Δ^9-Tetrahydrocannabinol Content in Cannabis Plants of Greek Origin

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The Δ^9-tetrahydrocannabinol (Δ^9-THC) content was identified and determined quantitatively using a Gas Chromatography Detector (Gas Chromatography - Electron Ion Detector) instrument in samples of illicit herbal cannabis. Law enforcement authorities sent the samples to the Department of Forensic Medicine and Toxicology, University of Athens, for toxicological analysis. The concentrations of Δ^9-THC in these samples ranged from 0.08% to 4.41%. Such concentrations suggest that Greece might be at high risk, as an area for the illicit cultivation of "pedigree" cannabis plants. The forensic aspects of cannabis classification are discussed.

Key words cannabis, Δ^9-tetrahydrocannabinol, Gas Chromatography Detector, forensic analysis, Greece

Marihuana continues to be the most readily available drug of abuse in Greece and the number and size of marihuana seizures have also increased. The major portion of cannabis seized is of Albanian origin, since Albania has become a major supplier of cannabis to Greece and Italy.[1] The fibre-type plants are legally cultivated in some regions of the Mediterranean area, including Greece, under specific permission.[2,3] However, significant illegal cannabis cultures do exist in various areas of Greece.

The aim of this study was the determination of Δ^9-tetrahydrocannabinol (Δ^9-THC) content in forensic cannabis samples seized last year by customs and police authorities in two areas of Greece (Ipiros and Lakonia).

Experimental

Thirty illicit herbal cannabis samples, seized last year from two different districts of Greece (the first located in the North–Ipiros and the second one in the South–Lakonia), were sent to the Department of Forensic Medicine and Toxicology of the University of Athens, for forensic chemical analysis. The samples were fresh upon receipt and dried until analysed.

The upper part of the main stem of each flowering cannabis plant was chosen and dried in the dark and then was stored for less than three months[4] in a paper box at room temperature. After removing seeds and stems, the dried leaves were ground to a powder.

Each sample consisted of 20 mg of the ground powder and was extracted overnight[5] with 1 ml of hexane, to ensure complete extraction of all the cannabinoids from the samples. The complete extraction was confirmed by the analysis of a second extract of the residue of each sample, which showed no detectable amounts of cannabinoids. Tetracosane was added to each sample as an internal standard, at a final concentration of 100 μg/ml. After centrifugation, 500 μl of the supernatant of each sample was evaporated and the dry residue was diluted with 500 μl of ethyl acetate, 1 μl of which was analysed by Gas Chromatography Detector (GCD). GCD is an advanced GC system introduced by Hewlett Packard in 1994 and consists of a chromatograph, an electron ionisation detector (EID) for m/z up to 420 and a data system. The EID generates retention time, abundance and the mass spectral data, so one can obtain results comparable with those obtained with a GC - MS instrument.

The conditions of the analysis were as follows: column: Hewlett - Packard HP-5 crosslinked 5% phenylmethylsilicone capillary column (30 m in length, 0.25-mm i.d., film thickness 0.25-μm); column temperature and initial temperature, 100 °C; initial time, 0 min; rate 15 °C/min, final temperature 300 °C, final time 8.00 min; injection port temperature, 280 °C; interface temperature, 300 °C. The helium flow rate was 1ml/min. Δ^9-THC content of the analysed samples was measured against a standard solution containing 100 μg/ml of Δ^9-THC.

Results

The Δ^9-THC content of cannabis samples was identified and determined quantitatively using GCD. The retention times of Δ^9-THC and tetracosane were determined to be 12.30 min and 11.44 min respectively. The total ion chromatogram of a cannabis sample is presented in Fig. 1.

The quantitation of Δ^9-THC was performed by using the areas of its major ions: (m/z 299, 314, 231). The percentage of the Δ^9-THC content of the analysed cannabis samples is presented in Table 1.

Discussion

Cannabis (Cannabis sativa L.) and its preparations remain by far the most popular and heavily used substances on the illicit market.[6—10]

As it is known, cannabis has been broadly classified into fibre-type or drug-type.[5,11—14] According to the European Union, the fibre-type plant does not exceed 0.3% of THC, while the drug-type plant usually contains up to 5% of THC, though higher percentages (up to 10%) have been reported.[15] It is widely accepted that only the presence of Δ^9-THC in substantial quantities (>0.3%) qualifies a sample of cannabis as a drug, since cannabidiol or cannabinol may be absent even from fresh samples.[16]

