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COMPARISON OF THE ANALYSIS OF Δ^9-TETRAHYDROCANNABINOL CAPSULES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The modification of a high-performance liquid chromatography (HPLC) system and the development of a capillary gas-chromatographic (GC) system for the analysis of Δ^9-tetrahydrocannabinol, encapsulated in soft gelatin capsules, are described. A photodiode array detector was used to evaluate peak homogeneity after each HPLC system modification. Sesame oil was separated from the extract by Sep-Pak® filtration prior to GC analysis. Quantitation by both systems had r values > 0.999 and R.S.D. values < 1.0%. Simultaneous capsule assays by both methods agreed within 1%.

INTRODUCTION

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the active ingredient in marijuana, has shown promise in the treatment of the nausea associated with certain chemotherapy regimens[1,2]. As an anti-emetic drug, it has been formulated in soft gelatin encapsulations containing sesame oil to overcome its poor solubility in water and its rapid decomposition in the presence of oxygen. The encapsulation of Δ^9-THC was also necessary for controlled administration[3].

The principal method for the analysis of nonformulated Δ^9-THC has been packed column gas chromatography (GC)[4]. However, the data from GC analysis of Δ^9-THC capsule extracts indicated that the Δ^9-THC peak area was dependent on the concentration of the sesame oil present in the sample. Several reversed-phase high-performance liquid chromatography (HPLC) systems have been reported for the analysis of cannabinoids[5–10]. Reversed-phase HPLC is not affected by the presence of the oil. Consequently, capsule analysis has relied primarily on this method.

Initial HPLC assays of reference samples and capsules stored at −15°C appeared to agree well with labeled values. However, assay values obtained on capsules stored at 37°C were highly variable, and a marked increase in the Δ^9-THC peak

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width was observed. The HPLC system was modified in order to explore the possibility of a non-homogeneous major peak (*i.e.*, an unresolved decomposition product). An alternative system using capillary GC was also investigated because of the high-resolution characteristics of this method and for use as a confirmatory assay method.

EXPERIMENTAL

Materials

The synthetic Δ^9-THC reference standard was supplied through the National Cancer Institute Division of Cancer Treatment (NCI, Silver Spring, MD, U.S.A.). The reference purity was determined by GC–flame ionization detection impurity profile analysis. Decanophenone (Pfaltz and Bauer, Stamford, CN, U.S.A.), N-phenylcarbazole (Aldrich, Milwaukee, WI, U.S.A.) and silicone GE SE-52 (Applied Science, Deerfield, IL, U.S.A.) were used as received. Absolute ethanol (U.S. Industrial Chemicals, Newark, NJ, U.S.A.) was degassed with argon. The HPLC mobile phase, acetonitrile (HPLC grade, Fisher Scientific, Fair Lawn, NJ, U.S.A.) and distilled water, containing acetic acid (Millinckrodt, Paris, KY, U.S.A.), were filtered through Nylon-66 0.45-μm filters (Rainin, Woburn, MA, U.S.A.). Standards of Δ^8-THC (10 mg/ml in ethanol) and cannabigerol (CBG) were purchased from Applied Science and olivetol was purchased from Aldrich. Cannabinol (CBN), Δ^{9-11}-THC (25 mg/ml in ethanol), cannabichromene (CBCh, 29 ml/ml in ethanol), and cannabidiol (CBD) were all supplied by the National Institute on Drug Abuse (NIDA, Rockville, MD, U.S.A.). These standards were all used as received and diluted to appropriate concentrations. The soft gelatin Δ^9-THC capsules along with soft gelatin placebo capsules were supplied by NCI.

Sample preparation

The Δ^9-THC reference standard solution was prepared in-house, under an argon atmosphere, in ethanolic solutions and stored at -15°C. The capsules were cut with a scalpel and the contents dissolved in absolute ethanol. Internal standard (decanophenone for HPLC or N-phenylcarbazole for GC) was added volumetrically in the final assay dilutions prior to analysis. Assay sample concentrations were approximately 0.15 mg/ml Δ^9-THC.

