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Rapid isolation procedure for Δ9-tetrahydrocannabinolic acid A (THCA) from *Cannabis sativa* using two flash chromatography systems

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ABSTRACT

Two isolation procedures for Δ9-tetrahydrocannabinolic acid A (THCA), the biogenetic precursor in the biosynthesis of the psychoactive Δ9-tetrahydrocannabinol (THC) in the cannabis plant, are presented. Two flash chromatography systems that can be used independently from each other were developed to separate THCA from other compounds of a crude cannabis extract. In both systems UV absorption at 209 and 270 nm was monitored. Purity was finally determined by HPLC-DAD, NMR and GC–MS analysis with a focus on the impurity THC. System 1 consisted of a normal phase silica column (120 g) as well as cyclohexane and acetone – both spiked with the modifier pyridine – as mobile phases. Gradient elution was performed over 15 min. After the chromatographic run the fractions containing THCA fractions were pooled, extracted with hydrochloric acid to eliminate pyridine and evaporated to dryness. Loading 1800 mg cannabis extract yielded 623 mg THCA with a purity of 99.8% and a THC concentration of 0.09%. System 2 was based on a reversed-phase C18 column (150 g) combined with 0.55% formic acid and methanol as mobile phases. A very flat gradient was set over 20 minutes. After pooling the THCA-containing fractions methanol was removed in a rotary evaporator. THCA was re-extracted from the remaining aqueous phase with methyl *tert*-butyl ether. The organic phase was finally evaporated under high vacuum conditions. Loading 300 mg cannabis extract yielded 51 mg THCA with a purity of 98.8% and a THC concentration of 0.67%.

1. Introduction

Cannabis is still the most frequently used illicit substance in the world. Globally, the number of people who had used cannabis at least once in 2008 is estimated between 129 and 191 million [1]. Besides of its status as a drug of abuse, cannabis has aroused increased interest as a medical product in the last years. Since the discovery of the primary psychoactive substance Δ9-tetrahydrocannbinol (THC) in 1964 cannabinoids and the endocannabinoid system have been extensively studied and turned out to exert pharmacological actions not only in the brain but also on the cardiovascular, immune and endocrine systems. In the beginning, research concentrated on THC and its synthetic analogue Dronabinol which has been approved for the control of nausea caused by cancer chemotherapy, as an appetite stimulant for AIDS patients and for the suppression of spasticity and neuropathic pain associated with multiple sclerosis. However, in several medical studies, the observed therapeutic effects of a cannabis preparation could not be ascribed to THC alone, pointing to the fact that there have to be other active components contributing to and modulating its action [2].

ElSohly et al. [3] reported about 489 compounds that have been found in the cannabis plant, more than 60 belonging to the class of cannabinoids – a group of biosynthetically related terpenophenolic substances. Depending on whether they carry a carboxyl function or not cannabinoids are classified into acidic and neutral cannabinoids. The highest amounts of acidic cannabinoid are accumulated in the resin of the flowering or the fruiting tops of the female plant. The principle cannabinoid components in the cannabis plant are THCA-A and cannabidiolic acid. To a minor extent the biosynthetic intermediate cannabigerolic acid as well as products of alternative biosynthetic pathways like cannabichromene and Δ9-tetrahydrocannabivarin are present.

Δ9-Tetrahydrocannabinolic acid A (THCA) is the most abundant compound of the cannabinoid fraction (presenting up to 95% of the cannabinoids in fresh, dried marihuana) of drug-type cannabis and the non-psychoactive biogenetic precursor of THC. Structures are shown in Fig. 1. THCA slowly decarboxylates during storage

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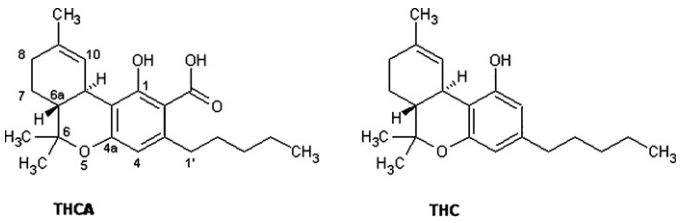

Fig. 1. Structures of Δ9-tetrahydrocannabinolic acid A (THCA) and Δ9-tetrahydrocannabinol (THC).

and fermentation. When heated or smoked and under alkaline conditions it is rapidly decomposed to its neutral form.

