*Journal of Chromatography*, 206 (1981) 117–123
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,372

# DETERMINATION OF $\Delta^9$-TETRAHYDROCANNABINOL IN PHARMACEUTICAL VEHICLES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

K. P. FLORA* and J. C. CRADOCK

*Analytical and Product Development Section, Pharmaceutical Resources Branch, National Cancer Institute, Building 37, Room 6D12, NIH, Bethesda, MD 20205 (U.S.A.)*

and

J. P. DAVIGNON

*Pharmaceutical Resources Branch, National Cancer Institute, NIH, Bethesda, MD 20205 (U.S.A.)*

(Received September 25th, 1980)

## SUMMARY

A procedure for the determination of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) in the presence of its degradation products in pharmaceutical vehicles by high-performance liquid chromatography (HPLC) is described. The method compares favorably with a standard gas–liquid chromatographic procedure used for the analysis of $\Delta^9$-THC in sesame oil USP. The HPLC method is suitable for quantitating $\Delta^9$-THC in the presence of several pharmaceutical vehicles and excipients including: sesame oil USP, polyvinylpyrrolidone, Emulphor EL620 and Cremophor EL. Extractions are not required and samples require little preparation. Only the addition of an internal standard in an appropriate solvent is necessary before injection. The procedure has been applied to stability studies of $\Delta^9$-THC in various pharmaceutical vehicles.

## INTRODUCTION

The preparation of dosage forms of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) suitable for human use presents interesting and challenging problems. The water solubility of $\Delta^9$-THC has been determined by Garrett and Hunt[1] to be 2.8 $\mu$g/ml. This poor solubility coupled with its viscous tar-like nature make routine handling and solubilization difficult. Additionally, $\Delta^9$-THC decomposes more readily in the presence of light or oxygen[2,3]. Much work has been directed toward the formulation of products of $\Delta^9$-THC that are useful in evaluating the pharmacological properties of the drug. However, more attention needs to be directed to the optimization of formulations suitable for routine therapeutic use in humans. Specifically of interest are products suitable for use in antiemetic therapy in combination with antitumor agents. The usefulness of $\Delta^9$-THC as an antiemetic was first reported by Sallan *et al.*[4]

in 1975. Since then the activity of $\Delta^9$-THC has been confirmed in several clinical trials *versus* chemotherapy induced emesis[5–8]. These trials were conducted with $\Delta^9$-THC products distributed by the National Institute on Drug Abuse, specifically a standardized marihuana cigarette and a soft gelatin capsule containing $\Delta^9$-THC in sesame oil. Some problems were encountered with the use of these dosage forms. There is reluctance in some older patients towards the use of marihuana cigarettes due to the associated social and legal implications. Also, the effectiveness of the cigarette as a dosage form depends heavily on the smoking process itself. Patients must be carefully trained in proper smoking techniques for administration to be successful. At best, only a fraction of the actual amount of $\Delta^9$-THC available is introduced to the lungs for absorption and the amounts of drug absorbed may be less predictable than desired[9]. Also, the use of the cigarette as a dosage form inherently involves the administration of various other marihuana components and combustion products which may present problems of their own. Likewise, the absorption of $\Delta^9$-THC from the gastrointestinal tract has been unpredictable[5–8]. Chang *et al.*[5] reported blood levels of $\Delta^9$-THC which suggested variable absorption of the drug after oral administration. Likewise, Frytak *et al.*[6] observed similar variations in plasma levels of $\Delta^9$-THC. These problems might be avoided by the use of alternate dosage forms.

Due to some of these drug delivery problems, formulation studies were begun to develop new dosage forms of $\Delta^9$-THC suitable for intravenous, intramuscular or oral administration to humans. Numerous methods for the quantitation of $\Delta^9$-THC in a variety of biological media and botanical products have been reported. Many have been summarized in monographs or reviews[10–14]. A gas–liquid chromatographic (GLC) method employing multiple extractions has been used for analysis of delta-9-THC in capsules containing the drug in sesame oil[15]. Our goal was to develop a reversed-phase high-performance liquid chromatographic (HPLC) method that would be generally applicable to the analysis of $\Delta^9$-THC in the presence of its decomposition products in a variety of pharmaceutical vehicles. A HPLC method was desirable to avoid laborious extractive work-up of samples. This non-destructive technique would also permit the collection and subsequent identification of degradation products. This report describes the development of such a method and its utility for the analysis of $\Delta^9$-THC in several potential pharmaceutical vehicles.

