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Isolation of Δ^9-THCA-A from hemp and analytical aspects concerning the determination of Δ^9-THC in cannabis products

Franz E. Dussy*, Cornelia Hamberg, Marco Luginbühl, Thomas Schwerzmann, Thomas A. Briellmann

Institute of Legal Medicine, Pestalozzistrasse 22, CH-4056 Basel, Switzerland

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Abstract

A simple procedure based on a common silica gel column chromatography for the isolation of Δ^9-tetrahydrocannabinolic acid A (Δ^9-THCA-A) from hemp in a multi-milligram scale is presented. Further, the decarboxylation reaction of Δ^9-THCA-A to the toxicologically active Δ^9-tetrahydrocannabinol (Δ^9-THC) at different analytical and under-smoking conditions is investigated. Maximal conversion in an optimised analytical equipment yields about 70% Δ^9-THC. In the simulation of the smoking process, only about 30 % of the spiked substance could be recovered as Δ^9-THC.

Keywords: Δ^9-THCA-A; Δ^9-THC; Total-Δ^9-THC; Decarboxylation; Isolation

1. Introduction

In Switzerland, the law concerning the agriculture, consumption and trafficking of cannabis is in a changing progress. Some more liberal forces claim that the today's widespread consumption of marihuana or hashish is harmless compared to alcohol or nicotine. On the other hand, more conservative parties set the focus on the observable circumstances in which the agriculture and trafficking of such products is already in the hand of the organised crime. As a consequence of these political discussions, the analysis of cannabis products is of increasing interest.

Swiss legislation, as well as the law in other European countries, requires the measurement of the total Δ^9-tetrahydrocannabinol (Δ^9-THC) content comprising the sum of the free Δ^9-THC and its precursor Δ^9-tetrahydrocannabinolic acid A (Δ^9-THCA-A) [1,2]. This is because Δ^9-THCA-A converts into the psychotropically active Δ^9-THC when heated, as it occurs when cannabis products are smoked (Fig. 1). If this total Δ^9-THC is higher than 0.3% of the dried material, then the cannabis product is assigned as drug stuff, whereas cannabis with less than 0.3% total Δ^9-THC is assigned as fibre hemp. The value of 0.3% is valid in Switzerland as well as in the European Community.

External quality control experiments organised by the European Network for Forensic Scientific Institutes (ENFSI) or the Swiss Society of Forensic Medicine (SGRM/SSML), focusing on that total Δ^9-THC, revealed that there is still a lot of work to do to bring the elaborated results of the different laboratories into an acceptable range.

Having in mind that the plant material mainly contains Δ^9-THCA-A besides free Δ^9-THC, it becomes obvious that this thermal conversion requires more investigation [3]. A problem arises as this precursor is not yet available in the market. Just now, Lipomed AG (http://www.lipomed.com) has announced to commercialise Δ^9-THCA-A as a standard. In the literature, there is described a method for the isolation of Δ^9-THCA-A from plant material by extraction and subsequent purification on a reversed phase medium pressure liquid chromatography system (MPLC), which makes it

* Corresponding author. Tel.: +41 61 2673885; fax: +41 61 2673907.
E-mail address: franz.dussy@bs.ch (F.E. Dussy).

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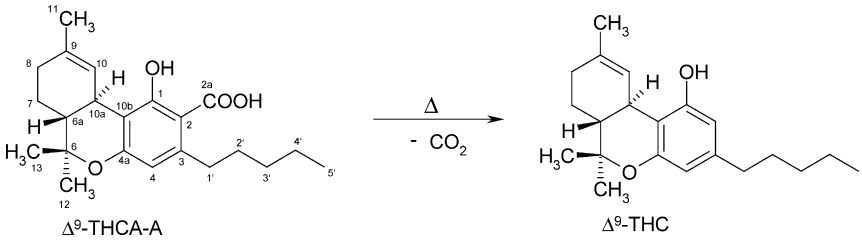

Fig. 1. Chemical structures for the decarboxylation reaction of Δ^9-THCA-A.

expensive [4]. We decided to develop a new purification procedure, based on a conventional preparative chromatography column, to gain Δ^9-THCA-A in a multi-milligram range.