Contrary to past beliefs THCA is not completely converted to THC during the smoking process. Dussy et al. [4] carried out experiments to investigate the temperature dependence of the decarboxylation of THCA under various analytical and smoking conditions. They demonstrated that at most 70% were recovered as THC at optimized conditions (140 °C). THCA is therefore available for inhalative absorption and could be detected in serum and urine samples of cannabis users in 2006 by Jung et al. [5].

In fact, THCA is commercially available as a reference standard since 2004. However, for medical studies several grams of highly pure THCA can be necessary. To our knowledge, four methods for the isolation and purification of THCA have been published in the past:

The first method was published in 1992 by Lehmann and Brenneisen [6]. They extracted the plant material with acidified petroleum ether followed by two reextraction steps using a basic aqueous solution and diethylether. Afterwards, the residue was submitted two times to MPLC separation on a reversed phase. Finally, 50 mg of THCA with a purity of 99.6% were gained from 50 g crude extract.

The patented procedure developed by Flockhart et al. [7] involves chromatographic separation of a hexane pre-extract on a Sephadex LH20 column with a mixture of chloroform/dichlormethane and subsequent crystallization and removal of impurities by taking advantage of different solubilities in two different solvents.

Another method for large-scale isolation of cannabinoids was published in 2004 by Hazekamp et al. [8]. The hexane-containing crude extract is coated on a washed sea sand bed. After that the acidic cannabinoids are eluted with sodium hydroxide solution, precipitated and filtered. Centrifugal partition chromatography using a two-phase system of hexane/methanol/water follows yielding THCA with a purity of 94%.

Dussy et al. [4] slightly modified the extraction procedure of Lehmann et al. by replacing the diethylether with MTBE while maintaining all other steps. Instead of MPLC the extract was then chromatographed on a manually packed silica column with an elution solvent mixture of hexane, toluene, acetone and acetic acid. The dry residue of the combined fractions yielded THCA with a purity of 96%.

All methods are either inefficient, time-consuming, use harmful organic solvent, require rather uncommon equipment or yield THCA in insufficient purity. We therefore decided to establish a simple and rapid procedure for the isolation of THCA from hemp which should not use harmful solvents and should yield THCA with very high purity (>98%, THC < 1%) in a milligram range. Flash chromatography is the method of choice since it is easy to handle and very rapid. If needed it can be transferred to traditional column chromatography using silica gel which does only require standard laboratory equipment.

2. Materials and methods

2.1. Chemicals and materials

Reference Δ9-tetrahydrocannabinolic acid A (1 mg) and the methanolic solution (1 mg/mL) of Δ9-tetrahydrocannbinol were obtained from Lipomed (Bad Säckingen, Germany). The methanolic solution (100 μg/mL) of 11-OH-Δ9-tetrahydrocannbinol (11-OH-THC) was purchased from Cerilliant (Wesel, Germany). Rotisol, cyclohexane, pyridine and formic acid were obtained from Carl Roth (Karlsruhe, Germany), acetone from VWR Prolabo (Briare, France), methanol from J.T.Baker (Deventer, The Netherlands), sodium sulphate from Merck (Darmstadt, Germany), hydrochloric acid, ethyl acetate and methyl *tert*-butyl ether (MTBE) from Sigma–Aldrich (Steinheim, Germany). N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was purchased from Macherey-Nagel (Düren, Germany). Deionized water was prepared on a cartridge deionizer from Memtech (Moorenweis, Germany).

