Mass Fragmentographic Assay for Δ^9-Tetrahydrocannabinol in Plasma

J. J. Rosenfeld,[1] Bob Bowins,[2] Jeff Roberts,[3] John Perkins,[3] and A. S. Macpherson[4]

McMaster University, Hamilton, Ontario, Canada

The wide-spread use of marihuana, the interest in its clinical application (*1, 2*), and the development of cannabinoid-like drugs (*3, 4*) make necessary a definition of parameters of effect. As with certain other drugs, knowledge of the plasma levels of the active compound may prove useful in this respect (*5*). The active compound in cannabis-based preparations is Δ^9-tetrahydrocannabinol (Δ^9-THC) (*6*). Cannabinoid-like drugs are usually modifications of Δ^9-THC (*3*).

To clarify the complex pharmacology of cannabis-based preparations in man, sensitive and selective assays of Δ^9-THC are being developed. Both radioimmunoassay (*7*) and fluorometric methods (*8*) have been proposed. However, the methods based on gas chromatography seem to give the best combination of sensitivity and selectivity. A procedure using flame ionization detection of phosphate esters of Δ^9-THC affords an assay with a sensitivity of 1 ng/ml of plasma (*9*). An electron capture technique based on the derivatization of the phenol with halogen-containing reagents yields a sensitivity of 1 picogram injected on column (*10*). The sensitivity of both these methods is impressive but the selectivity is not as good as the mass fragmentographic assay described by Agurell *et al.* (*11*).

In general, mass fragmentographic assays are among the most selective analytical methods available to date (*12*). However, development of mass fragmentographic assays gives rise to certain inherent difficulties. The first is the availability of appropriate isotopically labeled compounds to be used as internal standards (*12*). This often requires incorporation of deuterium or other isotopes into the skeleton of the drug which can present problems in chemical synthesis (*13, 14*). The cannabinoids represent a class of compounds which have pharmacological importance. To develop assays for this class, each individual compound would have to be synthesized with deuterium in an appropriate position, perhaps by the method of Agurell (*11*). This procedure could prove to be a problem in development of assays for these drugs and possibly pose a hindrance in the exploitation of their pharmacological properties.

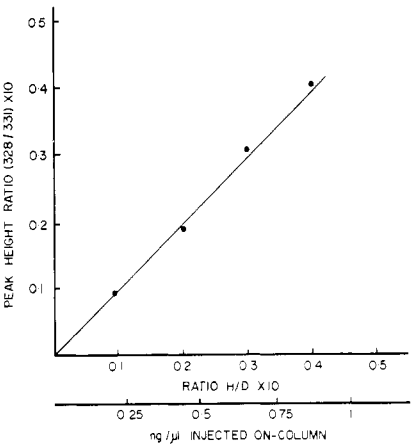

Figure 1. ● Experimental points by on-column methylation. —— "Theoretical" Δ^9-THC-OCH_3/Δ^9-THC-OCD_3

The second problem in developing plasma level assays arises from the contamination of the extract of plasma by endogenous compounds. This problem is particularly severe for lipid-soluble compounds since lipid extract of plasma is heavily contaminated by endogenous lipids. The cannabinoids fall into the class of lipid-soluble compounds; as a result, two chromatographic procedures have been published regarding the removal of extraneous lipids prior to analysis. A packed fore-column between injector port of the gas chromatograph and the analytical column has been used to separate Δ^9-THC from contaminating lipids prior to analysis (*10*). This technique requires modification of a gas chromatograph and, furthermore, the possibility exists that more polar, or less stable compounds such as metabolites (*15, 16*) might be lost on the fore-column.

The second procedure requires preparation of a Sephadex LH-20 column and maintaining proper chromatographic conditions such as temperature, flow rate of solvent, etc. (*11*). In addition, the proper chromatographic separation would have to be worked out for all new compounds.