Having large amounts of Δ^9-THCA-A in our hands, we investigated the parameters influencing the decarboxylation reaction in the liner of the injector of a gas chromatography system (GC), and compared it to the primer decarboxylation in a glass vessel with subsequent analyses by high pressure liquid chromatography (HPLC) and GC.

With an optimised analysis procedure, we investigated the content of Δ^9-THC and Δ^9-THCA-A in dried fresh plant material, hemp flowers and hashish seized in different hemp stores. According to the guidelines of the Swiss Society of Forensic Medicine, the leaves and flowers of the plant are mixed together and homogenised for analysis. The major stalks of the plants are sorted out.

Finally, commercially available cigarettes were spiked with Δ^9-THC and Δ^9-THCA-A, and the amount of recollected Δ^9-THC after the smoking process was measured.

2. Experimental

2.1. Reagents and materials

Reference cannabinol and Δ^9-THC were supplied by Lipomed (Arlesheim, Switzerland). All solvents were purchased by Macherey Nagel (Oensingen, Switzerland), and chemicals by Fluka (Buchs, Switzerland).

2.2. Extraction of Δ^9-THCA-A

The extraction was done according to the procedure described by Lehmann and Brenneisen [4]. In brief, 100 g pulverised plant material with a total Δ^9-THC content of 5% was extracted with 500 mL petrol ether acidified with acetic acid (0.5 mL CH_3COOH in 500 mL PE). The filtrated extract was re-extracted three times with 400 mL of an aqueous solution of NaOH and Na_2SO_3 (2% each). These combined extracts were acidified with ca. 500 mL of glacial 5 % sulfuric acid until pH reached 3, and immediately extracted with three times 400 mL TBME. These combined organic extracts were dried with Na_2SO_4, filtrated and concentrated in a rotary evaporator at 25–30 °C by aid of cryostatic cooling of the vapours. The concentrate was dried overnight at vacuum conditions, yielding 1.71 g brown amorphous material.

2.3. Purification of the crude extract by column chromatography

One hundred and seventy-seven milligrams of the crude extract was chromatographed on a 30 mm × 400 mm conventional column filled with 100 g silica 60 (0.063–0.2 mm). The elution solvent consisted of a mixture of hexane (650 mL), toluene (215 mL), acetone (135 mL) and acetic acid (20 drops), which were passed through the column at 1.5 mL/min. The fractions, sized 20 mL and in the critical moments of the chromatography 10 mL, were controlled by thin layer chromatography (TLC). Elution of Δ^9-THCA-A began at 280 mL. The fractions from 290 mL until 510 mL were collected and concentrated in a rotary evaporator at 25–30 °C. The concentrate was dried overnight at vacuum conditions to yield 99 mg pale yellow amorphous material. The purity of this material was assigned to 96% Δ^9-THCA-A by ^{1}H-NMR, the main impurity being the cannabinol analogue of Δ^9-THCA-A. All NMR analyses were measured at the Institute of Organic Chemistry (Basel, Switzerland) on a Bruker DRX500. The chemical shifts were assigned by several two-dimensional NMR techniques (HMBC, HMQC, COSY, DEPT 135 and NOE). The NMR data are listed in the annexe.

2.4. GC analysis

GC analyses were carried out on a Carlo Erba GC8000Top equipped with a flame ionisation detector (FID) and a CTC Combi PAL autosampler. Separation is achieved on a DB-5MS column from J&W Scientific, 15 m × 0.25 mm i.d., 0.25 μm film thickness. The GC operates with constant flow at 0.76 mL helium per minute and a split ratio of 50 to 1. The FID is fed with 40 mL/min H_2 and 300 mL air, and for a higher sensitivity, 30 mL/min N_2 as make-up gas is added. Temperature program: 120 °C, hold for 2 min, 20 °C/min up to 300 °C, hold for 3 min. The system was calibrated with cannabinol according the publication of Poortman-van der Meer and Huizer [5]. A typical chromatogram of a GC analysis is shown in Fig. 2.