2.2. Plant material and pre-extraction of THCA

Marijuana was provided by the Central Police Department of Nordrhein-Westfalen for research purposes. The quantitation of the dried plant material using gas chromatography–flame ionisation detection (GC–FID) yielded a total Δ9-THC content of 14.5%.

740 g marijuana were frozen, pulverized and extracted by occasional shaking with 3 L Rotisol at 4 °C for 96 h. After filtration, the crude extract was treated with 20 g activated carbon at 4 °C for 72 h. Refiltration followed. Finally the extract was concentrated in a rotary evaporator at 30 °C yielding a brown and viscous residue.

2.3. Purification with normal phase flash chromatography

A Combiflash Rf apparatus (Combiflash Rf version 1.5.14, fraction collector version 00.00.49) served for flash chromatography which was carried out with RediSep Rf Gold silica columns (120 g), both from Teledyne Isco (Lincoln, USA). All separations were carried out at room temperature.

1800 mg of brown viscous cannabis extract (1.25% of the column size) turned out to be a reasonable amount ensuring an acceptable baseline separation of THCA and its main impurity THC. 3600 μL of the extract solution in cyclohexane (500 mg residue/mL cyclohexane) were added to silica powder as absorbent matrix and all remaining solvent was removed by a rotary evaporator providing the sample coated on a silica bed ("dried solid sample load technique"). The dry solid residue was finally filled into an empty cartridge.

After equilibration the extract was loaded from the cartridge on the column and chromatographed with gradient elution over 15 min with a flow rate of 95 mL/min. As mobile phase A we used cyclohexane containing 0.1% pyridine, as mobile phase B a mixture of mobile phase A and acetone (v/v, 2:1). The gradient was as follows: 0–2 min: 5% B, then continuously rising up to 15% B over 13 min.

UV absorption of THC shows a maximum at 209 nm whereas UV absorption maxima of THCA are seen at 220 nm, 270 nm and 305 nm. As a consequence detection wavelengths were set to 209 nm and 270 nm (THC shows practically no absorption at 270 nm). This way THC and THCA could be distinguished from each other unambiguously. Based on the signal intensities of these two wavelengths, the Combiflash Rf collected fractions of THCA while cutting off THC and other non-specified impurities.

The THCA fractions were eventually pooled and extracted three times with 0.5 M hydrochloric acid (5 mL/100 mL eluate) to eliminate pyridine by transferring it into the aqueous phase. The remaining organic phase was evaporated in the rotary evaporator.

An oil pump finally removed any remaining traces of solvents yielding THCA in form of white amorphous powder. Its purity was tested with HPLC-DAD, GC–MS and NMR analysis.

2.4. Purification with reversed phase flash chromatography

Again, the solid sample load technique as described above was applied: 600 μl of the same solution of raw cannabis extract in cyclohexane were absorbed onto silica corresponding to 300 mg sample load. Cyclohexane was removed by evaporation and the dry solid residue was loaded in the same fashion as described for the normal phase run.

This time a RediSep Rf Gold C18 column (150 g) served as chromatographic column. The gradient started with a mobile phase composition of 85% methanol/15% formic acid (0.554%, pH 2.3) which was held for 2 min. The methanol concentration then slowly rose up to 95% in 20 min. The flow rate was set at 80 mL/min. The same two wavelengths as in the normal phase run were set for detection.

The fractions containing THCA were collected, pooled and evaporated. Most of the methanol of the mobile phase was easily removable at 30 °C in the rotary evaporator under vacuum. A small volume of aqueous phase remained containing THCA in its protonated form since the pH was already set to 2.3 by the formic acid. The aqueous phase was extracted five times with 25 mL methyl *tert*-butyl ether (MTBE). After pooling, the MTBE phases were dried with sodium sulphate and evaporated to dryness at 30 °C in the vacuum rotary evaporator. Before final evaporation under high vacuum 5 mL methanol were added to redissolve the residue. When THCA was completely dry, purity was determined by HPLC-DAD, GC-MS and NMR analysis.

