GC–MS Analysis of the Total Δ^9-THC Content of Both Drug- and Fiber-Type Cannabis Seeds

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Abstract

A GC–MS method was performed to determine the total Δ^9-THC content in both drug- and fiber-type cannabis seeds. Drug-type seeds were found to contain much higher levels of Δ^9-THC (35.6–124 µg/g) than fiber (hemp) seeds (0–12 µg/g). The majority of Δ^9-THC was found to be located on the surface of the seeds. Approximately 90% of the total Δ^9-THC was removed by a simple, quick wash with chloroform. Washed drug-type seeds contained less than 10 µg/g. Separation of the seeds into the kernel and testa showed that the bulk of Δ^9-THC is located in the testa, mainly on the outside. The kernels of drug- and fiber-type cannabis seeds contained less than 2 and 0.5 µg Δ^9-THC/g seeds, respectively. Fluctuations in the Δ^9-THC content of different replicates of the same type of seeds could be the result of the degree of contamination on the outside of the seeds.

Introduction

Cannabis seeds (hemp seeds) have long been used as a major component of bird seed mix. Although all of the areal parts of the cannabis plant are prohibited by law worldwide, the seeds are specifically excluded when separate from the rest of the plant.

Recently, interest in cannabis seeds and the oil derived from the seeds, which is used in food and cosmetic products, has increased. The seed oil is known to be a rich source for unsaturated fatty acids (1,2). As a result of the ever increasing consumption of hemp seed products, particularly the oil, there have been some reports that ingestion of hemp seed oil could result in a positive drug test for marijuana use (3–5). Analysis of cannabis seeds by different investigators showed that the seeds do contain Δ^9-THC in a wide range of concentrations (6–12), whereas others reported the absence of Δ^9-THC in ungerminated seeds (13,14).

This investigation was carried out to determine the Δ^9-THC level in cannabis seeds of different geographical origin belonging to both the drug- and fiber-type plants. Furthermore, it was an objective of this report to determine the location of the Δ^9-THC within the seeds.

Experimental

Plant material

The seeds of *Cannabis sativa L.* (Mexican, Jamaican, Colombian, and hybrid-mix) were obtained from the 1995 harvest of plants grown in the research facility at the University of Mississippi and kept in the vault at a temperature of 20°C. Fiber-type seeds (hemp seeds) were obtained from The International Hemp Association (Amsterdam, The Netherlands). Four different hemp seeds were used: Dioecious, "Kompalti" (Hungary, 1995); Dioecious, "Novosadska" (Yugoslavia, 1995); Unisex , "Uniko-B" (Hungary, 1995); and Monoecious, "Futura" (France, 1994). Fiber-type seeds are obtained from marijuana plants that contain < 1% Δ^9-THC and CBD > THC and drug type seeds are obtained from marijuana plants in which the percentage of THC is > CBD.

Reagents and standards

Corn oil was obtained from Sigma Chemical Co. (St. Louis, MO). Δ^9-THC-d_9 (100 µg/mL, internal standard) was obtained in vials from ElSohly Laboratories (Oxford, MS). Solutions of Δ^9-THC in 100-µg/mL and 10-µg/mL concentrations were prepared from standard Δ^9-THC solution (1 mg/mL) by 1:10 and 1:100 dilutions, respectively, with methanol (stock Δ^9-THC from Research Triangle Institute, Research Triangle Park, NC). Solvents were ACS or high-performance liquid chromatography (HPLC) grade.

Instrumentation

A Hewlett-Packard (HP) 5890A gas chromatograph (GC) interfaced with an HP 5970A mass selective detector (MSD) and equipped with a 7673 autosampler was used for the analysis. The GC–MS was fitted with a DB-1 column (15-m length, 0.25-mm i.d., and 0.25-µm film thickness, J&W Scientific, Folsom, CA). Operating conditions: GC splitless mode, initial temperature was 170°C (1 min), then raised to 250°C at 10°C/min and held for 10 min. The data were acquired in the selected ion monitoring mode. The ions monitored were at *m/z* 314, 299, and 231 for Δ^9-THC and *m/z* 323, 305, and 234 for Δ^9-THC-d_9. Electron multiplier voltage was maintained at 200 V above autotune value. Carrier gas was helium at a linear velocity of 34 cm/s.

