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Note

On-column silylation of cannabinoids after injection of solid plant material and cold trapping

KNUT E. RASMUSSEN

Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Oslo, Blindern, Oslo 3 (Norway)

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Trimethylsilylation is a well known method of obtaining volatile derivatives for gas–liquid chromatography (GLC) of compounds that contain reactive hydrogen[1]. The method has been widely applied to cannabinoids and, apart from permitting separations at lower temperatures, the formation of trimethylsilyl (TMS) derivatives provides additional data for identification of the cannabinoids[2,3]. In studies of plant material, trimethylsilylation has been performed on plant extracts prior to the GLC analysis. The reaction mixtures are then injected into the gas chromatograph by means of a syringe. Injection of solid plant material, however, eliminates the need for extraction. A solids injector has been used to introduce a small sample of plant material into the flash heater of the gas chromatograph[4,5]. Volatile constituents evaporate in the flash heater and are brought on to the column by the carrier gas. Methods of derivatization of compounds evaporated during injection of solids have not been reported. Cold trapping was recently described in combination with the solid-injection technique[6]. By use of this technique it seemed possible to form derivatives of the trapped compounds on the column. This paper describes such a method for the formation of TMS derivatives of the cannabinoids after injection of solid plant material and cold trapping.

EXPERIMENTAL

Chemicals and plant material

n-Heptane (E. Merck, Darmstadt, G.F.R.) was used to prepare the test solutions. *n*-Tetradecane (C_{24}) (Koch-Light, Colnbrook, Great Britain) was used as internal standard for the determination of the trimethylsilyl derivative of tetrahydrocannabinol (THC-TMS) in the studies which were carried out to optimize the reaction conditions and the yields. Bis(trimethylsilyl)acetamide (BSA) (Supelco, Bellefonte, Pa., U.S.A.) was used as silylating reagent. Plant material from *Cannabis sativa* L. grown in Norway and samples of tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) were kindly supplied by Prof. Dr. A. Nordal, Institute of Pharmacy, University of Oslo (Norway).

Apparatus

A Fractovap 2300 gas chromatograph (Carlo Erba, Milan, Italy) with a flame ionization detector was used. The column was a glass coil (2 m × 4 mm I.D.), filled with 3% SE-30 on Supelcoport (80–100 mesh). Nitrogen was used as carrier gas at a flow-rate of 30 ml/min. The sensitivity setting was 128 × 10 respectively 64 × 10. The flash heater temperature was 300° and the compounds were trapped in the column by use of a column temperature of 40° during the injection. After the injection the column temperature was increased to 230° in 3 min and held isothermally.

Reaction rate analysis

A 1-μl test solution containing 3 μg of THC and 1.5 μg of C_{24} was injected into the gas chromatograph by means of a syringe; 6 μl of BSA were then injected. In one experiment the column temperature was increased immediately after the injection of BSA and in the other experiments 3, 5, 10 and 20 min after the injection of BSA. The peak height ratio, THC-TMS: C_{24}, was calculated and the yield of THC-TMS determined by use of a calibration graph. The retention time of THC-TMS was noted in all these experiments.

Influence of the amount of BSA

A 1-μl test solution containing 1–20 μg of THC and 0.5–10 μg of C_{24} was injected into the gas chromatograph. Each test solution was separately studied by injecting 0.5, 1, 2, 3, 4, 5, 6 and 7 μl of BSA. The column temperature was increased immediately after each injection of BSA. The peak height ratio, THC-TMS: C_{24}, was calculated and the yield of THC-TMS determined by use of a calibration graph.

Reproducibility

A 1-μl test solution containing 3 μg of THC and 1.5 μg of C_{24} and 6 μl of BSA were separately injected into the gas chromatograph. The column temperature was increased immediately after the injection of BSA and the peak height ratio, THC-TMS: C_{24}, was calculated. Ten such analyses were carried out and the coefficient of variation was calculated.

Procedure for analysis of plant material

A 1-mg amount of plant material was placed in the basket of the injector as described elsewhere[4,5]. The injector was screwed on to the injection port inlet of the gas chromatograph and when equilibrium had been attained the plant material was inserted in the flash heater of the gas chromatograph and retained there for 1 min. 2 min after the injection of plant material the injector was removed and the septum placed on the injection port inlet. On re-attainment of equilibrium 6 μl of BSA were injected and the column temperature was increased immediately.

RESULTS AND DISCUSSION

Injection of solids permits analysis of milligram quantities of plant material and the method is well suited for systematic studies of the cannabinoids in a Cannabis plant[7] as well as for the analysis of illicit samples when only milligram quantities of

TABLE I

GLC ANALYSIS OF THE SYSTEM THC–*n*-HEPTANE–*n*-TETRADECANE AFTER INJECTION OF BSA

Column (2 m × 4 mm I.D.) filled with 3% SE-30. Carrier gas (nitrogen) flow-rate, 30 ml/min; temperature, 40° increased to 230° after the given interval.

Time before increase in column temperature (min)	*Yield of THC-TMS (%)*	*Retention time of THC-TMS (sec)*
0	99.7	646
3	100.9	654
5	98.7	650
10	101.9	642
20	100.9	648

the material are available. By use of the method of on-column silylation reported here, additional data for identification of the cannabinoids can be rapidly obtained.