We now report an analytical technique for Δ^9-THC based on the chemistry of the phenolic group which is common to all cannabinoids, cannabinoid-like drugs, and their metabolites. The internal standard is Δ^9-THC perdeuteriomethyl ether (Δ^9-THC-OCD_3) which is easily synthesized (see procedure) (*17*). The analytical technique is

[1] Department of Pathology.
[2] Central Drug Analysis Laboratory, McMaster University Medical Centre.
[3] Department of Psychology.
[4] Department of Psychiatry.

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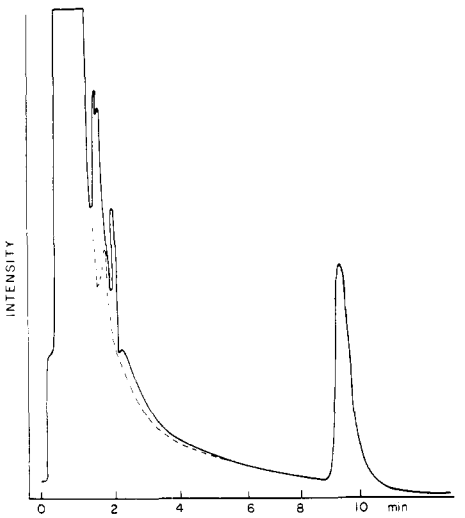

Figure 2. Detected by TIC monitor. Phenolic fraction of plasma extract——; superimposed, 23 ng of Δ^9-THC–OCD_3- - -

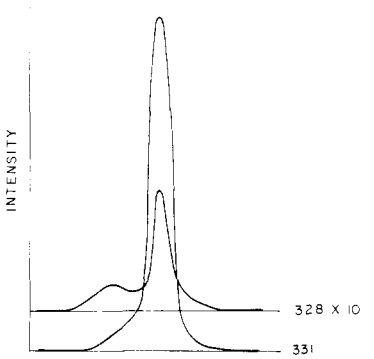

Figure 3. Mass fragmentographic detection of 0.88 ng Δ^9-THC–OCH_3 (328) and of 23 ng Δ^9-THC–OCD_3 (331)

based on the analysis of Δ^9-THC as its *O*-methyl ether (Δ^9-THC–OCH_3) which is prepared by the on-column methylation technique of Brochmann-Hanssen and Oke (*18*). The basis of the purification technique is the selective extractability of lipid-soluble phenols from hexane by Claisen's alkali.

EXPERIMENTAL

Assays were performed on a Varian CH 7 gas chromatograph–mass spectrometer unit (GC/MS) equipped with an accelerating voltage alternator for multiple ion detection. Mass fragmentographs were recorded on a Honeywell 4408-A Oscillograph. Ionizing voltages and emission current were 70 eV and 300 μA, respectively. The ion source was kept at a temperature of 290 °C. Gas chromatography was performed on a coiled 6-ft column containing 1.5% SE 30 on Chromosorb W 100–120 mesh at a temperature of 230 °C. Injector port and interface to the mass spectrometer was kept at 300 °C.

Reagents. Solvents were all analytical reagent grade. *N,N,N*-Trimethylanilinium hydroxide was prepared as needed by the procedure of Brochmann-Hanssen and Oke (*18*). Claisen's alkali (*19*) was prepared fresh using $\frac{1}{10}$ the normal amount of KOH.

Procedure. The perdeuteriomethyl ether of Δ^9-THC was prepared by the procedure used to synthesize Δ^8-THC–OCH_3 (*17*). A mixture of finely-ground K_2CO_3 in *N,N*-dimethyl formamide containing Δ^9-THC and perdeuteriomethyl iodide (isotopic purity 99.5%) was stirred overnight under argon. The mixture was worked up in the usual way and the product was purified by thick layer chromatography. This procedure gave Δ^9-THC–OCD_3 of 99.5% isotopic purity.