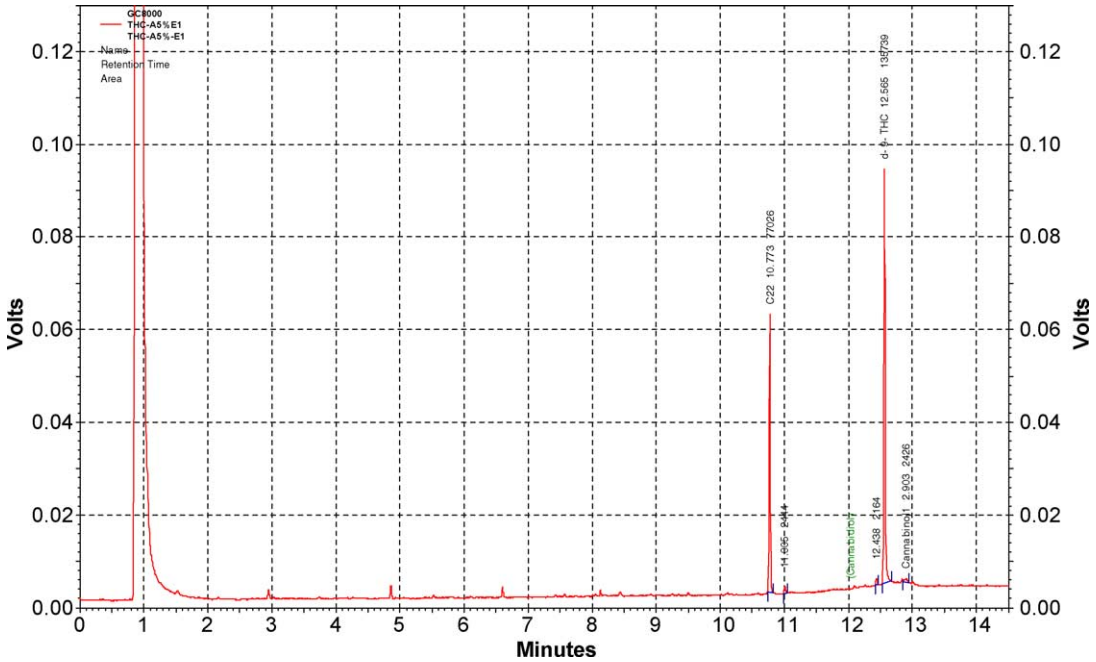

Fig. 2. GC chromatogram of a standard solution of Δ^9-THCA-A analysed at an injector temperature of 300 °C.

2.5. HPLC analysis

For HPLC analyses, a quaternary pump (P4000), a photo diode array (UV6000LP) and an autosampler (AS3000) from Thermo Finnigan were used. Chromatography was achieved on a 150 mm × 3.2 mm, 5 μm Allure C18 reversed phase column from Restek. The PDA was operated at 228 nm. The mobile phase was changed with a linear gradient: (A) buffer 0.05 mM acetic acid (pH 4.75), (B) acetonitrile and (C) methanol, keeping the starting mixture constant for 2 min (A 13%, B 22%, C 65%) and increasing B to 35% towards 25 min whilst decreasing A.

2.6. Decarboxylation of Δ^9-THCA-A

A solution containing 500 μg Δ^9-THCA-A was put into a gas tight vial. The solvent was evaporated under a gentle stream of nitrogen and the vial was closed. The prepared vial was then put in a GC oven-heated to the desired temperature for 15 min. The reaction product was taken up in 500 μL methanol. 1 μL was analysed by HPLC. Fig. 3 shows stacked HPLC chromatograms of the reaction mixtures from the decarboxylation at different temperatures.

