Journal of Chromatography, 226 (1981) 99—105
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 977

DETERMINATION OF CANNABIDIOL IN PLASMA BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

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(First received February 19th, 1981; revised manuscript received May 21st, 1981)

SUMMARY

A procedure was developed for the analysis of cannabidiol (CBD) in blood plasma. Tetrahydrocannabidiol was used as an internal standard and was added prior to extraction. The plasma extracts were derivatized with pentafluorobenzyl bromide and the product purified on a mini-column of Florisil. The pentafluorobenzyl derivatives were then analyzed by gas chromatography on a 5% OV-225 column using an electron-capture detector. A detection limit of 50 ng CBD per ml of plasma was observed. The procedure was used to study the plasma level of CBD after its oral and intravenous administration to monkeys.

INTRODUCTION

Accurate methods for the quantitative determination of cannabinoids in biological samples have been in demand for the past several years. (—)-*trans*-Δ^9-Tetrahydrocannabinol (Δ^9-THC) has received the most attention in this area since it was originally reported to be the major component of most *Cannabis* samples and has psychotomimetic activity. The methodologies employed for the quantitative detection of Δ^9-THC in biological samples include derivatization followed by single-column gas chromatography (GC) using an alkaline flame ionization detector [1,2], derivatization followed by dual-column GC using an electron-capture detector [3], derivatization followed by high-performance liquid chromatographic separation and GC detection with an elec-

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tron-capture detector [4], gas chromatography—mass spectrometry (GC—MS) methods [5,6], radioactive tracer methods [7—9] and radioimmunoassay methods [10,11].

Cannabidiol (CBD) is the most abundant cannabinoid in hashish and in the fiber type *Cannabis* [12]. In addition, CBD has interesting biological activities. One of its more important actions is the anticonvulsant activity seen in laboratory animals which suggests that CBD may be a useful antiepileptic agent in humans [13—15]. Studies are currently underway to establish the activity of CBD in humans [16]. In spite of these developments, there are no reports in the literature for the analysis of CBD in blood other than tracer methods [17—19]. Thus a simple analytical procedure is found necessary for determining CBD levels in biological fluids. Reported here are the procedures developed for the quantitative analysis of plasma samples for CBD utilizing the selectivity of electron-capture detectors by forming the dipentafluorobenzyl derivative of CBD.

EXPERIMENTAL

Reagents

All solvents were either chromatographic quality or redistilled in glass. Pentafluorobenzyl bromide (Pierce, Rockford, IL, U.S.A.) was used without further purification. Other reagents were analytical grade. Cannabidiol (CBD) was obtained from the National Institute on Drug Abuse (NIDA). Tetrahydrocannabidiol (THCBD) was prepared from CBD by hydrogenation over 10% palladium—charcoal catalyst and purified by column chromatography on Florisil (100—200 mesh).

Glassware

All glassware utilized in the recovery processes was previously silanized by soaking in a 10% solution of dichlorodimethylsilane in toluene for 4 h at room temperature.

Gas chromatograph

The analyses were performed on a Varian 1400 gas chromatograph with a ^{63}Ni electron-capture detector. A 1.8 m × 2 mm I.D. glass column packed with 5% OV-225 on Chromosorb W (100—120 mesh) was used at 230°C with nitrogen flow-rate of approximately 50 ml/min.

Preparation of CBD and THCBD derivatives for calibration

Ethanol solutions of both CBD and THCBD were prepared at concentrations of 2.0 μg/ml and stored at 5°C. Appropriate aliquots of the CBD solution were withdrawn to obtain 0.5, 1.0, 2.0, and 4.0 μg CBD and placed into separate 5-ml round-bottom flasks. To each flask were added 2.0 μg of THCBD (1.0 ml of the THCBD solution). The ethanol was evaporated under nitrogen at 60°C. Approximately 20 mg of anhydrous potassium carbonate were added to each flask, followed by the addition of approximately 3 ml of dry acetone (dried over anhydrous calcium chloride immediately prior to use). To this mixture, 50 μl of pentafluorobenzyl bromide were added and the reaction mixture re-

fluxed overnight with stirring. The reaction mixture was allowed to cool to room temperature then filtered. The flask was washed with three 3-ml portions of acetone and these washes passed through the filter paper. The filtrate, collected in a 15-ml conical centrifuge tube, was evaporated to dryness under nitrogen at 60°C. The residue was dissolved in 3.0 ml of hexane and aliquots of this injected into the chromatograph.

Analysis of plasma samples

Plasma samples (1.0 ml) were used throughout the standardization procedure. The basic extraction procedure is a modification of the protocol reported by Fenimore et al. [3] for Δ^9-THC. To a 50-ml conical centrifuge tube, 2.0 μg of THCBD (1.0 ml of ethanol solution of THCBD) were added. The ethanol was evaporated under nitrogen and 1.0 ml plasma added. The mixture was then vortexed and 10 ml of hexane—1.5% isoamyl alcohol were added, followed by vortexing for 30 sec and centrifuging at 400 *g* for 5 min. The hexane layer and most of the emulsified layer were transferred to a 15-ml conical centrifuge tube and the volume reduced under nitrogen by heating at 60°C being careful not to take to dryness. The plasma was reextracted with an additional 10-ml volume of hexane—1.5% isoamyl alcohol by vortexing and centrifuging as above. The volume of the combined hexane extracts was reduced to approximately 4 ml as before. The hexane extract was then washed successively with 2 ml of 0.1 *N* sodium hydroxide and 2 ml of 0.1 *N* hydrochloric acid and transferred to a 5-ml round-bottom flask, evaporated to dryness under nitrogen and derivatized as above.

