Journal of Chromatography, 171 (1979) 504–508

CHROM. 11,642

Note

Quantitative determination of Δ^9-tetrahydrocannabinol and Δ^9-tetrahydrocannabinolic acid in marihuana by high-pressure liquid chromatography

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(Received November 28th, 1978)

Measurement of Δ^9-tetrahydrocannabinol (THC) content of marihuana by high-pressure liquid chromatography (HPLC) is confounded by the fact that variable amounts of the THC are in the form of Δ^9-tetrahydrocannabinolic acid (THCA). Failure to convert the latter material to the former by decarboxylation could lead to erroneously low determinations of THC content in marihuana samples, unlike the situation with ordinary gas–liquid chromatography (GLC), in which the heat in the column is more than adequate to assure total decarboxylation. Decarboxylation procedures mentioned in the literature did not work in our hands[1]. This report describes the decarboxylation procedure that we developed for measuring THC content of marihuana using normal-phase HPLC.

EXPERIMENTAL

Method

All reagents (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.) were distilled in glass for UV. All glassware was silylated[2] and all stoppers were PTFE-lined screw caps.

A 100-mg amount of marihuana from a cigarette containing a putative 2% THC content in a stoppered 20 × 125 mm screw cap culture tube (SCCT) was extracted at room temperature by vigorous shaking at 10-min. intervals for 1 h (refs. 3 and 4). The extracted material was separated by filtering through a Whatman Phase separator into a 25 × 150 mm SCCT and washed with 2-ml volumes of chloroform until the combined volume was approximately 25 ml. The chloroform was volatized in a stream of nitrogen with heat, not exceeding 50° (ref. 5). The residue was dissolved in 10 ml of chloroform by vortexing for approximately 2 min. Duplicate 1-ml aliquots were transferred to 13 × 100 mm SCCTs and the chloroform volatilized as above. During evaporation, in order to minimize adsorption of THC and THCA to the residue, the SCCTs were rotated in a manner that dispersed the dried residue in a ring around the bottom of the tube. As each tube was removed from the stream of nitrogen, it was immediately stoppered in order to maintain an atmosphere of nitrogen within the tube. Residues to be assayed for total THC (neutral and acid) were incubated in a sand bath at 200° for 3 min to decarboxylate the THC acid(s). The decarboxylated residue, after cooling to room temperature, as well as the unincubated residue, were

dissolved in 4 ml of filtered (0.5-μm Millipore filter) 0.3% isopropanol (IPA) in heptane, by sonicating for 3 min and vortexing for approximately 1 min. During the vortexing, the tubes were inverted to dissolve any residue on the cap or upper walls. The THC was then measured by HPLC comparing peak heights to the THC standard solutions. Normal-phase HPLC was performed as previously described[6] with the following exceptions. The eluting solvent was changed to heptane–1% IPA in heptane (70:30) flowing at 1.4 ml/min, and the detector was set at 215 nm and 0.2 a.u.f.s.

The unincubated sample was analyzed using the same conditions because the regression of a standard curve at these conditions was almost linear. Greater precision for small amounts of neutral THC is easily obtainable by using smaller volumes to dissolve the residue, a more sensitive range selection of the detector and appropriate concentrations of standards.

Experiments

Our major task in using HPLC to measure THC content of marihuana was to determine the optimum conditions for the pyrolytic decarboxylation of THCA.

Extracts of marihuana from standardized marihuana cigarettes were prepared and subjected to various conditions to decarboxylate the THC acids. Initially low temperatures and continuous purging with nitrogen was used. At 70–75° little if any decarboxylation occurred. The content of THC for incubated material was 0.24 and for non-incubated 0.21%, clearly far below the presumed 2% THC content.

We next tried a temperature of 125°, with continual purging with nitrogen as before. At this temperature decarboxylation occurred but was still far less than expected and decreased as incubation time increased. We surmised this anomaly to be due to volatilization and not due to decomposition of the neutral THC as it was formed.

