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High-performance liquid chromatographic determination of Δ^9-tetrahydrocannabinol and the corresponding acid in hemp containing foods with special regard to the fluorescence properties of Δ^9-tetrahydrocannabinol

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Abstract

A solvent programmed reversed-phase HPLC method with UV detection for the determination of Δ^9-tetrahydrocannabinol (THC) and Δ^9-tetrahydrocannabinolic acid A (THCA-A) in foods containing parts of hemp such as edible oil, herb-teas (infusion), herbal hemp or hempseed is presented. The THC peak is also detected by fluorescence. The detection limits with UV detection are 0.01 ng for THC and 0.05 ng for THCA-A and with fluorescence detection 0.1 ng for THC. The relative standard deviation under repeatability conditions of the chromatographic procedure is about 0.5% and that of the over-all analytical procedure for THC in vegetable oils 2% (concentration range of 10–100 mg/kg).

Keywords: Hemp; Food analysis; Tea; Tetrahydrocannabinol; Tetrahydrocannabinolic acid

1. Introduction

Δ^9-Tetrahydrocannabinol (CAS Reg. No. 1972-08-3, 3-pentyl-6*a*,7,8,10*a*-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b,d*]pyran-1-ol, THC) is a natural constituent of hemp (*Cannabis sativa* L.). Its main precursor in the plant is Δ^9-tetrahydrocannabinolic acid A[1] (CAS Reg. No. 23978-85-0; 2-carboxy-Δ^9-tetrahydrocannabinol, 1-hydroxy-3-pentyl-6*a*,7,8,10*a*-tetrahydro-6, 6, 9-trimethyl-6*H*-dibenzo[*b, d*]pyran-2-carboxylic acid, THCA-A) which decarboxylates to THC when heated. One of THC's well known pharmacological actions in humans either inhaled by smoking or after oral administration consists of psychotropic effects. The cultivation of hemp is therefore prohibited in several countries, except of THC-poor cultivars. In Switzerland the cultivation of hemp is not prohibited, only its abuse as a psychotropic drug is a criminal offence. Since the demand of Swiss consumers for ''alternative'' and/or ''biological'' food is steadily growing, hemp or parts of it have been used to prepare foodstuffs, such as hempseed oil, hemp tea and hemp beer. Etheric oils isolated from hemp by steam-distillation with water are used in cosmetic preparations [1].

To produce a psychotropic effect in adults within

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[1]Not to be confounded with 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, the major urinary metabolite of THC often abbreviated as THCCOOH; CAS Reg. No. 56354-06-4.

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1 to 4 h a single oral dose of 15–20 mg THC is required. In 1995 the Swiss Federal Office of Public Health assessed the health risks of THC in foods, especially in view of unsuspecting consumers [1–3]. Based on the lowest observable physiological effect level of orally administered THC of 5 mg per adult [3–7] and applying an uncertainty factor of 10, a provisional tolerable daily THC intake of 7 μg/kg body mass (b.m.) was assessed [1]. Thereby it was assumed, that THCA-A has no pharmacological action in humans when orally administered. The German health authorities estimated a provisional tolerable THC intake of 1–2 μg/kg b.m./day [25]. Subsequently the following Swiss maximum legal limits for the THC concentration in foods were established: e.g., hempseed oil 50 mg/kg, hempseed 20 mg/kg (dry matter, d.m.), pasta and bakery products 5 mg/kg (d.m.), other foods of plant origin 2 mg/kg (d.m.), spirit drinks 5 mg/kg, beverages 0.2 mg/kg and herb teas 0.2 mg/kg (infusion) [1,8,9]. Nevertheless during the years 1996/97 some cases of intoxications became known due to the use of hempseed oil containing about 1500 mg/kg THC to prepare salad dressings as well as the drinking of a hemp infusion [10,11].

Analytical methods for the routine determination of THC in foods are not available. Most of the described analytical methods are based on gas chromatography that have been developed for forensic application to detect cannabis and its derivatives marihuana, hashish oil, etc. [12–14]. These methods usually yield the so-called ''total-THC'' concentration, that comprises the sum of the amount of original THC and that amount which is formed from the THCA-A by decarboxylation during smoking or during the injection into the gas chromatograph, respectively. However, a differentiation between THC and THCA-A is possible by previous methylation or silylation of THCA-A [15]. Similar methods are routinely used for the detection of THC and its metabolites in plasma and urine in connection with the smoking of cannabis products as illicit psychotropic drugs [16–18].

The aim of this work was to develop a simple analytical method for food control purposes allowing the reliable determination of THC and THCA-A in foods containing hemp or parts of it. The reason for including THCA-A, not psychotropic itself, in this procedure for foods was the possibility of its quantitation without further analytical effort and the possibility that subsequent heat treatment of the examined foodstuffs may give rise to an increase of the respective THC content. The method is based on a high-performance liquid chromatography (HPLC) procedure with UV detection originally developed to study the chemical composition of hemp and to identify thereby different cultivars [19]. In order to increase the sensitivity of the procedure and to reduce solvent consumption HPLC columns with a reduced diameter, 2 mm instead of 4.5 mm, were applied. The luminescence properties of THC were utilised to additionally characterize the compound [20]. A provisional protocol of this method to determine THC in hempseed oil was first distributed to the official Swiss laboratories of food control in 1996 [21]. The possibility to detect THC by fluorescence (FD) was first mentioned in the annual report of our institute for the year 1997 [22].