2.7. Recollection of Δ^9-THC following smoking

Commercially available cigarettes without filter were spiked with a solution of Δ^9-THCA-A and Δ^9-THC in methanol, and dried by sucking through a gentle stream of air. For the recollection of Δ^9-THC following smoking, an assembly similar to the one described by Huang et al. [6] was used. The cigarettes were connected to two serially mounted gas washing bottles by a short tubing (Fig. 4). The bottles were filled with 200 mL of ethyl acetate each and connected to a vacuum pump. After ignition, a slight stream was sucked through and the evolved gases were captured in the solvent inside the bottles. The solvents were concentrated separately and analysed by GC analogously to the cannabis extracts.

2.8. Extraction of cannabis products for GC and HPLC analyses

Fifty to hundred milligram cannabis product (dried fresh hemp plant, dried hemp flowers and hashish) was extracted with 2 mL ethyl acetate containing 1 mg/mL $C_{22}H_{46}$ as internal standard. Sonication was applied for 15 min. 10 μL of the supernatant was diluted with 390 μL ethyl acetate for direct GC analysis, or with 990 μL of methanol for direct HPLC analysis. The internal standard has no function in HPLC analyses.

3. Results and discussion

3.1. Purification of Δ^9-THCA-A

Compared to the previously published method [4], the herein presented purification is easier because it uses a

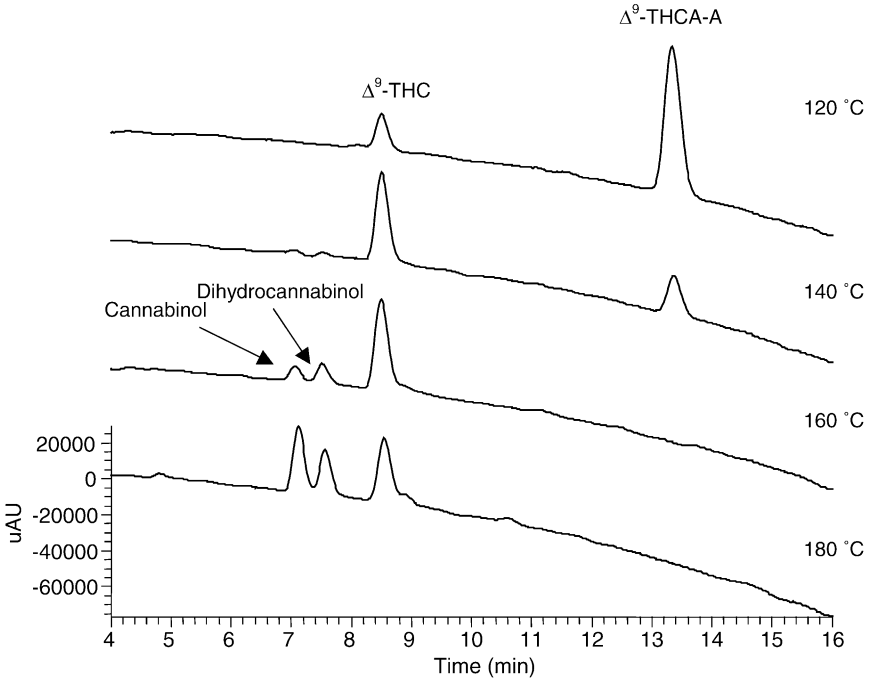

Fig. 3. HPLC chromatograms recorded at 220 nm for the conversion of Δ^9-THCA-A to Δ^9-THC at different temperatures prior to analysis.

Fig. 4. Assembly for the collection of Δ^9-THC in smoking experiments. The smoke and the gases are sucked first through the right bottle and subsequently through the second left bottle.

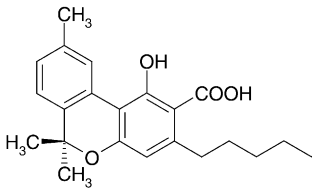

Fig. 5. Chemical structure of cannabinolic acid.

simple chromatographic column with silica available in every laboratory instead of highly sophisticated preparative MPLC. The Δ^9-THCA-A obtained by a single chromatography was about 96% pure. The main impurity present is cannabinolic acid, the further oxidised Δ^9-THCA-A (Fig. 5). The quality of this material was sufficient for our further purposes.