Chromatographic systems

For the liquid chromatography analyses, a Waters Assoc. (Milford, MA, U.S.A.) system with a WISP 710B autosampler, 440 detector and M6000-A pumps was used. A Varian UV50 variable-wavelength detector (Palo Alto, CA, U.S.A.) and a Hewlett-Packard 1040A photodiode array detector (Palo Alto, CA, U.S.A.) were also employed. The columns used were an Altex Ultrasphere ODS (PJ Cobert, St. Louis, MO, U.S.A.) 5 μm, 250 × 4.6 mm I.D. in series with an Alltech Spherisorb ODS II (Deerfield, IL, U.S.A.) 3 μm, 150 × 4.6 mm I.D. The mobile phase consisted of acetonitrile–1% acetic acid (85:15, v/v), 0.5 ml/min, ambient conditions. The analysis was monitored at 220 and 280 nm. The injection volume was 30 μl. Relative retention volumes were: components from sesame oil placebo (0.21–0.39); olivetol (0.26); CBG (0.51); CBD (0.54); CBN (0.81); Δ^{9-11}-THC (0.96); Δ^9-THC (1.00); Δ^8-THC (1.04); CBCh (1.24).

For the GC analyses, a Varian 3700 gas chromatograph, equipped with a capillary splitter, flame-ionization detector and a Model 8000 autosampler was used. The fused-silica capillary column, 27 m × 0.31 mm I.D., was purchased from Hewlett-Packard and coated in-house with SE-52. The calculated film thickness was 0.5 μm. Temperature settings were: detector and injector 300°C, oven 220°C isothermal for assay and 175°C to 275°C, 3°C/min program with a 10-min final hold for impurity profile. The carrier gas was helium, 33 cm/sec; the split ratio was set at 3:8. The injection volume was 1.0 μl and the attenuation was $8 \cdot 10^{-12}$ A/mV. Relative retention times under impurity profile conditions were: olivetol (0.23); CBD (0.85); CBCh (0.86); Δ^{9-11}-THC (0.91); Δ^8-THC (0.95); Δ^9-THC (1.00); CBG (1.04–1.05); CBN (1.06); components from sesame oil placebo (1.8–2.1).

Quantitation

The chromatography data system utilized for all analyses was the Nelson Analytical 4400 (Cupertino, CA, U.S.A.). The concentration of Δ^9-THC in the capsules was calculated by using the linear, least-squares fit, obtained from peak-height ratio analysis of the reference sample solutions. The plots of concentration *versus* the ratio of the reference sample peak height to the internal standard peak height were linear, $r > 0.999$.

RESULTS AND DISCUSSION

The data obtained from the analysis of capsules stored at elevated temperatures indicated that the peak for Δ^9-THC was symmetrical but broader (15–17% increased peak width) than that obtained for capsule stored at −15°C (Fig. 1). A wavelength ratio plot (230:280) of the major peak from the HP 1040A detector curved on the leading edge of the Δ^9-THC peak; the non-linear region is indicative of possible unresolved impurity(ies)[12] (Fig. 2a). This was confirmed by comparison with the uniform absorbance ratio plots, obtained for a capsule stored at −15°C (Fig. 2b) and for the reference standard.

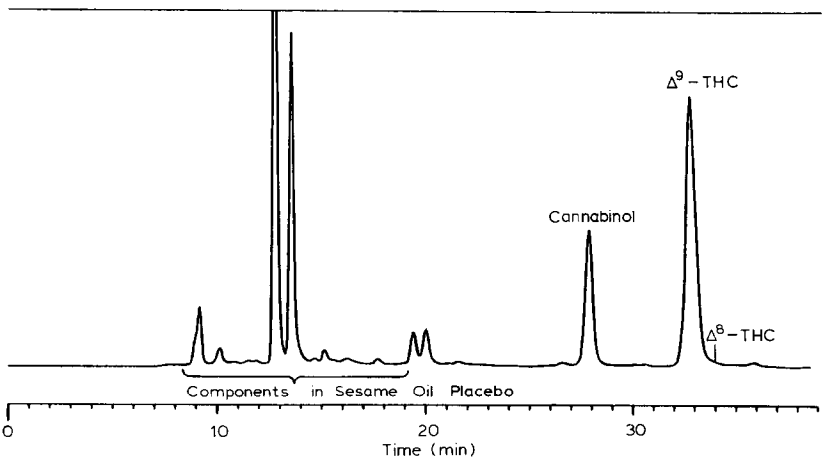

Fig. 1. HPLC chromatogram from a capsule, stored at 37°C for 24 months, with the initial system. Conditions: Altex Ultrasphere 5-μm column; mobile phase, acetonitrile–1% acetic acid (70:30, v/v); flow-rate, 1.0 ml/min.

