J. Pharm. Pharmac., 1973, **25**, 554–558 *Received February* 19, 1973

Quantitation of Δ^1-tetrahydrocannabinol in plasma from cannabis smokers

STIG AGURELL, BERTIL GUSTAFSSON, BO HOLMSTEDT*, KURT LEANDER**, JAN-ERIK LINDGREN*, INGER NILSSON, FINN SANDBERG AND MARIE ASBERG***

*Faculty of Pharmacy, Box 6804, S-113 86 Stockholm; * Department of Toxicology, Swedish Medical Research Council, Karolinska Institute, S-104 01 Stockholm 60; ** Department of Organic Chemistry, University of Stockholm, Sandåsgatan 2, S-113 27 Stockholm; *** Department of Psychiatry, Karolinska Hospital, S-104 01 Stockholm 60, Sweden.*

A method to identify and accurately measure non-labelled Δ^1-tetrahydrocannabinol (Δ^1-THC) in blood of cannabis smokers has been developed. It consists of the following steps: To a 5 ml plasma sample is added deuterated Δ^1-THC (Δ^1-THC-d_2) as internal standard. After extraction with light petroleum and evaporation, the Δ^1-THC containing fraction is separated by chromatography on Sephadex LH-20 (1 × 40 cm) using light petroleum–chloroform–ethanol (10:10:1) as eluant. A fraction containing Δ^1-THC is collected and subjected to mass fragmentography (LKB 9000; 3% OV-17/Gas-Chrom Q; 230°). The mass spectrometer was adjusted to record the intensities of *m/e* 299 and 314 of Δ^1-THC and *m/e* 301 and 316 of Δ^1-THC-d_2. The standard curve was made by plotting peak height *m/e* 299/*m/e* 301. Peak levels of 19–26 ng ml^{-1} were reached within 10 min after smoking a cigarette containing 10 mg Δ^1-THC.

A technique for the identification and quantitative determination of non-labelled Δ^1-tetrahydrocannabinol (Δ^1-THC) and/or its metabolites in blood, urine and other body fluids of persons taking cannabis has long been desired both for pharmacokinetic and forensic purposes.

Early reports claiming that identification of cannabis smokers could be effected by a simple t.l.c. analysis of a urine extract have not been verified (*cf.* Fish, 1972). However, recently it was shown (Andersen, Nielsen & others, 1971) that cannabinol was formed after *p*-toluenesulphonic acid treatment of a urinary extract from volunteers taking cannabis orally. Immunoassay procedures have been discussed (Grant, Gross & others, 1972) but so far no technique of sufficient sensitivity and selectivity has been published. Recently, it was proposed that the formation of dansyl derivatives followed by fluorimetry would be a possible means of detecting Δ^1-THC in body fluids (Just, Werner & Wiechmann, 1972).

Studies with [^{14}C] labelled Δ^1-THC in man (Galanter, Wyatt & others, 1972) indicate that Δ^1-THC levels of 25–50 ng ml^{-1} plasma may be reached during cannabis smoking. This would normally be within the limit of g.l.c. procedures, particularly those using EC-sensitive derivatives (Schou, Steentoft & others, 1971). The absence of such methods may be due to the difficulties in purifying Δ^1-THC sufficiently from naturally occurring lipophilic components in blood.

Mass fragmentography (Hammar, Holmstedt & Ryhage, 1968) has found an increasing use in the determination of small amounts of drugs and endogenous com-

pounds (*cf.* Borgå, Palmér & others, 1972). We now report a specific procedure that allows the quantitative determination of Δ^1-THC in blood samples down to at least 1 ng ml^{-1}.

MATERIALS AND METHODS

Three cannabis smokers, one woman and two men, volunteered for the study, which was approved both by the National Board of Health and Welfare and the ethical committee of the Karolinska Institute. The volunteers were casual cannabis users, and none had been exposed to the drug during the month preceding the experiment. Except for contraceptive steroids in the woman's case, none had received any medication for at least a year. They were physically healthy and a psychiatric examination showed no evidence of mental disturbances.