As is seen from Table I, the reaction between the 1-μl test solution (containing 3 μg of THC and 1.5 μg of C_{24}) and the 6 μl of BSA was instantaneous and complete on the column. There was no change in the yield or in the retention time of THC-TMS with a time lag of up to 20 min before the increase in column temperature. Fig. 1 shows, however, that the yield of THC-TMS was highly dependent on the volume of BSA injected. With the column used in this study, it was necessary to inject 6 μl of BSA in order to obtain complete reaction. The volume of BSA required was independent of the amount of THC when 1–20 μg of the latter were used. This range was studied as solid samples of plant material for injection usually contain 1–20 μg of the cannabinoids. Further experiments, however, must be carried out to study how the column parameters (I.D. and the column packing) influence the volume of BSA needed for complete reaction. The result of the reproducibility test is shown in Table II. A coefficient of variation of 3.4% was obtained for the peak height ratio,

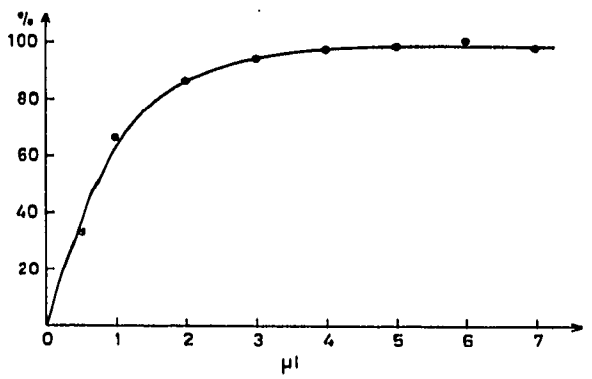


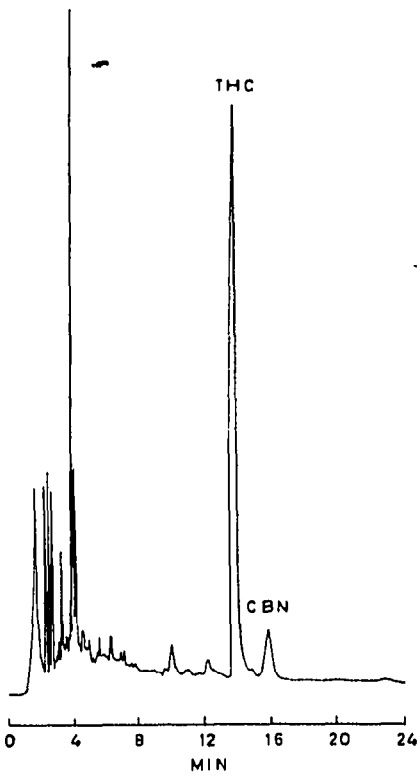
Fig. 1. Graph of percentage yield of THC-TMS against volume of BSA injected for a 1-μl solution containing 1–20 μg of THC and 0.5–10 μg of C_{24}. Column (2 m × 4 mm I.D.) filled with 3% SE-30. Carrier gas (nitrogen) flow-rate, 30 ml/min; temperature, 40° increased to 230° immediately after injection of BSA; detection, flame ionization.

TABLE II

REPRODUCIBILITY OF THE PEAK HEIGHT RATIO, THC-TMS: C_{24}, OBTAINED ON GLC ANALYSIS OF THE SYSTEM THC–*n*-HEPTANE–*n*-TETRADECANE AFTER INJECTION OF BSA

The column temperature was increased immediately after injection of BSA. Other conditions as in Table I.

Peak height ratio THC-TMS: C_{24}
0.888
0.874
0.885
0.875
0.864
0.883
0.892
0.884
0.864
0.873
Coefficient of variation: 3.4%



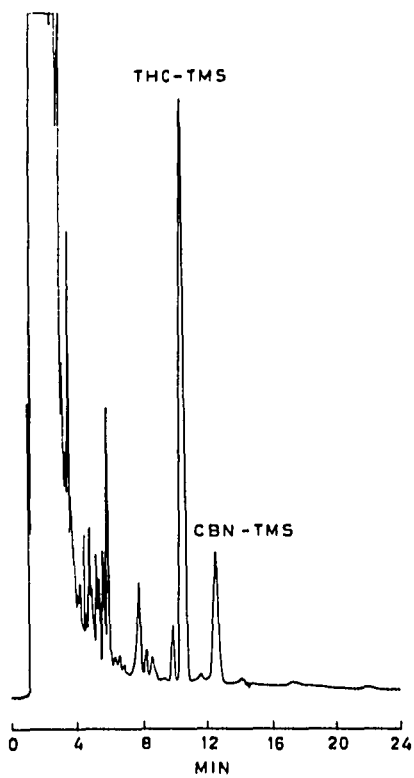


Fig. 2. Chromatogram of the volatile components of 1 mg of plant material analyzed by solid injection and cold trapping. Column parameters as in Fig. 1.

Fig. 3. Chromatogram of the volatile components in 1 mg of plant material analyzed by solid injection, cold trapping and on-column silylation.

THC-TMS: C_{24}, and this result is considered satisfactory for qualitative and quantitative use of the method.

Injection of solid plant material requires the injector to be screwed on to the injection port inlet of the gas chromatograph. This process interrupts the carrier gas stream through the column. However, equilibrium was re-attained 1–2 min after the injector was fastened. Fig. 2 shows a chromatogram of the volatile components of 1 mg of plant material analyzed by solid injection and cold trapping. Fig. 3 shows a chromatogram of the same components after solid injection, cold trapping and on-column silylation. The characteristic changes in the retention times confirm the identity of the major cannabinoids THC and CBN which were present in the plant material.

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