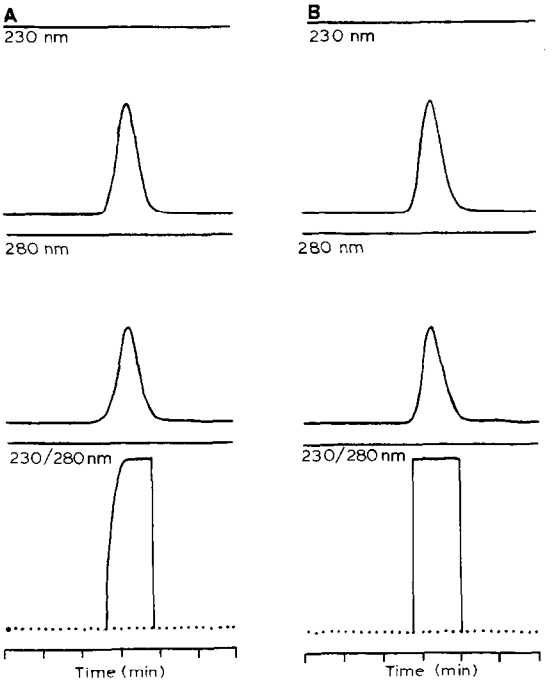

Fig. 2. A wavelength ratio plot (230:280) of the Δ^9-THC peak from capsules stored at 37°C (A) and at −15°C (B).

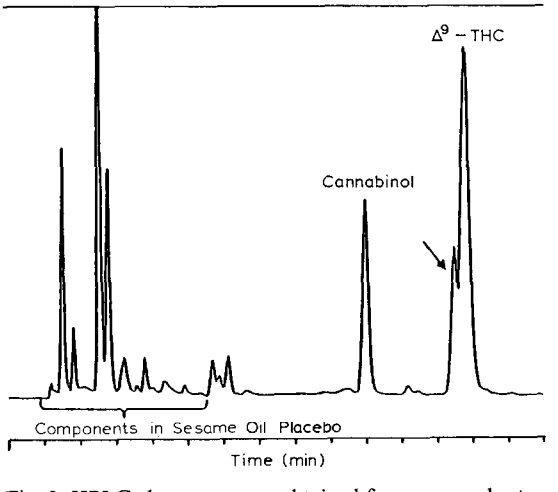

Fig. 3. HPLC chromatogram obtained from a capsule stored at 37°C, after the addition of a Spherisorb ODS II 3-μm column in series with the initial system. The arrow indicates the position of the partially separated impurity.

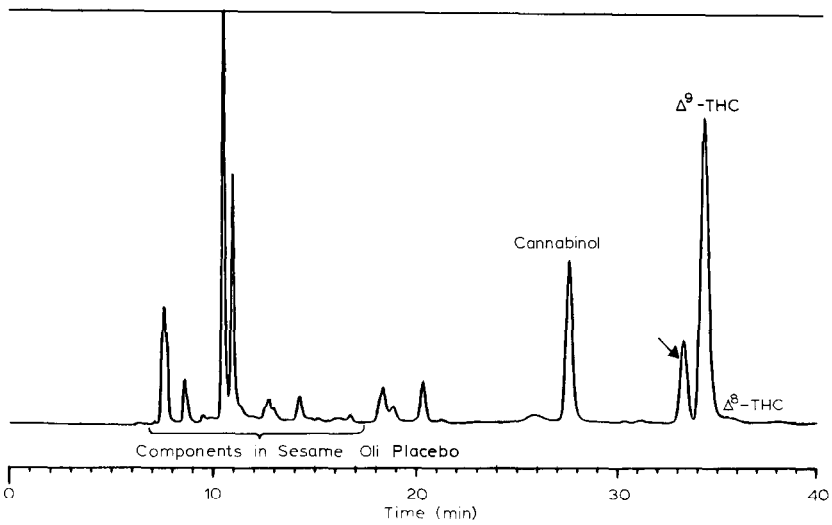

Fig. 4. HPLC chromatogram with the modified system, showing complete separation of the Δ^9-THC and the impurity peak. Conditions: Altex Ultrasphere 5-μm column in series with an Alltech Spherisorb 3-μm column; mobile phase, acetonitrile–1% acetic acid (85:15, v/v); flow-rate, 0.5 ml/min.