2.5. Quantitation of THCA and determination of purity

2.5.1. HPLC-DAD analysis

Purity of THCA was determined on an analytical HPLC system consisting of a Shimadzu LC-10AD pump and a SIL-10A injector which were controlled by a Shimadzu CBM-10A communications bus module. As a detector, a Shimadzu SPD-M10A diode array detector was used (Shimadzu, Duisburg, Germany). The chromatographic column was a Synergi Hydro RP column (150 mm × 2 mm internal diameter, 4 μm, Phenomenex, Aschaffenburg, Germany) with a corresponding guard column.

Separation was performed at room temperature using isocratic elution with a mixture of 0.55% formic acid (pH 2.3) and methanol (v/v, 15:85). The flow rate was 0.4 mL/min, one run took 20 min.

Purified THCA was redissolved in methanol to obtain sample solutions of 1 mg/mL and 100 μg/mL.

For quantitative analysis of THCA, a calibration curve was generated using solutions of THCA reference material in methanol with concentrations of 85, 90, 95, 100 and 105 μg/mL. Calibrators were prepared as follows: 50 μl 11-OH-THC (100 μg/mL) and the corresponding volume of THCA reference standard solution were evaporated to dryness in an autosampler vial, reconstituted in 100 μl mobile phase and 10 μl injected into the LC-system. Solutions of purified THCA were prepared likewise with 50 μl internal standard solution and 100 μl of the THCA solution (100 μg/mL).

For quantitative analysis of THC only 25 μl of the internal standard were used. This time, 100 μl of the THCA solutions with a concentration of 1 mg/mL were added. After evaporation of methanol and reconstitution in 100 μl mobile phase 20 μl were injected.

Presuming an impurity of 1% THC the calibration was performed using THC concentrations of 2.5, 5.0, 7.5, 10.0 and 12.5 μg/mL (which are equivalent to 0.25, 0.5, 0.75, 1 and 1.25% THC).

For THCA, absorption at 220 nm was monitored, for 11-OH-THC and THC at 209 nm. In general, the highest absorption maximum of a substance was used for quantification.

2.5.2. NMR analysis:

The white powder we had obtained after final evaporation of the solvents was analyzed by nuclear magnetic resonance in order to define its identity and to detect any remaining impurities. 20 mg of powder were dissolved in 650 μl deuterated chloroform.

Spectra were recorded with a Bruker Avance DRX-400 NMR spectrometer operating at a resonance frequency of 400.13 MHz for ^{1}H NMR spectra and 100.62 MHz for ^{13}C NMR spectra.

2.5.3. GC–MS analysis

GC–MS analysis was performed on a 7890A gas chromatograph combined with a HP 5975 C mass spectrometer and a 7683B injector (Agilent, Waldbronn, Germany) with a Gerstel MAS Controller C506 (Gerstel, Mülheim, Germany) and MSD chemstation software (version E.02.00.493). GC conditions were as follows: splitless injection mode; Agilent 19091 S-433 HP-5MS column (30 m × 0.25 mm × 0.25 μm), injection port temperature 250 °C; carrier gas helium, flow-rate 1.48 mL/min; column temperature, programmed from 140 °C for 2 min, rising at 60 °C/min for 1 min up to 200 °C, rising at 2.5 °C/min for 12 min up to 230 °C, rising at 60 °C/min up to 310 °C, hold time 4.3 min. The MS conditions were as follows: full-scan mode, *m*/*z* 50–550 amu; EI mode, ionization energy, 70 eV; ion source temperature, 230 °C.

5 μl of THCA solution (100 μg/mL in methanol) were evaporated and derivatized by silylation with 25 μl MSTFA and 25 μl ethyl acetate for 45 min at 90 °C. 1 μl was finally injected into the GC–MS system.

3. Results and discussion

3.1. Sample loading

Especially when separation is critical, the ideal loading technique is the “dried solid load cartridge technique”. In contrast to a direct syringe injection there is no solvent that could influence analyte–sorbent-interactions. Additionally, the column length is used to its full capacity. Judging from the dark brown colour in the loading cartridge silica was a very useful sorbent in the normal phase run because it already retained more polar compounds. Hence, these compounds could not occupy binding sites in the chromatographic column that remained free for THC and THCA.