Each volunteer smoked a cigarette containing 10 mg of Δ^1-THC during 5 min. A blood sample was taken before smoking and 10, 30, 60 and 120 min after the smoking was completed. The heparinized blood samples were centrifuged immediately and plasma stored at $-20°$ in glass tubes until analysed.

Nanograde (Fisher Scientific Co.) redistilled light petroleum was used for extraction and chromatography. All other solvents employed were of reagent grade and redistilled. All glass ware was thoroughly cleaned with chromic acid, glass redistilled water and ethanol before use.

Synthesis of deuterated Δ^1-THC

A mixture of 1-(3,5-dimethoxyphenyl)-1-pentanone (2·22 g) (Bäckström & Sundström, 1970) and sodium borodeuteride (0·42 g) in tetrahydrofuran (25 ml) was refluxed for 26 h. The resulting mono-deuterated alcohol (2·20 g) was dissolved in tetradeuteriomethanol and stirred over Pd/C (20%, 50 mg) in an atmosphere of deuterium. The reduction was accelerated by the addition of a small amount of deuterium chloride in deuterium oxide. After 3 h, one molar equivalent of deuterium had been consumed. The catalyst was filtered off and the solvent evaporated. The crude dimethylolivetol and hydroiodic acid (57%, 25 ml) was stirred for 3 h at 110° under nitrogen. The mixture was poured into ice water and extracted with chloroform. The extract was subjected to preparative thin-layer chromatography (Silica gel G; chloroform–methanol; 19:1) giving pure olivetol (1·3 g), which was reacted with (+) *trans-p*-menthadien-(2,8)-ol-(1). The resulting (−)-$\Delta^{1(6)}$-THC was then isomerized (Petrzilka, Haefliger & Siekemeier, 1969) to (−)-Δ^1-THC, which was purified by preparative thin-layer chromatography (Silica gel G; ether–light petroleum; 1:9). The plates were developed three times.

Both non-labelled and deuterium labelled Δ^1-THC were carefully dried before use and stored dissolved in ethanol (1–2 mg ml^{-1}) at 4° in the dark. The concentration of Δ^1-THC in the solutions was checked periodically by g.l.c. using triphenyl carbinol as internal standard (Agurell & Leander, 1971).

Procedure

To a sample of human plasma (5·0 ml) was added deuterated Δ^1-THC (1000 ng) (Δ^1-THC-d_2) as internal standard. The plasma was extracted (Super-Mixer, Lab Line Instruments, Melrose Park, USA) four times with an equal amount of light petroleum containing 1·5% isoamyl alcohol in a glass stoppered centrifuge tube.

After centrifugation for 10 min at 1500 *g* the light petroleum layers were drawn off and evaporated to dryness under a stream of nitrogen. The residue was chromatographed on a Sephadex LH-20 column (1 × 40 cm—void volume 15 ml) using light petroleum–chloroform–ethanol (10:10:1) as eluant (0·15–0·18 ml min^{-1}). The fraction corresponding to the elution volume of Δ^1-THC (28–34 ml) was collected and evaporated to dryness under nitrogen. The residue was transferred to a conic vial (0·3 ml) and dissolved in absolute ethanol (25–50 μl).

This solution was subjected to mass fragmentography (3% OV-17/Gas-Chrom Q 100–120 mesh, 230°) using a flexible gas chromatography—mass spectrometry-laboratory computer system for on line data collection and processing. A program to sample simultaneously four different masses and record the resulting data is part of this system (Elkin, Pierrou & others, unpublished). In this case the mass spectrometer was adjusted to record (Fig. 1) the intensities of *m/e* 299 and 314 of Δ^1-THC and *m/e* 301 and 316 of Δ^1-THC-d_2.