The surmised loss due to volatilization was demonstrated by adding 100-μg amounts of an ethanolic solution of THC to a series of 13 × 100 mm SCCTs and, after evaporating the alcohol in a stream of nitrogen, incubating the tubes at 125° for 5, 10, 15, 20, 30, 45 and 60 min, while continuously purging with nitrogen. Each residue was dissolved in 4 ml of 0.3% IPA in heptane and the recovery of THC was measured by HPLC. Peak heights were 88, 86, 80, 78, 61, 40 and 28% of theoretical, showing increased loss with increasingly longer incubation periods. A similar experiment demonstrated that loss was not due to decomposition. Instead of continuous purging with nitrogen during the incubation, the tubes were stoppered immediately after volatilizing the alcohol. After incubating at 125° for 10, 20, 30 and 60 min, recoveries were 102, 104, 103 and 101% of theoretical, as measured by peak heights. The differences from theoretical were not statistically significant. These two experiments confirmed that excessive volatilization of THC was the result of continual purging with nitrogen and this practice was stopped.

With purging removed, the next step was to determine a temperature that was least likely to produce decomposition of THC but most likely to lead to maximal decarboxylation in a reasonable period of time. As shown in Table I, a number of experiments were done in which both the temperature and time of incubation were varied. We concluded that a maximum temperature of 200° with incubation for 3 min was most satisfactory.

TABLE I

EFFECT OF INCUBATION TEMPERATURE AND TIME ON THE DECARBOXYLATION OF Δ^9-THC ACIDS FROM MARIHUANA

Each value from an aliquot containing 10 mg of marihuana from a combined pool of extracts prepared as described under *Method* from standardized marihuana cigarettes.

175°		*200°*		*225°*	
Time (min)	*THC (%)*	*Time (min)*	*THC (%)*	*Time (min)*	*THC (%)*
1.5	1.4	1.0	1.3	1.5	1.7
3.0	1.7	1.5	1.7	3.0	1.4
5.0	1.7	3.0	1.7	5.0	1.4
15	1.7	5.0	1.7		
		15	1.5		
		30	1.2		
		60	1.0		

RESULTS

Two extracts of each of two cigarettes prepared and analyzed by HPLC on two different days contained 1.60 and 1.58%, and 1.69 and 1.69% total THC, respectively. The latter cigarette contained 1.63% THC as measured by GLC. HPLC analysis showed that each extract contained 0.12 and 0.11% neutral THC, respectively. Thus, approximately 93% of the THC in these cigarettes was in the acid form.

In a sample of street marihuana analyzed by HPLC, total and neutral THC content was 5.4 and 3.7%, respectively. Thus, in this marihuana, 31% of the THC was in the acidic form. This analysis was done using a guard column prepared with Whatman HC Pellosil. The guard column did not change retention time but did broaden the peaks slightly. Its protection of the analytical column from contamination by the myriad of components in the plant material makes it a valuable addition to the HPLC procedure.

A typical chromatogram of a decarboxylated sample of a marihuana cigarette is shown in Fig. 1A. Besides showing a large peak for THC compared to cannabidiol (CBD) and cannabinol (CBN), it shows the excellent separation obtained by these conditions. In addition to THC, the cigarette contained 0.23% total CBN of which 87% was acidic.

Fig. 1B demonstrates how a marihuana extract containing a large amount of THC and a small amount of CBN can be analyzed accurately for the CBN when at 215 nm the peak of the THC overlaps that of CBN but not at 280 nm. This is possible because of the absorbance characteristics of these compounds. At 280 nm the absorbances of THC and CBN are approximately a thirtieth and a half, respectively, of what they are at 215 nm thus reducing the interference due to THC by a factor of fifteen[7].