3.2. Optimisation of the GC system for the determination of the total Δ^9-THC content

For optimisation of the conversion of Δ^9-THCA-A into Δ^9-THC, three types of GC glass liners with a different geometry were compared (Fig. 6):

- an open glass liner (Restek #20939),
- an open glass liner filled with silanised glass wool (Restek #20939/ Supelco #20411) and
- a cup splitter (Restek #20950).

The open glass liner filled with glass wool proved to deliver the best results according to reproducibility and conversion. The empty open glass liner showed some lack in reproducibility, whereas the cup splitter was a little lower in conversion. The amount of glass wool inserted into the open liner had only a negligible effect on the conversion. More important is the fact that the glass wool needs to be compressed instead of just loosely filled. Maximal conversion was obtained at an injector temperature of 220 °C with no measurable amounts of Cannabinol being produced by further oxidation of Δ^9-THC. The value of the maximal conversion being about 67% is in the same range as described by Lehmann and Brenneisen [4]. A conversion versus injector temperature profile is shown in Fig. 7.

3.3. Optimisation of the decarboxylation temperature for the determination of the total Δ^9-THC content by HPLC

As Δ^9-THCA-A is commercially available only since this year, most laboratories measuring the total Δ^9-THC content by HPLC (a method without thermal stress) needed to convert Δ^9-THCA-A prior to analysis. Fig. 8 shows that optimum is reached at about 150 °C with about 70% yield. At higher temperature, Δ^9-THC is oxidised to form cannabinol. As the sum of Δ^9-THCA-A, Δ^9-THC and cannabinol does not reach 100%, it is assumed that polymeric material is formed also. If the time of exposure to temperature is changed, temperature needs to be adjusted to maximal conversion. With the chosen time, the maximal conversion is in the same range as it is in the injector of the GC system. Furthermore, in between 140 and 160 °C, there is no significant temperature dependence observed such that an exact temperature adjustment is not of importance in that range.

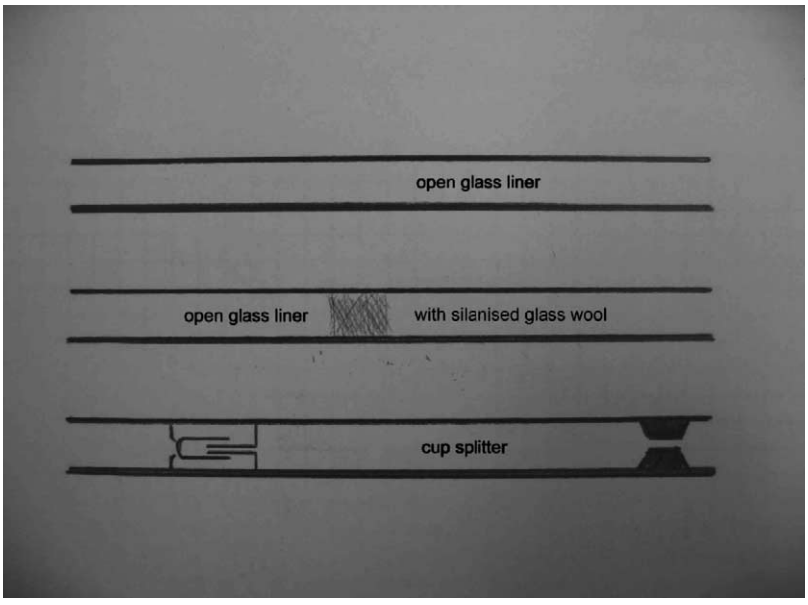

Fig. 6. Drawing of the three different GC injector types used.