Extraction of cannabis seeds for Δ^9-THC determination

To 0.5 g seeds were added 50 µL of the internal standard solution and 4 mL of a mixture of chloroform/methanol (99:1), and the mixture was homogenized for 5 min and centrifuged for 5 min (Fisher Centrific model 228, RPM 3400). The clear solution was separated and evaporated to yield an oily residue that was heated at 110°C (1 h), then vortex mixed with 4 mL methanol (1 min) and centrifuged. The clear solution was separated, then mixed with 0.8 mL of 1N KOH in methanol and 4 mL of a mixture of hexane/ethylacetate (9:1) and vortex mixed (1 min). The lower layer was separated, acidified with 1 mL of 1N HCl and 4 mL of water, and vortex mixed (1 min). The upper layer was saved. To the lower layer was added 4 mL of a mixture of hexane/ethylacetate (9:1), and the mixture was vortex mixed (1 min). The organic layer was separated and combined with the saved fraction. The combined organic extract was evaporated, and the residue was dissolved in 1 mL hexane and applied over a 0.5 g silica gel 60 column (packed in a pasteur pipette). The column was eluted with 4 mL hexane, and the eluate was discarded. Further elution with 3 mL of a mixture of hexane/ether (80:20) was carried out, and the volume of the eluate was reduced to 1 mL and then analyzed.

Table I. Concentration of Δ^9-THC in Cannabis Seeds

Seed type	Δ^9-THC Concentration (µg/g)
Fiber Type	
Hemp seeds (Dioecious), Hungary, 1995	8
Hemp seeds Yugoslavia, 1995	9
Hemp seeds Unisex, uniko-B, Hungary, 1995	12
Hemp seeds (Futura) France, 1994 with fungicide	0
Drug type	
Mexican seeds	66
Jamaican seeds	124
Colombian seeds	41
Hybrid seeds, mix (no plant particles)	79

Table II. Analysis of Other Replicates of Cannabis Seeds

	Δ^9-THC concentration (µg/g)	
Seed Type	Replicate # 1	Replicate # 2
Mexican	44.4	43.8
Jamaican	81.8	49.6
Colombian	35.6	38.0
Hybrid (no plant particles)	40.2	57.0
Hybrid seeds*	394.0	602.0

* Raw seeds with some plant debris.

Table III. Effect of Quick Washing of Cannabis Seeds with Chloroform

	% Δ^9-THC Concentration in Washes			
Seed Type	Wash 1	Wash 2	Wash 3	Washed seeds
Mexican	84	6	1	9
Jamaican	95	2.3	0.44	2.3
Colombian	92	3	0.67	4.3
Hybrid(mix)(no plant particles)	87	4	18	

Table IV. Location of Δ^9-THC Within Seeds

	Δ^9-THC Concentration (µg/g equivalent of seeds)		
Seed type	Seed surface*	Seed shell	Kernel
Washed Seeds			
Mexican (Drug type)	67.30	2.32	1.67
Unwashed Seeds			
Mexican (Drug type)	39.85	1.58	
Commercial Hemp seed (Sample 1)		2.03	less than 0.5
Commercial Hemp seed (Sample 2)		2.60	less than 0.1

* Determined by analysis of the rapid chloroform wash of the seeds prior to separation into shell and kernel. The separation of the shell from the kernel was carried out manually.

Standard curve

Calibration points at 2, 5, and 10 µg Δ^9-THC were prepared by adding 20, 50, and 100 µL of standard Δ^9-THC solution (100 µg/mL) to 0.25 g corn oil. The internal standard (50 µL of 100 µg/mL) was added to all the samples. The spiked corn oil samples along with a negative control were extracted and analyzed following the previously described procedure starting at the point after the oily extract of the seeds was heated at 110°C for 1 h.