In order to increase the number of theoretical plates, a Spherisorb ODS II 3-μm column was placed in series after the Ultrasphere 5-μm column. Analysis with these two columns in series resulted in a large shoulder on the leading side of the Δ^9-THC peak (Fig. 3). This system was modified by changes in the flow-rate, temperature and mobile phase composition to achieve baseline separation of both peaks. The effect of each change was monitored with the HP 1040A photodiode array detector by obtaining a spectral scan overlay of the upslope, apex and downslope of both the impurity and the Δ^9-THC peaks after each system change.

Baseline separation of the two peaks was achieved with minor changes in the solvent proportions and flow-rate (Fig. 4). The spectral scans obtained with the modified system indicated peak homogeneity for the Δ^9-THC peaks by the high degree of

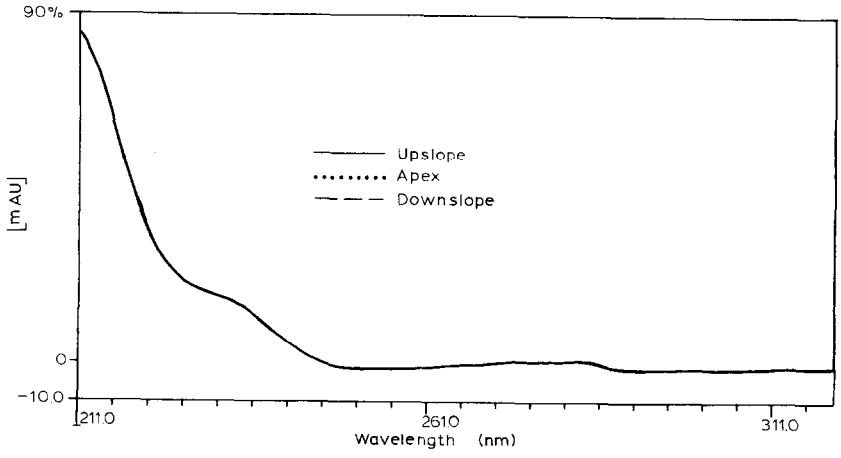

Fig. 5. Spectral scan overlay of the Δ^9-THC peak.

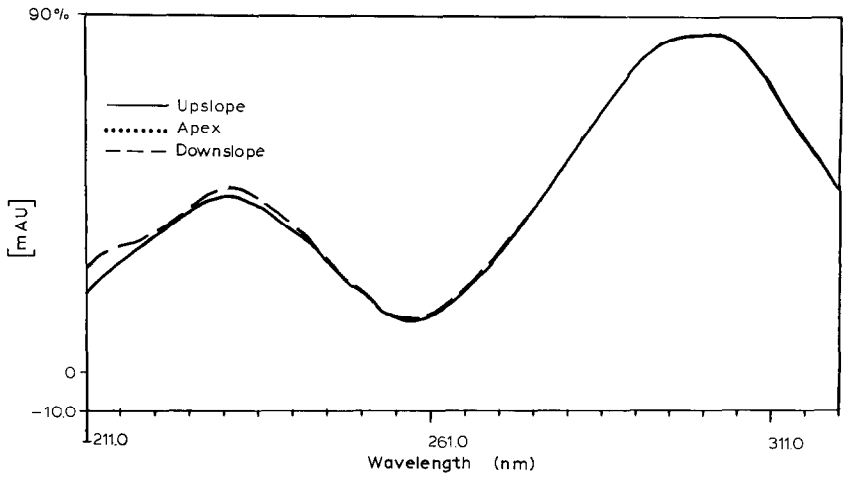

Fig. 6. Spectral scan overlay of the separated impurity peak.

spectral overlay (Fig. 5). The spectral scan of the separated impurity exhibited higher absorptivity values in the range of 260–320 nm (Fig. 6) than for Δ^9-THC. The modified system, however, did not improve the separation of Δ^8-THC from Δ^9-THC. Because of the lack of baseline separation of the Δ^8-THC and Δ^9-THC peaks, peak height data rather than peak areas were used for quantitation. The analyses of capsule content based on peak heights were linear, $r > 0.999$; the assay values had relative standard deviations of $< 1\%$.