## EXPERIMENTAL

### *HPLC apparatus and conditions*

*Method A.* A modular high-performance liquid chromatograph (Model 3500B, Spectra-Physics, Santa Clara, CA, U.S.A.), including a reciprocating piston pump with flow feedback control, delivered mobile phase at a constant rate (1 ml/min) to a stainless-steel column (250 × 4.6 mm I.D.) packed with 10-$\mu$m particles of silica gel chemically bonded to a $C_8$ hydrocarbon phase (Altex LiChrosorb RP-8, 10 $\mu$m, Altex Scientific, Berkeley, CA, U.S.A.). A fixed-wavelength (280 nm) ultraviolet detector (Model 8200, Spectra-Physics) with a sensitivity setting of 0.08 a.u.f.s. detected the eluted compounds. The detector output signal was recorded with a strip chart recorder equipped with variable chart speed controls (Model A5211-1, Omniscribe, Houston Instruments, Austin, TX, U.S.A.). Samples were introduced to the column

with a manual injection valve equipped with a 10-$\mu$l sample loop (Model CV-6-UHP-a-N60, Valco Instrument Company, Houston, TX, U.S.A.).

The mobile phase consisted of methanol–distilled water (78:22). The column pressure was about 630 p.s.i. at a flow-rate of 1 ml/min. All separations were affected isocratically at ambient temperature.

*Method B.* This procedure is identical to Method A with the following exceptions: mobile phase consisted of methanol–distilled water (73:27). The column packing material consisted of a dimethylsilane phase chemically bonded to silica gel (Altex LiChrosorb RP-2, 10 $\mu$m).

Quantitation was performed using an internal standard method. Standard curves constructed from the ratio of peak heights of $\Delta^9$-THC to the internal standard tetraphenylethylene (I.S.) *versus* concentration were linear ($r > 0.99$).

*Reagents*

$\Delta^9$-THC was supplied by the National Institute on Drug Abuse (Rockville, MD, U.S.A.). Tetraphenylethylene (Aldrich, Milwaukee, WI, U.S.A.) was used as received. Methanol, HPLC grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and distilled water were filtered through 0.5-$\mu$m and 0.8-$\mu$m solvent-resistant filters, respectively (Millipore, Bedford, MA, U.S.A.). Reference samples of $\Delta^8$-THC, cannabinol and cannabidiol were used as received (Supelco, Bellefonte, PA, U.S.A.). Solutions of ethanol–Emulphor EL620 (1:1) and of ethanol–Cremophor EL (1:1) were supplied by the Division of Cancer Treatment, National Cancer Institute (National Institutes of Health, Bethesda, MD, U.S.A.). All other chemicals were reagent grade and were used as received.

Most $\Delta^9$-THC-containing samples were diluted or reconstituted with absolute ethanol to give a drug concentration of 5 or 10 mg/ml. An aliquot of this solution (100 $\mu$l) was diluted with 600 $\mu$l of a solution of tetraphenylethylene in ethanol (150 $\mu$g/ml). This solution (10 $\mu$l) was injected directly for HPLC analysis. $\Delta^9$-THC in seame oil USP was quantitated by weighing individual samples (25–40 mg) of the drug in sesame oil (3% $\Delta^9$-THC, w/w) and then dissolving the sample in 600 $\mu$l of *n*-butanol containing tetraphenylethylene (128.5 $\mu$g/ml). The resulting solution was injected directly for HPLC analysis. Samples for GLC analysis were prepared in ethanol to contain about 0.3 mg/ml of $\Delta^9$-THC and 0.45 mg/ml androst-4-ene-3,17-dione (internal standard) according to a modification of a standard method[13,15].