1800 mg cannabis extract dissolved in cyclohexane were loaded on a 120 g normal phase column. After evaporation 623 mg THCA remained. Compared to silica gel that can be loaded up to 10% the typical loading on a C18 column is limited to about 1% of the column volume. To avoid overloading we decided to use an even lower percentage: only 300 mg were loaded on the 150 g RP column and finally yielded 51 mg pure THCA. With regard to the good separation a higher sample load seems feasible.

3.2. Normal phase chromatography

During development of the method several columns (normal, diol, acidic alumina and amine phase) were tested in combination with different solvent systems (cyclohexane/ethyl acetate, cyclohexane/toluene, cyclohexane/ether, cyclohexane/acetone). Formic acid and ammonia were tried as modifiers.

Successful separation of THC and THCA on a normal phase column could be achieved only with a cyclohexane/acetone solvent system spiked with the modifier pyridine for the first time. The major impurity THC eluted between 8.9 and 10.4 min and was almost baseline-separated from THCA with a retention time from

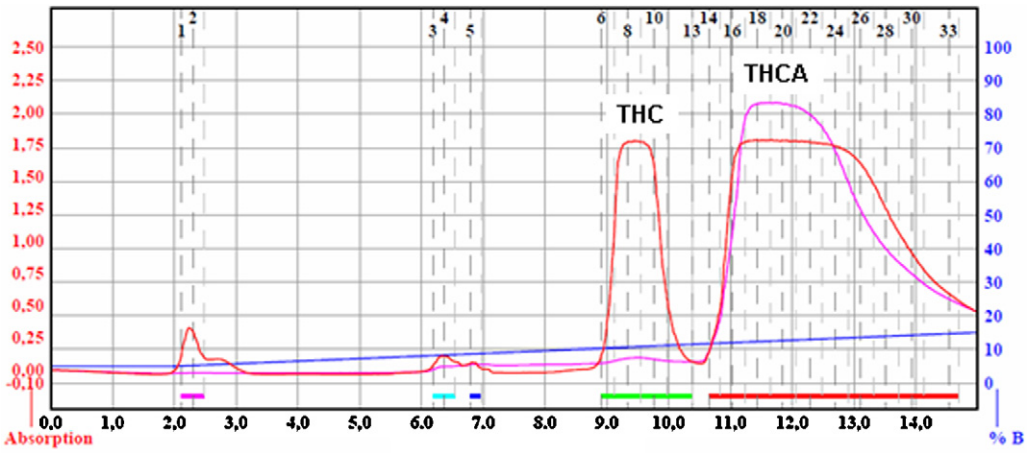

Fig. 2. Chromatogram of a run with normal phase silica gel (mobile phase A: cyclohexane containing 0.1% pyridine, mobile phase B: mobile phase A and acetone 2:1).

10.6 to 15 min (see Fig. 2). Pyridine is an organic base that easily dissolves in these mobile phases and possesses an optimal pKs of 5.3 to quantitatively deprotonate THCA (pKs 3.3) without setting alkaline conditions that would favour decarboxylation. This way the only difference between THCA and its main impurity THC – which is the polarity of the deprotonated carboxyl function intensifying interactions with the silica sorbent – is optimally exploited.

Of course, pyridine is undesirable after that. It is therefore removed immediately after pooling all THCA fractions by extraction with 0.5 M hydrochloric acid (5 mL per 100 mL eluate). Pyridium chloride is highly soluble in aqueous medium (85 g/100 mL water). Apart from the elimination of pyridine this acidification has a beneficial side effect: it transfers THCA into its more stable protonated form. In the final analysis with NMR no pyridine was found.

Acetone has a UV cutoff at 330 nm. Therefore it did not influence the absorption at 270 nm during gradient elution.