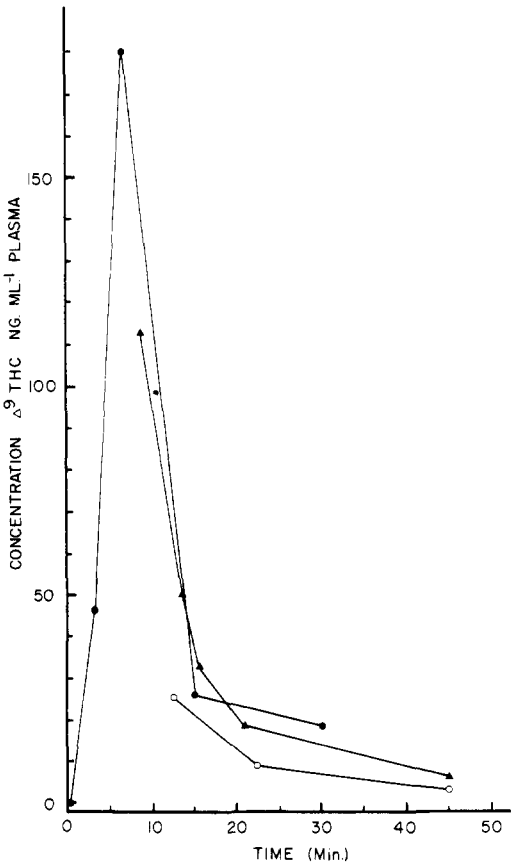

Figure 4. Subject 1 O, Subject 2 ▲, Subject 3 ●

Blood was collected by scalp vein needle in the hand veins and transferred to a 10-ml heparinized vacutainer tube. The plasma was prepared immediately after sampling and stored under argon at −30 °C until use. Two ml of plasma were extracted with 4 × 2 ml of hexane containing 5% isoamyl alcohol (V/V). The hexane isoamyl alcohol layers were collected and then extracted 4 times with 1 ml of the modified Claisen's alkali. The combined Claisen's alkali layers were acidified with 0.2 ml of concentrated HCl and the precipitated solid KCl was dissolved by adding 1 ml of H_2O. The aqueous phase was extracted with hexane. The hexane was evaporated under a stream of nitrogen to dryness and 50 μl of solution containing 1.15 μg of internal standard, Δ^9-THC–OCD_3, was added. This was again evaporated, the residue re-constituted with 50 μl of 0.2M *N,N,N*-trimethyl anilinium hydroxide in methanol. An aliquot of 1 μl of this solution was injected for analysis. The ions monitored were the molecular ions, 328 for Δ^9-THC–OCH_3, and 331 for Δ^9-THC–OCD_3, and peak heights were used for quantitation. All analyses were corrected for a background corresponding to 300 picograms of Δ^9-THC which is attributable to, in part, hydrogen impurities in the deuterium and in part to column bleed.

RESULTS AND DISCUSSION

In order for this technique to be viable, it was necessary to determine the relationship between the ratio of Δ^9-THC–OCH_3 to Δ^9-THC–OCD_3 injected on column and the ratio of the intensities of the corresponding ions, *i.e.*, 328 to 331. It was also necessary to show that the on-column methylation was quantitative. Figure 1 shows that there is a 1:1 relationship between the protium/deuterium ratio injected on column and the ratio of the intensities of the corresponding ions. This indicates that there is no isotope effect on the ionization of Δ^9-THC methyl ethers. Figure 1 also shows that Δ^9-THC is quantitatively methylated by the on-column methylating reagent.