*Preparation of experimental formulations*

Several formulations were investigated. The following vehicle and packaging variations were prepared and the contents subjected to HPLC analysis:

(A) An evacuated vial containing 10 mg $\Delta^9$-THC prepared by a low-temperature vacuum drying procedure[16].

(B) A solution containing 10 mg/ml $\Delta^9$-THC in ethanol–Emulphor EL620–sodium chloride injection USP (5:5:90) was prepared as described previously[17]. Aliquots (0.5 ml) of this solution were dispensed and sealed under room air in clear glass ampules.

(C) A solution containing 10 mg/ml $\Delta^9$-THC in ethanol–Cremophor EL–sodium chloride injection USP (5:5:90) was prepared and dispensed in a manner similar to formulation B.

(D) A solution containing 100 mg/ml $\Delta^9$-THC in ethanol–Emulphor EL620 (1:1) sealed under nitrogen in a glass ampule.

(E) A solution containing 100 mg/ml $\Delta^9$-THC in ethanol–Cremophor EL (1:1) sealed under nitrogen in a glass ampule.

(F) Adsorbates with polyvinylpyrrolidone containing 3.5% (w/w) $\Delta^9$-THC.

(G) A solution of 3% (w/w) of $\Delta^9$-THC in sesame oil USP.

## RESULTS AND DISCUSSION

### *Chromatography*

$\Delta^9$-THC and several related cannabinoids: $\Delta^8$-THC, cannabinol and cannabidiol could be resolved by either HPLC method (Fig. 1). Preliminary studies of $\Delta^9$-THC decomposition in formulation B under accelerated conditions of heat and light in the presence of air indicated cannabinol, cannabidiol and several relatively polar products were formed. Little or no $\Delta^8$-THC was detected. The elution of the various cannabinoids was more prolonged from the $C_8$ column as compared to the $C_2$ column with equivalent mobile phases. Increasing the organic component in either system resulted in faster elution of all components; however, $\Delta^9$-THC and $\Delta^8$-THC could no longer be resolved. Mobile phases containing less organic component required more time for elution of the components with subsequent peak broadening and tailing. Ultraviolet detection at 280 nm was satisfactory as both $\Delta^9$-THC and $\Delta^8$-THC have absorbance maxima in that region ($\Delta^9$-THC, $UV_{max.}$ in ethanol: 282 and 278 nm ($\varepsilon$, 2075 and 2040 $l \cdot mol^{-1} \cdot cm^{-1}$; $\Delta^8$-THC, $UV_{max.}$ in ethanol: 283, 276

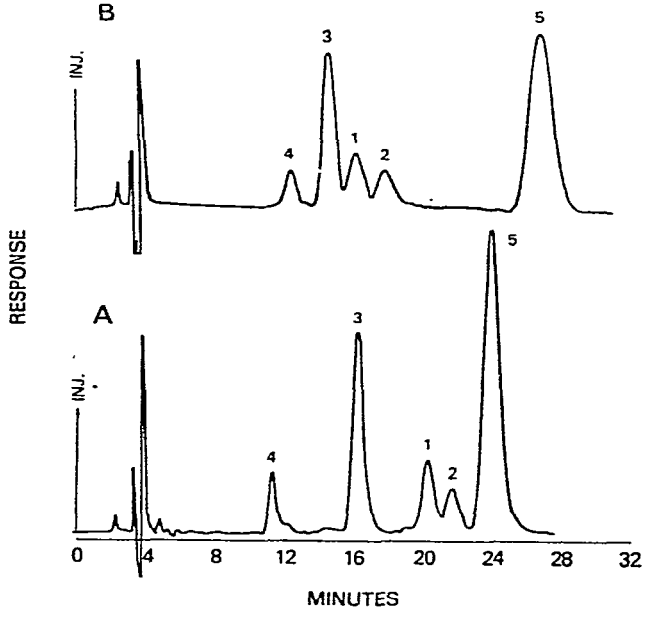


Fig. 1. HPLC chromatograms of a mixture of $\Delta^9$-THC (1), $\Delta^8$-THC (2), cannabinol (3), cannabidiol (4) and the internal standard tetraphenylethylene (5) using Method B and Method A.

and 209 nm ($\varepsilon$, 1390, 1330 and 41,000 $l \cdot mol^{-1} \cdot cm^{-1}$; ref. 18). Cannabinol, the aromatic degradation product of $\triangle^9$-THC, has a much greater molar absorptivity than $\triangle^9$-THC or $\triangle^8$-THC ($UV_{max.}$ in ethanol: 286 nm ($\varepsilon$, 18,300 $l \cdot mol^{-1} \cdot cm^{-1}$; ref. 19) and consequently displays peaks which are disproportionately large for the amount present.