Technical restrictions of the pumps deem it necessary to dilute mobile phase B. If the amount of one solvent remains below 10% and changes only minimally during the run the pumps are not precise enough to ensure reproducible results. In order to guarantee the exact composition of the mobile phases during the run the volume of mobile phase B had to be increased.

During method development columns loaded with 4 g adsorbent were used for first experiments. After successful separation on a 4-g-column, all parameters were scaled up to 12-g, 40-g and finally 120-g-columns using a Combiflash software feature. The proposed gradient, flow rate and run time had to be only slightly modified to achieve similar good separations.

3.3. Reversed-phase chromatography

The reversed phase purification method was performed on the same apparatus but used a completely different chromatographic system. Again, impurities and THCA could be separated. The principal composition of the solvent system was similar to the system used for the HPLC quantification procedure: Using a C18 RP column and a gently inclining gradient with the mobile phases 0.55% formic acid (A) and methanol (B) we were able to separate four minor and major impurities before THCA was eluted after approximately 17 min (see Fig. 3). Mobile phase A was simply adopted from the HPLC-DAD method. In the quantification setting the pH needs to be adjusted to 2.3 in order to guarantee a reproducible UV spectrum as depicted in the library of Pragst et al. [9]. Though that specification is negligible in the purification setting there was no reason to change it in turn. Two aspects had to be considered regarding the choice of the organic mobile phase. First, it had to be easily vaporizable in the vacuum rotation evaporator to prevent the thermolabile THCA from degradation and, second, it should be immiscible with MTBE in the later extraction step. Methanol fulfils these criteria and was therefore the solvent of choice.

After pooling the THCA fractions methanol was evaporated until the remaining aqueous phase turned cloudy. MTBE proved to be

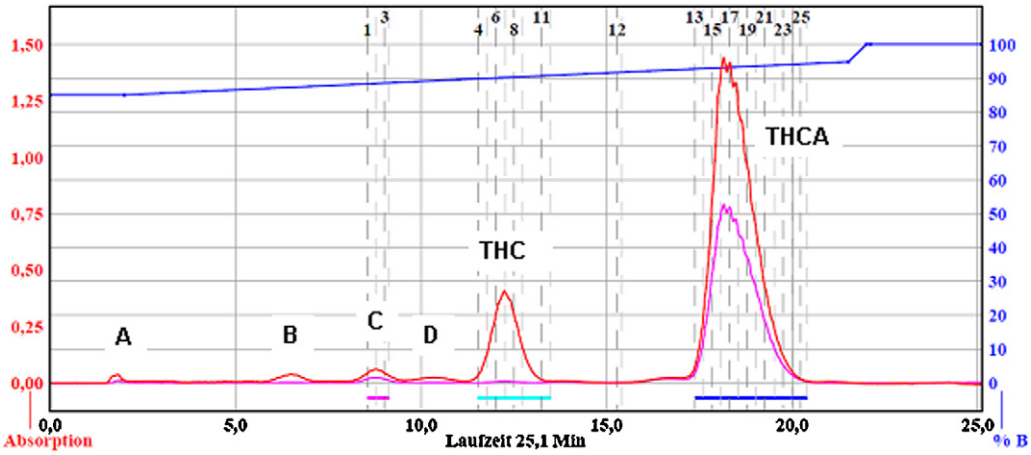

Fig. 3. Chromatogram of a run with reversed phase silica gel (mobile phase A: 0.55% formic acid, mobile phase B: methanol), A–D: minor impurities.

suitable for effective re-extraction of THCA from that medium. During method development MTBE showed a tendency to incorporate in THCA crystals. Once included it could not be removed from there even under high vacuum conditions. Since methanol forms an azeotrope with MTBE it was added to draw out small amounts of remaining MTBE during final evaporation under high vacuum conditions.