The residue obtained after derivatization was dissolved in 0.3 ml hexane, then chromatographed with hexane on 1 g Florisil packed in a glass champagne column (Supelco, Bellefonte, PA, U.S.A.) with a 30-ml reservoir. The first 30 ml were collected, evaporated to dryness under nitrogen and the residue redissolved in 3.0 ml hexane. Aliquots of this sample were then injected into the chromatograph.

Spiked plasma samples for calibration purposes were treated in an identical manner with the addition of CBD and at the same time the internal standard (THCBD) was added to the sample.

Animal studies

Three male rhesus monkeys weighing 7—8 kg were utilized for the preliminary animal studies. The subjects were fasted overnight and anesthetized with 100 mg ketamine·HCl. CBD was administered orally via a nasal gastric tube in sesame oil or intravenously in ethanol. Blood samples were obtained via femoral puncture and collected in Vacutainer tubes containing disodium EDTA. The blood samples were centrifuged and plasma removed and stored in silanized containers at —20°C until analysis.

RESULTS AND DISCUSSION

The analysis of cannabinoids in biological fluids has drawn a lot of attention in the past decade because of the increased health problems associated with marihuana use as well as the increased number of clinical investigations

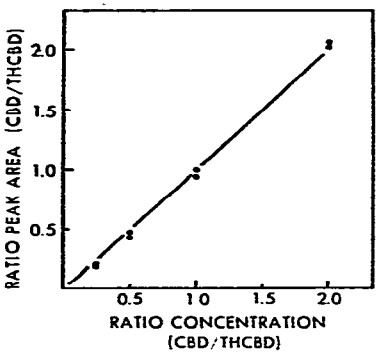
on certain cannabinoids. In this communication, we report a simple GC procedure for the analysis of CBD, a major cannabinoid which has received little attention in this respect. The selection of another cannabinoid to be used as an internal standard for the analysis of CBD was difficult since there are 61 cannabinoids known to exist naturally in *Cannabis* [20] and there are 22 known metabolites of CBD [21]. The use of THCBD as an internal standard was found to be satisfactory since the latter is not known to occur naturally or as a metabolite of CBD. THCBD was prepared by hydrogenation of CBD over palladium—charcoal catalyst. The product of the hydrogenation procedure was purified by column chromatography and then dissolved in ethanol. Quantitative hydrogenation was obtained as indicated by GC analysis with flame ionization detection [11]. The concentration of THCBD was calculated assuming the response factor to be the same as that utilized for CBD. The ethanol solution of THCBD became the stock solution of internal standard and was stored at 5°C. Appropriate dilutions were made to obtain the desired concentration of THCBD employed in the analyses.

Preliminary experiments to evaluate the procedure outlined above included monitoring of the derivatization and evaluating the extraction and recovery. Initially, the pentafluorobenzyl derivatives of CBD and THCBD were produced on milligram quantities of the materials and the reactions followed by thin-layer chromatography. No underivatized CBD or THCBD could be detected in the reaction mixture after 14 h and the conversion was quantitative. Thus, the overnight refluxing of the samples was selected out of convenience rather than necessity for the samples could be extracted one day, derivatized overnight and analyzed the next day.

Samples of the CBD and THCBD derivatives were analyzed by GC—MS to confirm the formation of the diether derivatives. The CBD and THCBD derivatives showed molecular ions of *m/e* 674 and 678 respectively indicating the formation of the desired products.

Detector response to CBD and THCBD derivatives was evaluated by adding varying amounts of CBD to 2.0 μg THCBD in the reaction vessel and derivatizing these samples. Fig. 1 illustrates the data obtained which demonstrate that the relative response factor for the CBD derivative and THCBD derivative remains constant over the range examined and is approximately 1.0.

The analysis of plasma samples spiked only with CBD at a level of 2 μg/ml and THCBD added after extraction (prior to derivatization) indicated the recovery of the CBD to be approximately 55%. The efficiency of this extraction did not remain constant over the concentration range examined; therefore, it was necessary to establish calibration data using plasma samples spiked with both CBD and THCBD. This variation in extraction and recovery efficiency is illustrated in Fig. 2. Even though these data exhibit non-linearity over the concentration range examined, a smooth curve can be drawn and the curve utilized to estimate the plasma concentration of CBD in the animal experiments. Fig. 3 illustrates a typical chromatogram of one of the samples from the animal studies illustrating the baseline resolution attainable. No peaks were seen in this region of the chromatograms from samples which contained no CBD or THCBD indicating the absence of interference by components extracted from the plasma.