Method A was chosen for stability studies because of the somewhat better resolution of the cannabinol and $\Delta^9$-THC peaks. This was important because cannabinol is produced from the decomposition of $\Delta^9$-THC and possesses a strong chromophore. The resolution of $\Delta^9$-THC and $\Delta^8$-THC was of lesser concern because $\Delta^8$-THC was not produced in significant amounts in preliminary studies and contains a weaker chromophore than that of $\Delta^9$-THC.

The HPLC system (Method A) was compared to a standard GLC method which has been used for the analytical evaluation of the $\Delta^9$-THC sesame oil capsules produced for the National Institute on Drug Abuse[13,15]. Duplicate shelf life samples of formulation A were subjected to HPLC and GLC analysis. Results of these comparative analyses are presented in Table I. These data show reasonable agreement for the two methods for samples stored at 25° or 50°C.

TABLE I

COMPARISON OF GLC AND HPLC METHODS ON ANALYSIS OF LOW-TEMPERATURE VACUUM DRIED $\Delta^9$-THC (FORMULATION A)

| *Time (weeks)* | *Percent of initial assay* | | | |
|---|---|---|---|---|
| | *50°C* | | *25°C* | |
| | *HPLC* | *GLC* | *HPLC* | *GLC* |
| 4 | 100.0 | 101.4 | 101.1 | 100.7 |
| 8 | 92.0 | 93.5 | 100.0 | 102.2 |
| 12 | 82.1 | 80.4 | 98.0 | 97.8 |
| 20 | 76.2 | 74.6 | — | — |

$\Delta^9$-THC in sesame oil (formulation G) was also determined by HPLC and GLC. The GLC procedure required multiple extraction (six times) and an evaporation step[15]. Six individual samples of formulation G were weighed (250–350 mg for GLC analysis and 25–40 mg for HPLC analysis) for analysis by each method. The results were in agreement. The precision, as measured by percent relative standard deviation, of the HPLC method was somewhat better (2.25% as compared to 3.05% for the GLC method.)

The analysis of other formulations some of which contain various surfactants and oils can also be accomplished directly with this HPLC method. Direct injection offers distinct advantages over the GLC method which would require multiple extractions in most instances. Emulphor EL620 and Cremophor EL do not exhibit UV absorbance at 280 nm and are thus not detected during HPLC analysis. However, detection at 220 nm indicated two large peaks eluted after the injection of samples containing either Emulphor EL620 (retention volume, $V_R \approx$ 27–37 ml) or Cremophor EL ($V_R \approx$ 28–35 ml). Several peaks in sesame oil USP are eluted and detected

at 280 nm with retention volumes ranging from 3.8 to 8.1 ml. Polyvinylpyrrolidone produced no peaks that were detectable at 280 nm. At 220 nm, a large tailing peak was observed between $V_R$ 1.8 and 12.0 ml. Fortunately none of the peaks eluted from the various vehicles interfered with the $\Delta^9$-THC peak or the internal standard peak at 280 nm. Repeated injections of any of the formulations described did not affect resolution, retention volumes or overall column performance. As a precaution, however, the column was routinely flushed with methanol for 1–2 h after each 2–3 days of analysis.

This HPLC method was suitable for stability studies of $\Delta^9$-THC in any of the vehicles described. Typical stability data in several vehicles are seen in Table II. Formulations B and C demonstrate the more rapid decomposition of $\Delta^9$-THC in the presence of air and water. Other vehicles with the product stored an inert nitrogen atmosphere (formulations D and E) are considerably more stable. Day-to-day variation based on the analysis of a standard solution of $\Delta^9$-THC in ethanol over a period of 6 months resulted in a relative standard deviation of 4.40%.