3.4. Quantification and proof of purity:

An HPLC-DAD method was established for the quantification of THCA as well as of THC. The starting point was the standard procedure of Pragst et al. [9] that uses acetonitrile and phosphate buffer pH 2.3 and a reversed-phase C_8 column. In a first modification acetonitrile was replaced by the less expensive methanol. This led to incompatibilities with the phosphate buffer (during sonication precipitation occurred). Phosphate buffer was therefore substituted by 0.554% formic acid with a pH of exactly 2.3. It was essential to maintain pH 2.3 in order to reproduce the UV spectra in the database of Pragst et al. [9]. Afterwards, the relative composition of the two solvents was varied to achieve good separation using isocratic elution. A ratio of 85% methanol and 15% formic acid proved to be most suitable and resulted in satisfactory baseline separation of the internal standard 11-OH-THC (2.9 min), THC (6.1 min) and THCA (13.8 min) (see Fig. 4).

Since THCA should contain only small amounts of THC for our purposes the maximal percentage allowed was limited to 1%. The two different preparation procedures for quantification of the impurity THC and the main compound THCA are owed to the enormous concentration differences of both substances.

Three calibration curves were established and proved to be linear and reproducible (r^2 = 0.9928 for THCA and r^2 = 0.9976 for THC).

Since the certificate of analysis for the purchased THCA declared a purity of 98.751 ± 0.064%, the calculated values for THCA were corrected by applying this factor. Purity of THCA separated with normal phase chromatography (99.8%) was slightly higher than purity of THCA obtained after the reversed-phase run (98.8%). Still, one chromatographic run is sufficient to obtain preparations that can be regarded as "substantially pure" per definition (purity >95%). THCA that was isolated by the normal phase run already complies the most preferably purity grade greater than 99.5% [7]. No impurities, especially no pyridine, were found with NMR and GC–MS analysis.

0.09% THC was found in the "normal phase batch" whereas in the "reversed-phase batch" 0.67% THC could be detected. Both

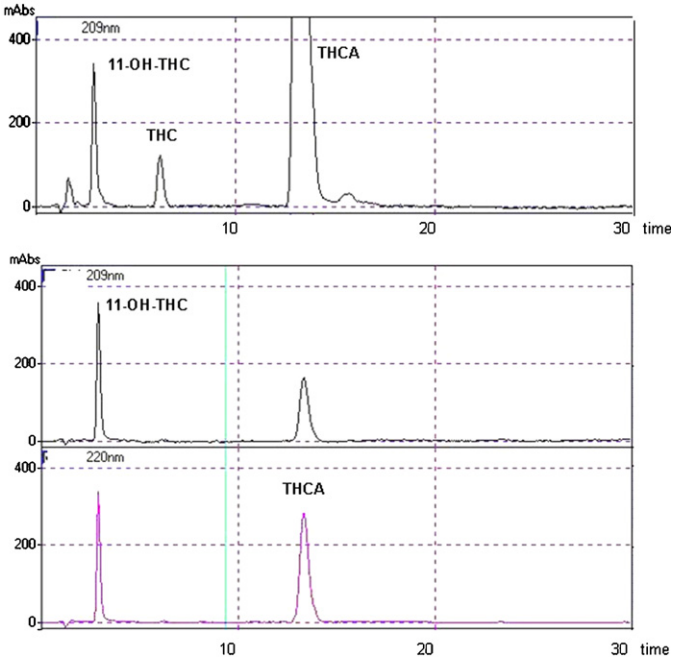

Fig. 4. (A) Quantification of THCA with HPLC-DAD: chromatogram of a sample of isolated THCA (c = 100 μg/mL) containing 50 μg 11-OH-THC, injection volume: 10 μl. (B) Quantification of THC with HPLC-DAD: sample of isolated THCA (c = 1 mg/mL) containing 25 μg 11-OH-THC, injection volume: 20 μl, calculated THC concentration 0.67%.

methods are therefore suitable for reducing the THC concentration to a minimum.

NMR analysis identified the white powder as THCA. All peaks in the ^{1}H NMR are consistent with the data published by Dussy et al. [4]. Furthermore all correlations in the heteronuclear multiple bond correlation spectrum were consistent with the structure of THCA.

