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Note

Rapid high-performance liquid chromatographic assay with pharmacokinetic applications for monitoring cannabidiol in plasma

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Cannabidiol (CBD) is one of the major neutral non-psychoactive cannabinoids produced by *Cannabis sativa* L. [1]. It was first isolated in 1940, but its structure was elucidated only 23 years later [2]. The availability of CBD in its pure form makes possible the study of its pharmacological properties. It was thus shown that CBD possesses anticonvulsant and antiepileptic activity [3, 4]. Recent studies have shown that CBD has a high protective index comparable with that of phenobarbital and phenytoin [4–6]. In spite of the fact that CBD is a main constituent of cannabis preparation, and the increasing interest in its medical application, few reports have been published concerning its pharmacokinetics [7, 8].

During the past decade various methods for the determination of CBD have been described. These methods include gas chromatography (GC) with electron-capture detection (ECD) [9], GC with mass spectrometry (GC–MS) [7, 8], radioimmunoassay [10, 11], high-performance liquid chromatography (HPLC) using a fluorescent dansyl derivative of CBD [12] and colorimetric analysis by HPLC [13].

The GC–ECD assay has a low recovery (55%) and requires derivatization with pentafluorobenzylbromide. The GC–MS assay requires expensive equipment and some expertise in the synthesis and purification of a suitable deuterated internal standard in order to achieve radiolabeled quantitation. Radioimmunoassay for CBD has the disadvantage of misidentification and misquantitation because of cross-reactivity with other cannabinoid compounds and metabolites. The HPLC assay based on the chromatography and detection of a fluorescent dansyl derivative of CBD might be inaccurate because at least two different derivatives of

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CBD are formed, as indicated by two distinct peaks on the chromatogram.

The aim of this work was to develop a sensitive and selective assay for the routine quantitative determination of CBD in plasma. The assay is essential to pharmacokinetic and stability studies of CBD. Its in vivo application is illustrated by a pharmacokinetic experiment with dog plasma.

EXPERIMENTAL

Reagents and standards

CBD was synthesized in our laboratory. An experimental stock solution of CBD was prepared by dissolving the pure substance in methanol. Tetrahydrocannabinol (THC) was used as an internal standard (I.S), and the pure substance was dissolved in methanol at a concentration of 30 μg/ml. All solutions were stored at 4°C. Methanol and acetonitrile were obtained from Merck (Darmstadt, F.R.G.) and octadecyl (C_{18}) HPLC sorbent from Sigma (St. Louis, MO, U.S.A.).

Apparatus and conditions

An HPLC Tracor Model 970A (Austin, TX, U.S.A.) with a variable-wavelength UV detector equipped with a Linear 1200 recorder (Linear Instrument, Irvine, CA, U.S.A.) was used. The HPLC column was RP-18 (Alltech., Deerfield, IL, U.S.A.; 5 μm, 25×0.46 cm I.D.) with a pre-column CO:Pell ODS 30–38 μm (Whatman, Clifton, NJ, U.S.A.; 4.2 ×0.3 cm I.D.). The mobile phase was acetonitrile–methanol–doubly distilled water (7:1:2), and the flow-rate was 2 ml/min. The detection was made at 220 nm.

Extraction and clean-up

Extraction of plasma. To 0.5 ml of plasma spiked with an appropriate aliquot of CBD (or taken from the dog), were added 25 μl of I.S. solution and 2 ml of acetonitrile. The sample was vortexed for 120 s and centrifuged at 2000 *g* for 5 min. The supernatant was transferred to another tube, and concentrated to dryness by vacuum evaporation.

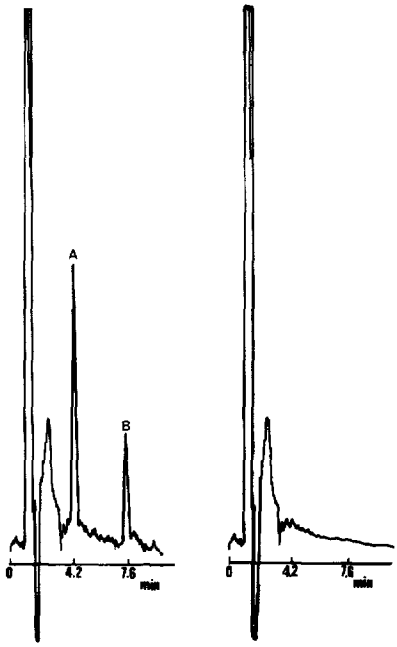
Clean-up procedure. The C_{18} (octadecyl) column was prepared as follows: a small ball of glass-wool was tamped at the bottom of the column and 0.4 g of (C_{18}) HPLC sorbent (40 μm) was added, followed by another ball of glass-wool. The column was rinsed in sequence with 2 ml of acetonitrile and 2 ml of water. Another 2 ml of water were used to wash the plasma extract from the tube, and transferred to the column. After the volumes that accumulated in the column had passed through the C_{18} sorbent, another 2 ml of acetonitrile–water (1:1) were used to wash the extract. The CBD was then eluted with 3 ml of acetonitrile into an 8-ml test-tube. The eluate was concentrated to dryness by vacuum evaporation. The dry residue was dissolved in 200 μl of methanol, and 100 μl were injected into the HPLC instrument. In order to determine the precision of the assay, 5 ml of plasma were spiked with appropriate aliquots of the stock solution of CBD and were stored at −20°C for one month during the precision study. On different days, 0.5-ml samples were taken from the various stored samples and analysed

with a fresh calibration curve constructed according to the extraction procedure on the same day.