TABLE II

STABILITY OF $\Delta^9$-THC IN VARIOUS VEHICLES AT 25°C

| *Time* | *Percent of initial assay* | | | |
|---|---|---|---|---|
| | *Formulation B* | *Formulation C* | *Formulation D* | *Formulation E* |
| 0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 2 Days | 86.0 | 91.6 | — | — |
| 7 Days | 82.9 | 86.5 | — | — |
| 10 Days | 81.1 | 84.2 | — | — |
| 6 Weeks | — | — | 96.2 | 98.1 |
| 14 Weeks | — | — | 97.0 | 96.0 |
| 20 Weeks | — | — | 96.2 | 97.5 |

HPLC has proven to be a useful method for formulation and stability studies with $\Delta^9$-THC. The procedure compares favorably to a standard GLC method used in other formulation related analysis of $\Delta^9$-THC. The method is simple and requires no extraction prior to analysis in the vehicles investigated.

ACKNOWLEDGEMENTS

The authors thank Mr. Larry M. Kleinman of the Pharmaceutical Resources Branch, National Cancer Institute, for helpful discussions and Mrs. Darlene Trageser for assistance in the preparation of this manuscript.

REFERENCES

1 E. R. Garrett and C. A. Hunt, *J. Pharm. Sci.*, 63 (1974) 1056.
2 J. W. Faubairn, J. A. Liebmann and M. G. Rowan, *J. Pharm. Pharmacol.*, 28 (1976) 1.
3 R. K. Razdan, A. J. Puttick, B. A. Zitko and G. R. Handrick, *Experientia*, 28 (1972) 121.
4 S. E. Sallan, N. E. Zinberg and E. Frei, *N. Engl. J. Med.*, 293 (1975) 795.
5 A. E. Chang, D. J. Shiling, R. C. Stillman, N. H. Goldberg, C. I. Seipp, I. Barofsky, R. M. Simon and S. A. Rosenberg, *Ann. Intern. Med.*, 91 (1979) 819.

6 S. Frytak, C. G. Moertel, J. R. O'Fallon, J. Rubin, E. T. Creagan, M. J. O'Connell, A. J. Schutt and N. W. Schwartau, *Ann. Intern. Med.*, 91 (1979) 825.
7 S. E. Sallan, C. C. Cronin, M. Zelen and N. Zinberg, *N. Engl. J. Med.*, 302 (1980) 135.
8 V. S. Lucas and J. Laszlo, *J. Amer. Med. Ass.*, 243 (1980) 1241.
9 E. B. Truitt, *Pharmacol. Rev.*, 23 (1971) 273.
10 R. E. Willette (Editor), *Cannabinoid Assays in Humans*, National Institute on Drug Abuse Research Monograph Series No. 7, National Institute on Drug Abuse, Rockville, MD, 1976, pp. 1–120.
11 J. A. Vinson (Editor), *Cannabinoid Analysis in Physiological Fluids*, American Chemical Society Symposium Series No. 98, American Chemical Society, Washington, DC, 1979, pp. 1–242.
12 J. M. Parker and B. L. Stembal, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 888.
13 K. H. Davis, N. H. Martin, C. G. Pitt, J. W. Wildes and M. E. Wall, *Lloydia*, 33 (1970) 453.
14 E. E. Knaus, R. T. Contts and C. W. Kazakoff, *J. Chromatogr. Sci.*, 14 (1976) 525.
15 K. H. Davis, Research Triangle Institute, Research Triangle Park, NC, personal communication, 1980.
16 D. W. Flamberg, D. L. Francis, S. L. Morgan and G. F. Wickes, *Bull. Parenteral Drug Assoc.*, 24 (1970) 209.
17 J. C. Cradock, J. P. Davignon, C. L. Litterst and A. M. Guarino, *J. Pharm. Pharmacol.*, 25 (1972) 345.
18 *Merck Index*, 8th ed., Merck and Co., Inc., Rahway, NJ, 1973, p.1206.
19 K. H. Davis and H. Setzman, Research Triangle Institute, Research Triangle Park, NC, personal communication, 1980.