Linearity (LOL) and limit of detection (LOD)

Aliquot volumes of standard Δ^9-THC solution (10 or 100 µg/mL) were added to 0.25 g corn oil to obtain a concentration range of 0.1–400 µg of Δ^9-THC. Fifty microliters of the internal standard solution (100 µg/mL) was added to all samples. The spiked corn oil samples were extracted and analyzed following the previously described procedure. LOD is defined as the lowest concentration of Δ^9-THC where the identity of the drug is established (ion ratios within ± 20% of standard) regardless of the accuracy of quantitation, and LOL is defined as the upper limit of the assay where the quantitation is within ± 20% of the expected value.

Results and Discussion

Analysis of cannabis seeds for their Δ^9-THC concentration has been carried out by a variety of techniques including thin-layer chromatography, GC, and GC–MS (6–12). The interest in the Δ^9-THC content of cannabis seeds and products derived from the seeds was intensified because of

the recent reports that ingestion of hempseed oil could result in positive urine test for marijuana (3–5). Because of the wide reported range in the Δ^9-THC concentration in cannabis seeds (6–12), this investigation was initiated to determine the Δ^9-THC content of seeds of drug-type cannabis versus fiber-type cannabis, the latter of which has always been presumed to be the source of commercial seeds and seed products. In addition, this investigation also examined the location of Δ^9-THC within cannabis seeds.

Table I shows the concentration of Δ^9-THC in four samples of hemp seeds of different origin and four samples of drug-type cannabis seeds. It was evident that, as predicted, the Δ^9-THC content of drug-type samples in general was much higher than that in fiber-type samples.

Replication of the analysis of the drug-type seeds showed that the Δ^9-THC content of the seeds was not always consistant. Table II showed that two replicate samples of Jamaican seeds had quite different Δ^9-THC content (81.8 vs. 49.6 µg/g). The table also showed that the hybrid cannabis seeds separated from any visible plant particles had only a fraction of the Δ^9-THC content of the seed of the same variety where plant particles (debris) were present in the seeds. This suggested that the cleanliness of the seeds plays a major role in the apparent concentration of Δ^9-THC in cannabis seeds.

In order to determine the location of Δ^9-THC within the seeds, two experiments were carried out. In one experiment, the seeds were washed quickly with chloroform three times and each wash analyzed for Δ^9-THC followed by analysis of the washed seeds. Table III shows that the majority of Δ^9-THC was recovered in the first wash, with less than 10% of Δ^9-THC remaining in the seeds after washing. This suggests that exterior contamination of the seeds by the resin in the leaves through mechanical rubbing of the seeds with the leaves during processing of the plant materials is a major source of Δ^9-THC in the seeds. This was further proved by determination of the Δ^9-THC content of the separate components of the chloroform-washed Mexican seeds, namely the washings, the kernel, and the outside testa (the seed's shell); the data showed 67.3, 1.67, and 2.32 µg Δ^9-THC/g seeds, respectively (Table IV). This suggested again that the bulk of Δ^9-THC in the cannabis seeds resides on the outside of the seeds with only small amounts in the seed coat (testa) or the kernel itself. The kernels of drug-type and fiber-type cannabis seeds contained less than 2 and 0.5 µg Δ^9-THC/g seeds, respectively.

Conclusions

This study shows that cannabis seeds do contain measurable amounts of Δ^9-THC. The concentration of Δ^9-THC in the seeds is a function of the type of seed (drug vs. fiber) and the extent of contamination of the seeds with plant debris. The location of Δ^9-THC within the seeds was found to be mainly on the outside surface of the seed coat, possibly the result of physical interaction with the plant leaves during processing. Only a small amount of Δ^9-THC is found inside the seeds (less than 2 µg/g for drug-type seeds and less than 0.5 µg/g for fiber-type seeds).

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