GC–MS analysis for cannabinoids was performed using an in-house SIM method validated according to international guidelines for forensic toxicological analysis and covered THC, 11-OH-THC, THC-COOH, cannabinol, cannabidiol and THCA. In order to detect other compounds which might have been extracted from the cannabis plant material, scan mode was applied. The chromatogram of the purified THCA showed no additional peaks apart from THCA itself and signals observed in the blank silylation reagent before. An exemplary chromatogram with labeled peaks can be seen in Fig. 5.

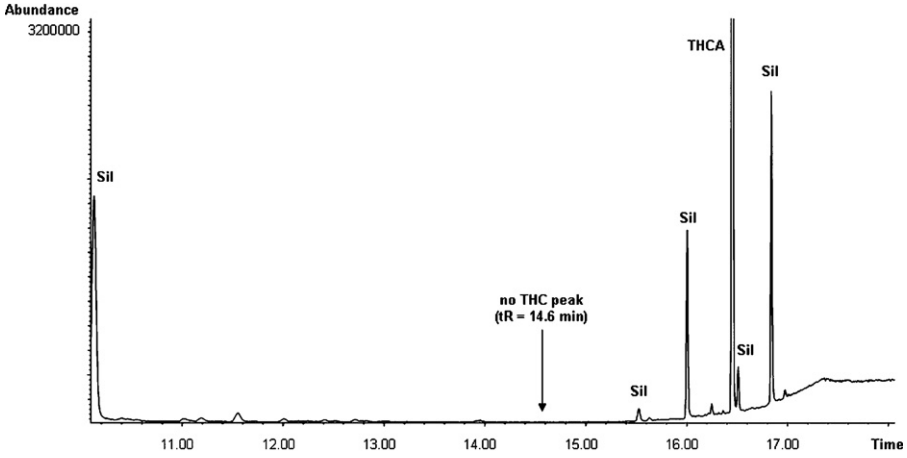

Fig. 5. GC–MS analysis: chromatogram of a sample containing 10 μg/mL isolated THCA, Sil: peaks that were also found in a blank sample containing MSTFA and ethyl acetate.

4. Conclusions

The described procedure presents a simple, non-hazardous and – compared to other methods – very rapid procedure for the isolation of THCA from hemp. Two flash chromatography systems proved to be suitable for preparation of larger amounts of THCA with a purity ≥98.8.

In a normal phase run 1800 mg crude cannabis extract resulted in 623 mg THCA consuming 2.0 L cyclohexane and 0.1 L acetone. In the reversed phase run 300 mg cannabis extract finally yielded 51 mg pure THCA consuming 2.2 L methanol, 0.3 L of 0.1% formic acid and 125 mL methyl *tert*-butyl ether.

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References

[1] UNODC, United Nations Publication, Sales No. E.10.XI.13 2010.
[2] E.M. Williamson, F.J. Evans, Drugs 60 (2000) 1303.
[3] M.A. ElSohly, D. Slade, Life Sci. 78 (2005) 539.
[4] F.E. Dussy, C. Hamberg, M. Luginbuhl, T. Schwerzmann, T.A. Briellmann, Forensic Sci. Int. 149 (2005) 3.
[5] J. Jung, J. Kempf, H. Mahler, W. Weinmann, J. Mass Spectrom. 42 (2007) 354.
[6] T. Lehmann, R. Brenneisen, Phytochem. Anal. 3 (1992) 88.
[7] I. Flockhart, G.W. Wheatley, S. Dring, L. Archer, patent no. WO/2004/026857 2004.
[8] A. Hazekamp, R. Simons, A. Peltenburg-Looman, M. Sengers, R. van Zweden, R. Verpoorte, J. Liq. Chromatogr. Rel. Technol. 27 (2004) 2421.
[9] F. Pragst, M. Herzler, S. Herre, B.-T. Erxleben, M. Rothe, UV Spectra of Toxic Compounds, Verlag Dr. Dieter Helm, Heppenheim 2001.