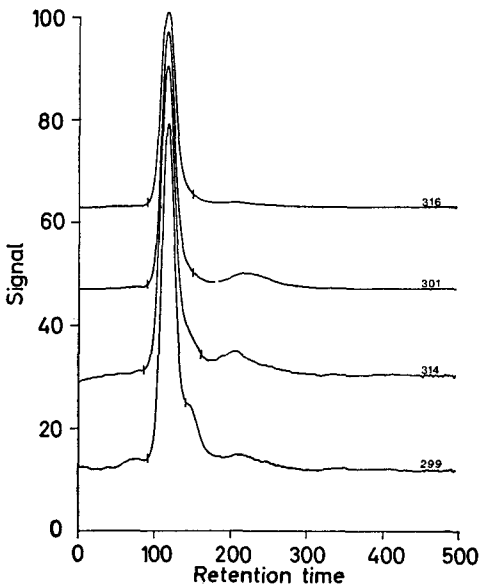

FIG. 1. Mass fragmentogram of internal standard (*m/e* 301, 316) and Δ^1-THC (*m/e* 299, 314) in purified extract of a plasma sample taken 10 min after smoking 10 mg Δ^1-THC. Plasma level of Δ^1-THC 26 ng ml^{-1}; retention time of Δ^1-THC 2·9 min.

A standard curve was prepared by adding known amounts (0, 10, 20, etc. ng ml^{-1}) of Δ^1-THC to blank plasma samples and carrying out the described procedure. The standard curve was made by plotting: peak height Δ^1-THC (*m/e* 299)/peak height Δ^1-THC-d_2 (*m*/e 301) against known amounts of added Δ^1-THC in ng ml^{-1} of plasma.

RESULTS AND DISCUSSION

Mass fragmentography was previously tested by Agurell (1970) as a possible means of measuring levels of Δ^1-THC in plasma of cannabis smokers. The technique of mass fragmentography was also used by Mikes, Hofmann & Waser (1971) to identify cannabinoid compounds after the administration of high doses (50–135 mg kg^{-1}) of Δ^1-THC to rats.

With metabolic work we have used Sephadex LH-20 columns for the purification of cannabinoids (*cf.* Paton & Crown, 1972). Separation on Sephadex LH-20 (Änggård & Bergkvist, 1970) combined with mass fragmentography has proved to be a sensitive and specific assay for Δ^1-THC in blood of cannabis smokers. The Sephadex LH-20 column procedure is reproducible and in separate experiments [^{3}H] Δ^1-THC added to samples of human plasma was recovered to $70 \pm 6\%$ ($n = 10$) using the described extraction and purification procedure.

The mixture of Δ^1-THC and Δ^1-THC-d_2 is analysed by mass fragmentography using the ratio: peak height *m/e* 299 (from Δ^1-THC) *vs* *m/e* 301 (from Δ^1-THC-d_2). The molecular peaks of non-labelled (*m/e* 314) and labelled (*m/e* 316) Δ^1-THC are also recorded (Fig. 1).

The specificity in determining Δ^1-THC is also ensured by the g.l.c. retention time and may, if necessary, be improved by the registration of further fragments in the mass spectrum of Δ^1-THC. In a limited number of control samples we have so far encountered no background interference at the retention time of Δ^1-THC when recording the selected four mass numbers.

As shown in Fig. 1 Δ^1-THC is readily identified in a plasma sample of a person who has smoked a cigarette containing 10 mg Δ^1-THC. Δ^1-THC, in radiolabelled form, has earlier been identified in human blood by t.l.c. (Galanter & others, 1972) and also from a large amount of plasma after oral administration of [^{3}H] Δ^1-THC, by gas chromatography-mass spectrometry (Wall, Brine & others, 1972). The latter authors, who tentatively identified an isomer of Δ^1-THC as a metabolite, stress the importance of an adequate identification of Δ^1-THC and question the use of t.l.c. in combination with radio-labelled material.

Fig. 2 shows the plasma levels of Δ^1-THC in three cannabis smokers who each smoked one cigarette containing 10 mg Δ^1-THC each. The peak levels (19–26 ng ml^{-1}) were apparently reached within 10 min followed by a rapid decline to 5 ng ml^{-1} or less within 2 h. The present plasma levels agree well with those found by Galanter & others (1972) after [^{14}C] Δ^1-THC had been smoked.