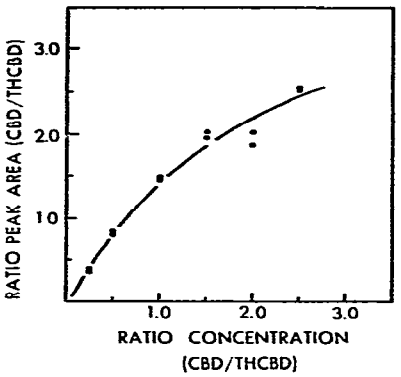


Fig. 1. Electron-capture detector response curve for the dipentafluorobenzyl derivatives of cannabidiol (CBD) and tetrahydrocannabidiol (THCBD).

Fig. 2. Calibration curve for cannabidiol (CBD) with tetrahydrocannabidiol (THCBD) added to plasma as an internal standard prior to extraction.

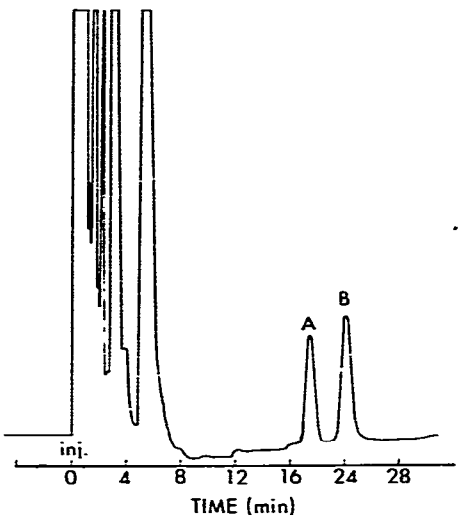


Fig. 3. Typical chromatogram from a plasma sample containing 2.0 μg internal standard (THCBD) and calculated to contain 1.5 μg cannabidiol (CBD). Peaks: A, THCBD derivative; B, CBD derivative.

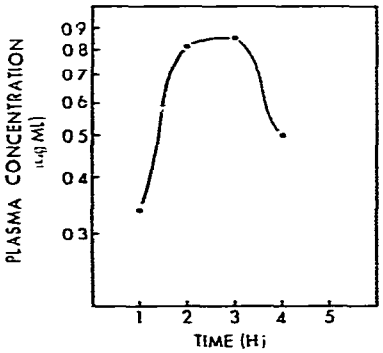
From the data utilized to generate Fig. 1, it is estimated that the absolute detection limit of the chromatographic system is approximately 80 pg. From the data utilized to generate Fig. 2, the detection limit of the total assay procedure as stated above is approximately 50 ng CBD per ml plasma.

Three monkeys were administered CBD either orally or intravenously and blood samples were withdrawn at selected times after administration. Fig. 4 illustrates the plasma concentrations of CBD detected after oral administration of 9.0 ml of a sesame oil solution of CBD (100 mg/ml) to a 7.9 kg male rhesus monkey (dose = 114 mg/kg). As is depicted in the figure, extremely low levels of CBD were detected over the time course of the experiment. The

two remaining animals were administered CBD intravenously in ethanol solution (10 mg/ml) at a 1.4 mg/kg dose. Fig. 5 illustrates the plasma concentrations of CBD detected in each of these experiments.

The actual fate of CBD in the body is not demonstrated in these preliminary animal experiments. However, it can be said that the free CBD rapidly disappears from the plasma with an apparent half-life in the order of 10 min. This is probably a distribution half-life rather than an elimination half-life for recently Siemens et al. [19] have shown the elimination half-life of CBD to be of the order of 11 h in rats. Their data indicated an initial rapid disappearance of CBD from the plasma and a multicompartmental system was necessary to describe the pharmacokinetics.

The results of these experiments indicate that the procedures presented can be successfully utilized for the quantitative detection of CBD in plasma samples by the formation of a high molecular weight fluorinated derivative. The procedure is applicable for the routine analysis of single or multiple samples with a standard gas chromatograph equipped with an electron-capture detector. The inclusion of the internal standard in the sample prior to extraction is a necessity.



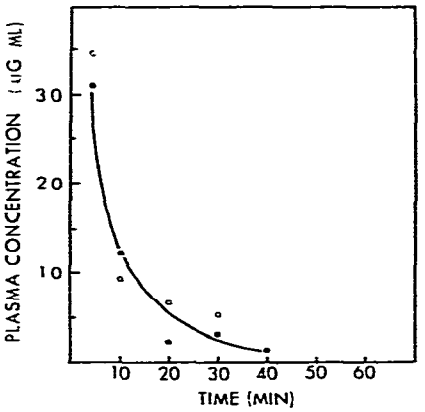


Fig. 4. Plasma concentration of free cannabidiol (CBD) following oral administration of the drug in sesame oil to a monkey. Dose = 114 mg/kg.

Fig. 5. Plasma concentration of free cannabidiol (CBD) following intravenous administration of the drug in ethanol to monkeys. Dose = 1.4 mg/kg; (o) experiment 1, (•) experiment 2.

ACKNOWLEDGEMENTS

The authors thank the National Institute on Drug Abuse (NIDA) for supplying the cannabidiol sample. Supported by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS, U.S.A.

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