There are wide variations in the relative amounts of

Table 1. The Percentage of Δ^9-THC in Cannabis Samples from Lakonia and Ipiros

Cannabis samples	% Δ^9-THC	
	Lakonia	Ipiros
1	0.75	1.31
2	1.57	1.20
3	2.73	0.24
4	0.69	1.97
5	1.58	3.56
6	0.41	1.34
7	2.53	0.55
8	0.09	4.41
9	3.83	3.40
10	0.08	3.74
11	3.28	1.42
12	0.18	0.73
13	1.16	2.35
14	3.47	1.22
15	0.09	—
16	0.38	—

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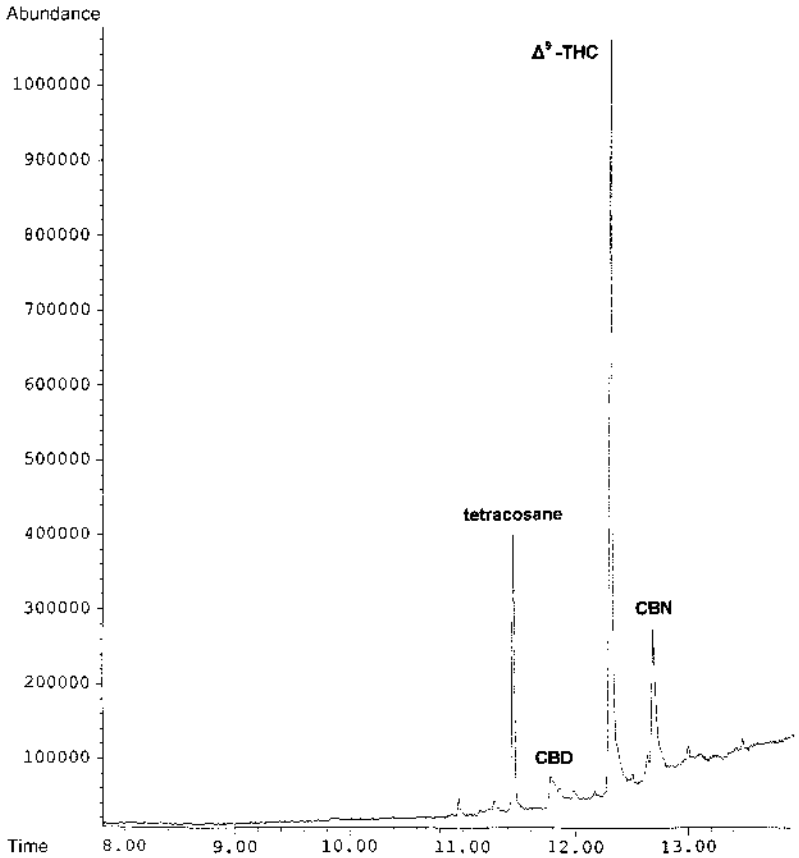

Fig. 1. Total Ionic Chromatogram of a Cannabis Sample, Extracted with Ethyl Acetate, which Contained Tetracosane as Internal Standard

cannabinoids in cannabis plants, depending on many factors. The predominant factors are the genetic characteristics of the seedstock,[17,18] the environment in which the plant is grown,[19,20] the maturity, sex, the part of the plant harvested, the time elapsed between harvesting and chemical analysis, and the conditions of storage of the plant.[21] Nevertheless, the generation plays a substantial role in the cannabinoid content, *e.g.* plants by the third generation have shown a significant increase in cannabinoid content and by the fifth generation all plants have become Δ^9- THC rich.[20] Since Δ^9-THC concentration of the plant gradually decreases over time as a result of oxidation to CBN,[22—24] it has been suggested that the formula Δ^9-THC+CBN could approximate the total Δ^9-THC content, regardless the degradative process. The quantitative determination of CBN in our samples showed insignificant amounts of this degradation product, less than 0.7%.

According to the literature,[3] the typical THC level of the three illicit cannabis products is 0.5—5% for herbal cannabis, 2—10% for the resin and 10—30% for the hashish oil.

It should be noted that these values are only a guide to levels likely to be encountered by the forensic analyst. Many samples of herbal, resin or liquid cannabis will have a THC content outside these limits.[3] Hybrid plants have a higher THC content than the traditional varieties and there are some reports, in the literature, that quantities of Δ^9-THC up to 17% have been determined.[1]

According to the present work the Δ^9-THC content varies from 0.24—4.41% in Ipiros samples and from 0.08—3.83% in Lakonia samples. The results suggest that the illicitly cultivated cannabis in Greece is of "good quality", with no major variations in cannabinoid content, at least in the two tested areas. The term "good quality" refers to the potency of the cannabis plants, which reflects the contained amount of Δ^9-THC.[5,10] These relatively high concentrations of active substances in cannabis plants may be due to the warm climate, the sun, the fertile soil of Greece, and possibly the quality of the seeds. This evidence suggests that Greece might be a high-risk area for the illicit cultivation of some new "pedigree" cannabis plants with high amounts of cannabinoid content.

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