RESULTS AND DISCUSSION

Typical chromatograms of dog plasma extracts are presented in Fig. 1. Under the assay conditions used, the following retention times were obtained: CBD, 4.2 min; THC 7.6 min. The k' values for CBD and THC were 5 and 6.4, respectively. There was no interference from endogenous plasma compounds. Calibration graphs from the plasma extract showed a linear correlation between the peak-height ratio of CBD to its respective I.S. (y) and plasma concentration of the substance (x). The linear regression equation was: $y=9.19\cdot10^{-3}x+0.05$ ($r=0.999$). The minimal detectable concentration for CBD was 25 ng/ml.

The recovery of CBD was determined as follows. Various amounts of CBD were dissolved in 0.5 ml of drug-free plasma, and the samples were analysed according to the extraction and clean-up procedures described above. In these instances, the I.S. solutions were added after the clean-up was terminated. A series of exter-



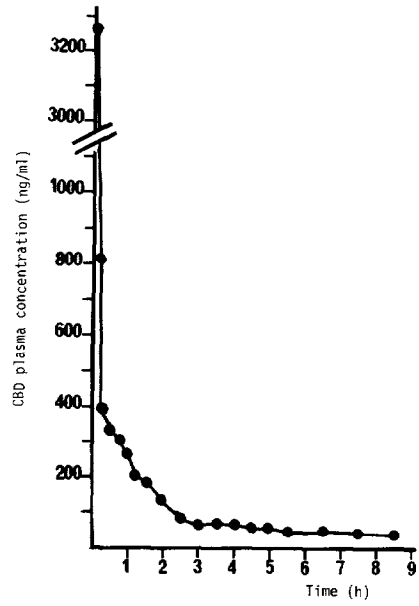


Fig. 1. Chromatograms of CBD (taken from a dog before and during the pharmacokinetic study presented in Fig. 2). (Right) Dog plasma blank; (left) CBD (A) 300 ng/ml and THC (B) 1.5 g/ml as I.S.

Fig. 2. Plasma levels of CBD obtained after i.v. administration of CBD (45 mg) to a dog.

TABLE I

RECOVERY AND REPRODUCIBILITY OF CBD IN DOG PLASMA

Concentration added (ng/ml)	Recovery (mean ± S.D., $n=7$) (%)	Coefficient of variation (%)
25	100.5 ± 13.6	13.5
50	99.2 ± 7.2	7.2
200	103.1 ± 6.1	5.9
Mean	100.9 ± 2.0	2.0

TABLE II

PRECISION OF THE ASSAY FOR CBD IN DOG PLASMA

Concentration added (ng/ml)	Concentration found (mean ± S.D., $n=8$) (ng/ml)	Coefficient of variation (%)
25	24.8 ± 3.8	7.9
50	48.0 ± 3.8	7.9
200	202.5 ± 8.6	4.2

nal standards was prepared by adding the same amounts of I.S. solutions to the various amounts of CBD, taken from the methanolic stock solutions. Analytical recoveries were calculated by comparing peak-height ratios of the extracted standard to the external standards (Table I). The standard deviations (S.D.) from the recovery studies serve as a good estimate of the reproducibility. The mean (± S.D.) recovery of CBD was 100.9 ± 2.0% (Table I). This complete recovery is higher than any from the previously reported GC and HPLC assays of CBD.

The precision of the assay was determined by performing eight replicate analyses of seven control samples containing 25, 50, 100, 200, 300, 400 and 500 ng/ml CBD on different days during a one-month period. The results of three are shown in Table II. The observed values of the various concentrations were not statistically different from the added concentration ($p > 0.05$).

A biomedical application of the HPLC method is illustrated by the following preliminary pharmacokinetic study. In this study, CBD (45 mg) was administered intravenously (i.v.) to a dog (mongrel, 20 kg). The plasma levels of CBD are shown in Fig. 2. After the i.v. administration, a rapid distribution of CBD occurred followed by a prolonged elimination with a terminal half-life of 5.8 h. Although CBD is usually administered orally or by smoking, the i.v. administration is fundamental and essential for pharmacokinetic studies of CBD after any mode of administration.

The proposed method is a very rapid, convenient and selective procedure for the routine assay of CBD in plasma. This assay should be very useful in pharmacokinetic studies of CBD and in clinics after the use of CBD as an antiepileptic agent. Using this assay, the pharmackinetic profile of CBD after i.v. administration of CBD to a dog was measured for the first time.

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