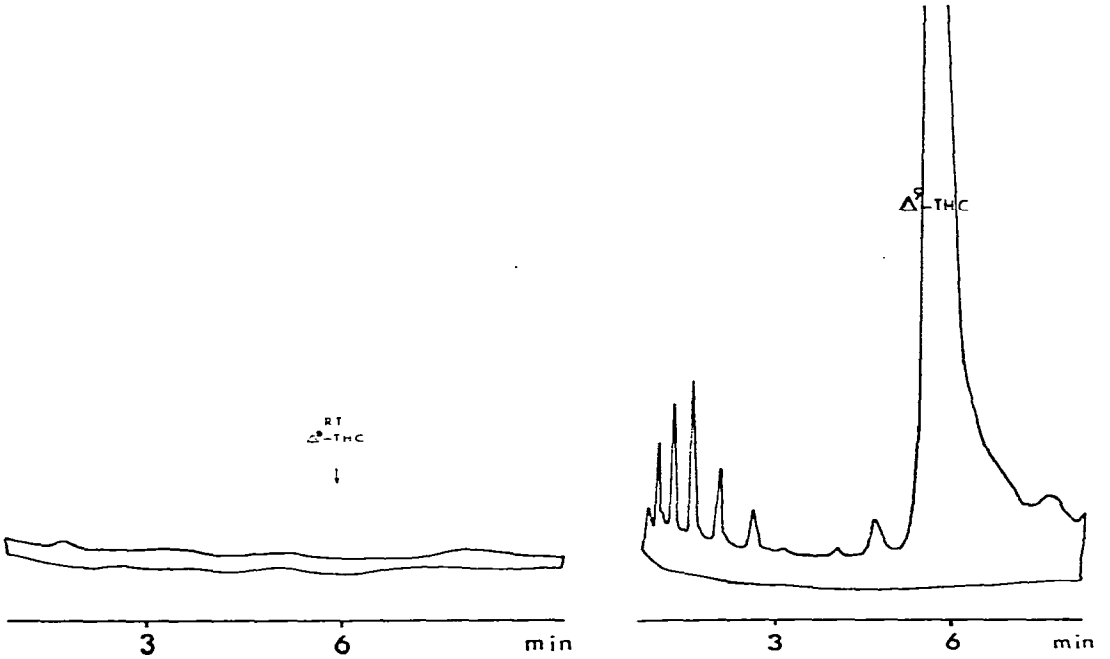

Fig. 3. Mass fragmentogram of human plasma before Δ^9-THC inhalation. Arrow indicates Δ^9-THC-HFB retention time.

Fig. 4. Mass fragmentogram of human plasma 5 min after Δ^9-THC inhalation.

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