The efficacy of the extraction procedure was determined by adding varying amounts of Δ^9-THC to plasma and per-

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forming the analytical procedure. The range investigated was 1–200 ng/ml of plasma. Recoveries were 65% with a relative standard deviation of 5.5%. The specificity of the method was shown by monitoring a gas chromatographic analysis of the lipid phenol fraction of plasma simultaneously using non-specific, total ion current detection (solid trace in Figure 2) and ion specific detection (Figure 3). The necessity for the specific ion detection was highlighted by the response of the total ion current detector to 23 ng of Δ^9-THC–OCD_3 (dashed trace in Figure 2) which is the amount of internal standard used in the analyses. It is apparent from Figure 2 that the signal due to 23 ng of Δ^9-THC–OCD_3 would be completely swamped using non-specific detection. Figure 3 shows clearly that the mass fragmentographic detection is very specific for both ions at an excellent signal to noise ratio. At present, a factor limiting the sensitivity of the assay is the background corresponding to 300 pg of Δ^9-THC–OCH_3 which arises from causes previously discussed.

The simplicity of the reported assay permitted the preliminary study of plasma levels of Δ^9-THC in human volunteers which established that the method was sufficiently sensitive and selective to allow for pharmacokinetic studies in humans. Subjects smoked one cigarette dosed to deliver 88 μg of Δ^9-THC per kg of body weight. Smoking was done in a rigidly controlled manner and the entire procedure lasted 6–6.5 minutes. Sessions were timed from the onset of smoking, and blood samples were drawn at various intervals.

The plasma was analyzed for Δ^9-THC and the concentration was calculated based on an extraction efficiency of 65%. The time course of the plasma levels in three subjects is shown in Figure 4. Plasma levels of Δ^9-THC for all subjects are similar to plasma levels reported by Agurell *et al.* (*11*), although two of the subjects from whom samples were drawn prior to 15 minutes after commencement of smoking show peak plasma levels earlier and of greater magnitude than levels previously reported (*11*).

It appears that the reported assay is feasible for and will be useful in further pharmacokinetic studies of Δ^9-THC in humans. Because of the reliance on the chemistry of the phenol group, it is possible that the assay can be adapted for the general class of cannabinoids, cannabinoid-like drugs, and the metabolites of these compounds which, while being oxidized to various alcohols (*15, 16*) and carboxylic acids (*20*), still maintain the phenolic function upon which this assay is based. Furthermore, since the on-column methylation technique was developed for use with a wide variety of drugs (*18*) (*e.g.*, barbiturates, xanthines, phenolic alkaloids), it is possible that this methodology may prove of general utility.

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New Soft X-Ray Method for Determining the Chemical Forms of Sulfur in Coal

Ronald G. Hurley[1] and Eugene W. White

Materials Research Laboratory, The Pennsylvania State University, University Park, Pa. 16802

Sulfur is ubiquitous to bituminous coals, varying in concentrations from a low of about 0.1% to as much as 10 or 11%. For most consumer markets, sulfur in coal is looked upon as an unwanted constituent. During combustion, it contributes to the formation of fireside boiler deposits that reduce boiler efficiency and lead to other corrosion problems. The steel industry is interested in low sulfur coking coals because of the deleterious effects of sulfur in the throughput of the blast furnaces and on the finished products (*1, 2*). Moreover, the growing concern over air pollution has recently aroused interest in gaseous sulfur products released during coal utilization and has led to studies designed to find ways of reducing the amounts of these products. This reduction depends upon the form or chemical state that the sulfur exists within the coal.

Sulfur occurs in three principal forms in coal: combined with organic substances, combined as sulfides of iron (pyrite or marcasite), and combined with calcium and iron as sulfates. These forms are commonly referred to as organic, pyritic, and sulfate sulfur.

Organically bonded sulfur is distributed throughout the coal, presumably occurring in all the macerals. This type of sulfur generally predominates in low sulfur coals. With increasing total sulfur, both pyritic and organic forms tend to increase although there is no direct relationship between these two forms (*3*).

Pyritic or sulfide sulfur, on the other hand, is present as the minerals pyrite and marcasite, and is distributed in coal in many ways. These minerals may occur as lenses, bands, or nodules, or as finely disseminated microscopic particles.

The percentage of sulfate sulfur in fresh coals generally is less than 0.05 wt % (*3*). Under moist conditions, sulfides may be oxidized to sulfates, and their increased concentra-

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