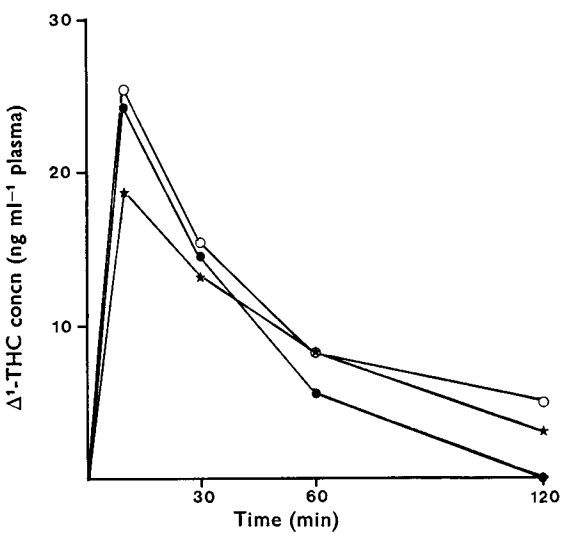

FIG. 2. Plasma levels of Δ^1-THC in three volunteers after each having smoked 10 mg of Δ^1-THC in a tobacco cigarette.

As far as we are aware this is the first method to identify and accurately measure non-labelled Δ^1-THC in the plasma of persons who have smoked cannabis. The accuracy in the low concentration range may be increased by decreasing the concentration of the internal standard.

The present method can be used for the correlation of plasma levels and pharmacological-psychological effects and for pharmacokinetic studies in man. This technique may also form the basis for the forensic identification of cannabis.

Acknowledgements

The support of the Swedish Medical Research Council (03X-2724) and Försvarsmedicinska Forskningsdelegationen is appreciated. The following grants to the department of toxicology are also acknowledged: the Swedish Medical Research Council (70E-3743 and 14X-4041), the Wallenberg Foundation, the Tri-Centennial Fund of the Bank of Sweden and the National Institute of Mental Health (MH 12007), and to M. Asberg from the Swedish Medical Research Council (14P-3647).

REFERENCES

AGURELL, S. (1970). In: *The Botany and Chemistry of Cannabis.* Editors: Joyce, C. R. B. and Curry, S. H., London: J. & A. Churchill Ltd.

AGURELL, S. & LEANDER, K. (1971). *Acta pharm. suecica*, **8**, 391–402.

ANDERSEN, J. M., NIELSEN, E., SCHOU, J., STEENTOFT, A. & WORM, K. (1971). *Acta pharmac. Tox.*, **29**, 111–112.

ÄNGGÅRD, E. & BERGKVIST, H. (1970). *J. Chromat.*, **48**, 542–544.

BÄCKSTRÖM, P. K. & SUNDSTRÖM, G. (1970). *Acta chem. scand.*, **24**, 716–717.

BORGÅ, O., PALMÉR, L., SJÖQVIST, F. & HOLMSTEDT, B. (1972). In: *Symposium on the basis of drug therapy in man.* Fifth International Pharmacology Congress, San Francisco. Vol. 3, C/9. Basel: S. Karger.

FISH, F. (1972). *Pharm. J.*, **209**, 343–347.

GALANTER, M., WYATT, R. J., LEMBERGER, L., WEINGARTNER, H., WAUGHAN, T. B. & ROTH, W. T. (1972). *Science* 5, **176**, 934–935.

GRANT, J. D., GROSS, S. J., LOMAX, P. & WONG, R. (1972). *Nature*, **236**, 217–218.

HAMMAR, C. G., HOLMSTEDT, B. & RYHAGE, R. (1968). *Analyt. Biochem.*, **25**, 532–548.

JUST, W. W., WERNER, G. & WIECHMANN, M. (1972). *Naturwiss.*, **59**, 222.

MIKES, F., HOFMANN, A. & WASER, P. G. (1971). *Biochem. Pharmac.*, **20**, 2469–2476.

PATON, W. D. M. & CROWN, J. (1972). *Cannabis and its derivatives.* London: Oxford University Press.

PETRZILKA, T., HAEFLIGER, W. & SIEKEMEIER, C. (1969). *Helv. chim. Acta*, **52**, 1102–1116.

SCHOU, J., STEENTOFT, A., WORM, K., ANDERSEN, J. M. & NIELSEN, E. (1971). *Acta Pharmac. Tox.*, **30**, 480–486.

WALL, M. E., BRINE, D. R., PITT, C. G. & PEREZ-REYES, M. (1972). *J. Am. chem. Soc.*, **94**, 8580–8581.