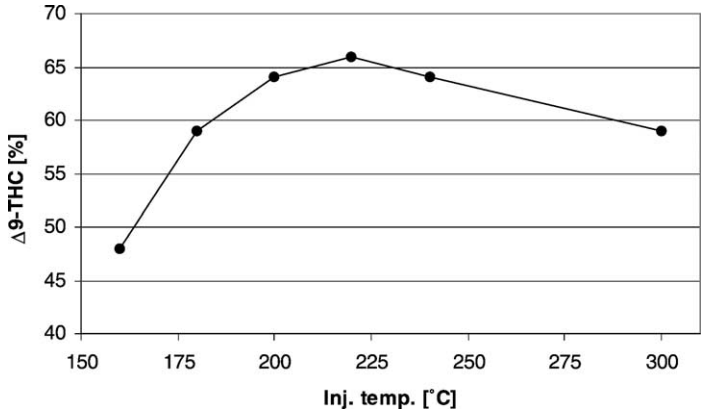

Fig. 7. Conversion of Δ^9-THCA-A into Δ^9-THC in the GC injector equipped with an open glass liner filled with silanised glass wool.

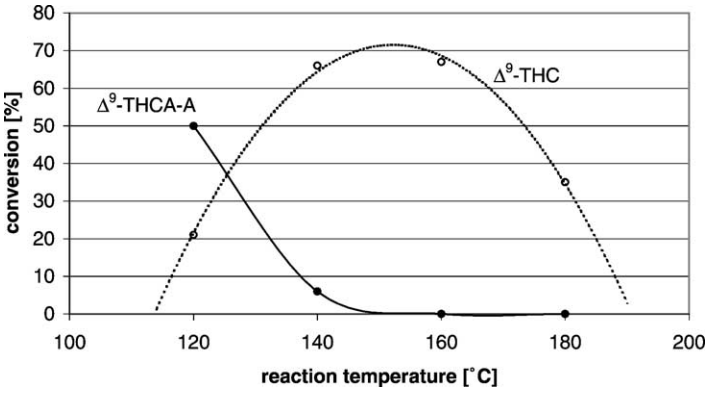

Fig. 8. Conversion of Δ^9-THCA-A into Δ^9-THC prior to HPLC analysis.

3.4. Analyses of cannabis products

With these optimised methods we investigated several hemp plants of Swiss indoor agriculture, some hemp flowers and hashish samples. After extraction with ethyl acetate analyses by GC and HPLC without prior decarboxylation were performed. As expected the sum of Δ^9-THCA-A and Δ^9-THC measured by HPLC was higher than the total Δ^9-THC content measured by GC in all cases (Table 1). The expected total Δ^9-THC content was calculated from the HPLC data based on a decarboxylation conversion of 70%. The correlation in-between the calculated and the measured total Δ^9-THC was very good for all hemp plants, a little worse for the hemp flowers and insufficient for hashish. It seems that with higher Δ^9-THCA-A concentration the decarboxylation pathway is favoured over other reaction pathways such that a higher total Δ^9-THC can be measured than expected.

3.5. Smoking experiments

For the smoking experiments, commercially available cigarettes without filters were spiked with 5 mg Δ^9-THCA-A and Δ^9-THC, respectively. The substances in the smoke were captured by the assembly described previously. GC analysis of the concentrated solvents showed that no Δ^9-THC was present in the second bottle, demonstrating that all Δ^9-THC was dissolved in the first one. Only about 30% Δ^9-THC of the spiked cannabinoid could be recovered in the first bottle and about 8% in the cigarette end, the vast majority being destroyed in the gloom (Table 2). As the cigarette was sucked by a gentle stream of air generated by a vacuum pump, only negligible amounts of Δ^9-THC are expected to be lost in the side smoke. In the literature, it is described that about 50% Δ^9-THC is available in the smoke [7].

4. Summary and conclusions

An easy method for the isolation of Δ^9-THCA-A in a multi-milligram scale is presented, enabling every laboratory interested in Δ^9-THC analysis to gain their own reference material from hemp plant.