DISCUSSION

Since our previous publication on the determination of THC in urine by HPLC[6], a mixture of heptane and IPA was found to be a very satisfactory eluting solvent for separating CBD, THC and CBN by normal-phase HPLC. Under the

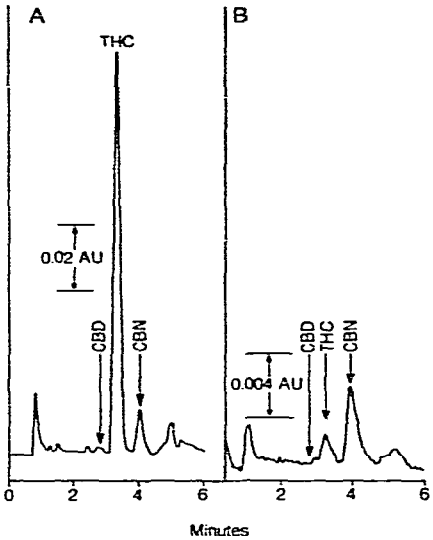

Fig. 1. Normal-phase high-pressure liquid chromatograms of 25 μg (measured by a 10-μl loop) of decarboxylated marihuana of a standard marihuana cigarette in 0.3% isopropanol in heptane. Column, 3 × 250 mm containing Spherisorb silica (5 μm). Solvent, heptane–1% isopropanol in heptane (70:30), flow-rate, 1.4 ml/min. A, Detector at 215 nm, 0.2 a.u.f.s.; B, detector at 280 nm, 0.04 a.u.f.s.

conditions described, the retention times were 2.8, 3.3 and 3.9 min, respectively. The mixture as a whole is more desirable for HPLC because it has a higher boiling point than the hexane, methanol, dichloromethane (DCM) mixture previously used without much, if any, change in viscosity as the column pressure remained about the same, *ca.* 1000 p.s.i.[8]. The elimination of DCM increased the sensitivity by improving the signal to noise ratio and allowing the working wavelength to be reduced to 215 nm. The replacement of methanol by the less polar IPA is also more desirable as minor changes in solvent composition have less effect. Finally, decreasing the concentration of IPA in heptane to 1% (B solvent) allows both reciprocating piston-type pumps to operate at conditions that maintain constant flow-rate better than previously described.

A reversed-phase HPLC method has been described for neutral and acidic cannabinoids that uses as acidic reference standards, acidic cannabinoids extracted from cannabis resin which, after some purification by HPLC, are quantitated by GLC[9]. Besides not having the GLC capability, the procedure appeared less practical for our immediate needs, and hence was not used.

The decarboxylation conditions, 200° for 3.0 min are considerably more intense than the 110° for 15 min previously described[1]. On careful examination of the latter work it was noted that it made little difference whether or not the THCA in the cannabis was decarboxylated during the incubation as any THCA in the extract which was prepared after the incubation, would be converted to THC during the GLC analysis[3]. While dehydrogenation of THC to CBN during decarboxylation appears less likely to occur under these conditions than by standard GLC analysis during which THC is exposed for as much as ten times longer to the same high temperature[10], the data of Table I show decomposition occurring after prolonged heating. After 15 min at 200°, the loss of THC approximates 16% and after 30 min, 34%. At the same time

there is an apparent increase of CBN of approximately 6 and 35%, respectively. The first value has a very large error as the difference in peak heights was only 1 mm, from 17 to 18 mm, but the latter value has some significance. This observation that CBN increases with burning of THC coincides with previous experience[11]. The loss of THC during prolonged heating also confirms the observation of Kinzer *et al.*[3], that 50% of THC in a marihuana cigarette decomposed during the mechanical smoking process used in their study.

The range of THCA in marihuana is apparently quite large. Fetterman *et al.* found 34.5, 80 and 93%, respectively, for three different samples[10], thus the wide difference between the values we obtained for the two different samples appears reasonable.

Now that it appears feasible to quantitatively decarboxylate the marihuana in a cigarette prior to smoking, it is possible to test whether cigarettes composed primarily of THCA or THC, respectively, have equal potency.

ACKNOWLEDGEMENTS

Standardized marihuana cigarettes made available by DHEW, Public Health Service, NIMH.

We thank Dr. Carlton F. Turner, Ph. D. and Ms. Mardi Russell of the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi for the gas chromatographic analysis.

This study was supported in part by grant DA 00424 and the Research Service, Veterans Administration.

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