No on-column decomposition was observed for Δ^9-THC when the HPLC system described was used, although rapid decomposition of Δ^9-THC in the presence of acid (a half-life of approximately 15 min at 37°C and pH 1) has been reported[13]. HPLC chromatograms of Δ^9-THC, obtained with and without 1% acetic acid in the mobile phase, showed no significant differences in the number or in the total area of the impurities. The presence of acid in the mobile phase did, however, sharpen the peak shape of the detected components.

Initial capillary GC studies indicated a diminished response of the Δ^9-THC peak area when the sesame oil concentration was varied. To minimize the effect of the sesame oil, ethanol solutions of the capsule contents were forced through Sep-Pak® C_{18} cartridges to extract the oil. The amount of oil removed by this filtration step for each extract was determined to be > 10 mg by gravimetric analysis of concentrated capsule solutions.

Simultaneous analysis of the filtered and non-filtered extracts from a common stock solution resulted in standard deviations of 0.9 and 3.9%, respectively. Good linearity, $r > 0.999$, and recoveries of Δ^9-THC $> 99\%$ were achieved with the addition of the filtration step. Good separation of Δ^8-THC from Δ^9-THC was achieved in the capillary GC impurity profiles (chromatographic analysis of samples with high concentration for impurity detection)[14] (Fig. 7). Automated injection was, however, critical for Δ^9-THC analysis. Standard deviations obtained from manual replicate injections were four to five times greater than those obtained by automated replicate injections.

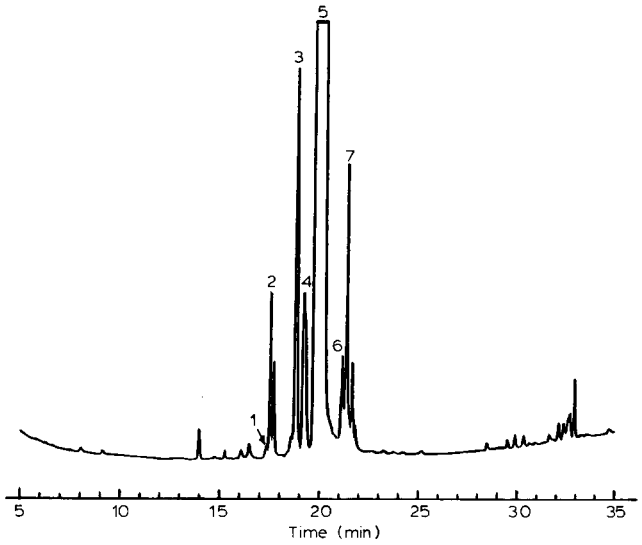

Fig. 7. Capillary GC impurity profile of synthesized Δ^9-THC. Peaks of various cannabinoids standards: 1 = CBD; 2 = CBCh; 3 = Δ^{9-11}-THC; 4 = Δ^8-THC; 5 = Δ^9-THC; 6 = CBG; 7 = CBN.

Absolute ethanol was an excellent sample solvent for both systems. No quantitative changes were observed by HPLC or capillary GC for the major peak or the detected impurities in the reference sample, stored at −15°C over a 12-month period. Both Δ^9-THC and sesame oil are very soluble in absolute ethanol, >500 and 25 mg/ml, respectively. The gelatin encapsulating material does not appear to be soluble in the solvent and therefore does not hinder the chromatography of the cannabinoids. Other sample solvents tried (*e.g.*, methanol, acetonitrile, tetrahydrofuran) had either much lower solubility for the sesame oil or tend to dissolve the gelatin capsule.

CONCLUSIONS

Simultaneous capsule assays with the modified HPLC and the capillary GC systems yielded similar assay values (average content difference, 1.1%) for capsules assayed to date. Both the modified HPLC and the capillary GC systems are highly reproducible. The HPLC system has the advantage of not requiring Sep-Pak® separation of the sesame oil; however, the HPLC assay time is twice as long as that of the capillary GC system. GC has the disadvantage of degrading some of the impurities observed in Δ^9-THC samples[15,16]. Therefore, changes in the impurity profiles *versus* time can only be accurately compared by HPLC.

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