It is shown that thermal conversion of Δ^9-THCA-A into Δ^9-THC is only partial in the used analysis equipments yielding about 70% at the maximum. Thus, laboratories

Table 1
Results of HPLC analyses without decarboxylation compared to the results of the GC analyses

	HPLC-PDA (Δ^9-THCA-A)	HPLC-PDA (Δ^9-THC)	Total HPLC-PDA (Δ^9-THCA-A × 314/358 + Δ^9-THC)	Total GC-FID (Δ^9-THC expected)	Total GC-FID (Δ^9-THC measured)
Hemp plant 1	10.2	0.7	9.6	7.0	7.0
Hemp plant 2	11.5	3.1	12.3	10.2	10.5
Hemp plant 3	9.4	0.8	9.0	6.6	6.6
Hemp plant 4	5.9	0.3	5.5	3.9	4.1
Hemp plant 5	12.7	1.8	12.9	9.6	10.0
Hemp plant 6	12.4	0.3	11.2	7.9	8.2
Hemp plant 7	10.5	0.4	9.7	6.8	7.2
Hemp flowers 1	18.1	0.5	16.4	11.6	13.2
Hemp flowers 2	18.9	0.6	17.1	12.2	14.2
Hemp flowers 3	14.8	1.2	14.2	10.3	12.1
Hemp flowers 4	13.2	1.3	12.9	9.4	10.7
Hemp flowers 5	16.8	3.0	17.8	13.3	14.0
Hashish 1	33.5	1.7	31.1	22.3	26.8
Hashish 2	34.9	1.5	32.2	22.9	28.9

Table 2
Results of the smoking experiments

	Δ^9-THC collected in the solvent of the first bottle (%)	Δ^9-THC in the cigarette end (%)
Cigarette spiked with 5 mg Δ^9-THCA-A	30	8
Cigarette spiked with 5 mg Δ^9-THC	24	8

quantifying the total Δ^9-THC, building the sum of Δ^9-THCA-A and the already present Δ^9-THC in the plant, get therefore a higher value than those who decarboxylate prior to analysis. It is proposed that each laboratory analysing total Δ^9-THC in cannabis products of forensic interest should evaluate their conversion yield. The only way to exactly determine the total Δ^9-THC content is to measure Δ^9-THCA-A and Δ^9-THC separately, and calculate the total Δ^9-THC based on those values. Every total Δ^9-THC value determined after decarboxylation gives a minimal content rather than an exact value. Nevertheless, the measurement of the total Δ^9-THC by GC without derivatisation is the procedure proposed by the authorities [2].

Finally, during smoking of a joint, most of Δ^9-THC is destroyed and only about 30% of the active substance can be taken up by the consumer.

5. Annexe

NMR-data of Δ^9-THCA-A ^{1}H-NMR ($CDCl_3$, ppm, 500 MHz):

δ 0.90 (3H, m, H-5′), 1.11 (3H, s, H-12), 1.34 (4H, m, H-3′ and H-4′), 1.44 (5H, m, H-7 and H-13), 1.58 (2H, m, H-2′), 1.68 (4H, m, H-6a and H-11), 1.92 (1H, m, H-7), 2.17 (2H, m, H-8), 2.78 (1H, d, d, d, $J = 6.4$ Hz, 9.5 Hz, 13.2 Hz, H-1′), 2.95 (1H, d, d, d, $J = 6.0$ Hz, 9.5 Hz, 13.2 Hz, H-1′), 3.23 (1H, m, H-10a), 6.26 (1H, s, H-4), 6.39 (1H, septett, $J = 1.6$ Hz, H-10), 12.20 (1H, s, OH).

^{13}C-NMR ($CDCl_3$, ppm, 500 MHz):

δ 14.1 (C-5′), 19.5 (C-12), 22.5 (C-4′), 23.4 (C-11), 25.0 (C-7), 27.4 (C-13), 31.2 (C-8), 31.3 (C-2′), 32.0 (C-4′), 33.5 (C-10a), 36.5 (C-1′), 45.6 (C-6a), 78.9 (C-6), 102.3 (C-2), 109.9 (C-10b), 112.6 (C-4), 123.6 (C-10), 133.9 (C-9), 146.9 (C-3), 159.8 (C-4a), 164.7 (C-1), 176.1 (C-2a).

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