2. Experimental

2.1. Instrumentation

Separations were carried out on a LC-10A HPLC system from Shimadzu (Burkard, Geroldswil, Switzerland) consisting of a high-pressure gradient system with two pumps LC-10AD, an autoinjector SIL-10A, a UV–Vis detector SPD-10AV, a spectrofluorescence detector RF-10A, a communication module CBM-10A and a workstation with CLASS-LC10 software. In addition a degasser from Gastorr (Omnilab, Mettmenstetten, Switzerland), a dynamic mixing chamber (75 μl) and a column oven (Pelcooler) from Portmann Instruments (Biel-Benken, Switzerland) were used. For the sample preparation a mixer Model B-400 from Büchi (Flawil, Switzerland), a Polytron PT10-35 homogenizer (Kinematica, Littau, Switzerland) and an ultrasonic bath 2210 from Branson (Bioblock, Frenkendorf, Switzerland) were employed.

2.2. Chemicals

Methanol (absolute supra gradient) and acetonitrile

(HPLC-S) were purchased from Biosolve (Brunschwig, Basel, Switzerland). Dichloromethane (for pesticide residue analysis) was obtained from Promochem (Wesel, Germany) and phosphoric acid (85%, analytical grade) from Merck-Darmstadt (Dietikon, Switzerland). The water used for the HPLC was from a Barnstead EASYpure-UV system (Bioblock).

2.3. Standards

THC in ethanolic solution (20 mg/ml) was kindly donated by the National Institute on Drug Abuse (delivered by Research Triangle Institute, NC, USA). On the other hand it was purchased at Sigma (Fluka, Switzerland; Product No. T 4764) as a methanolic solution (1 mg/ml). The THC concentrations given by the different deliverers were checked by HPLC (peak area) and agreed within 4.5%.

THCA-A was isolated, purified and identified [UV, IR, MS, gas chromatography–mass spectrometry (GC–MS) (silyl derivative)] in our laboratory according to the procedure and data given by Lehmann and Brenneisen [23]. The purity of the THCA-A standard was estimated at 97% by HPLC and GC–MS. The isolated substance showed the same retention times and spectra in HPLC and GC–MS as an authentic sample kindly donated by Lehmann (Institute of Pharmacy, University of Berne, Switzerland). For simple concentration measurements of THCA-A standards the following UV data (λ_{max} in ethanol) were used: 258 nm, ϵ=9717 l mol^{-1} cm^{-1} and 300 nm, ϵ=4914 l mol^{-1} cm^{-1}.

2.4. Chromatography

The separations were carried out on a Nucleosil 120-3 C_{18}, 125×2 mm column (Machery–Nagel, Oensingen, Switzerland) at a flow-rate of 0.2 ml/min at 26°C. The injection volume was always 10 μl. Solvent A was acetonitrile and solvent B water with 8.6 g 85% phosphoric acid per liter. A linear gradient program was used: 0 min, 55% A and 45% B, 25 min, 80% A and 20% B; flushing: 26 min, 90% A and 10% B, 30 min, 90% A and 10% B, 31 min, 55% A and 45% B; reequilibration 31–40 min.

2.5. Detection and quantitation

Monitoring with the UV detector was performed at 210 nm for THC and at 272 nm for THCA-A. The fluorometric detector for THC detection was operated at an excitation wavelength of 210 nm and an emission wavelength of 305 nm. Quantitation was performed by the external standard method, measuring the peak areas. The signals of standard solutions showed a linear behavior in the range of 0.1 ng to 90 ng for THC (UV and fluorescence detection) and in the range of 0.2 ng to 80 ng for THCA-A (UV detection). The linear model was evaluated by a simple analysis of residuals. For very precise calculations we usually recommend to use only calibration data over two-orders of magnitude, resulting in a narrower confidence interval.

2.6. Sample preparation

2.6.1. Hempseed oil

A 20–40-mg amount of hempseed oil was dissolved in 20 ml methanol and 10 μl injected into the HPLC system.

2.6.2. Hempseed

A 100-g amount of seeds was briefly blended (three times for 10 s) in the mixer. A portion of 3 g was placed into a 100-ml Erlenmeyer flask with stopper and extracted with 60 ml methanol–dichloromethane (9:1, v/v) by sonication during 15 min. A 1-ml volume of the extract was diluted with methanol up to 10 ml and used for HPLC.

2.6.3. Biscuits

A 50–100-g amount of biscuits (depending on the type) were briefly blended (three times for 10 s) in the mixer or homogenized in a mortar. A portion of 2 g was placed into a 100-ml Erlenmeyer flask and extracted with 30 ml methanol–dichloromethane (9:1, v/v) by homogenization with a Polytron mixer for 2 min. An aliquot of the supernatant was filtrated through a glass filter. A 100-μl volume of the filtrate was diluted with 500 μl methanol prior to injection.

2.6.4. Herb

A 2-g amount of dried, commercially pulverized herbal hemp was extracted with 60 ml methanol–

dichloromethane (9:1, v/v) by sonication during 15 min. For HPLC 1 ml of the extract was diluted with methanol up to 10 ml.

2.6.5. Hemp tea (infusion)

A 200-g amount of boiling water was poured on 3 g dried, commercially pulverized herbal hemp and the mixture was slightly stirred with a magnetic stirrer for 30 min at 90°C. Afterwards, a portion of the aqueous solution was decanted (not filtered) and cooled to room temperature. For analysis 2 ml of the infusion was diluted with methanol up to 10 ml, ready for HPLC. This procedure follows those given in the Swiss regulations [9].

3. Results and discussion

3.1. Stability of THC and THCA-A in solutions

3.1.1. THC

Stock solutions of THC in methanol stored at −20°C were stable for at least 1 year. Diluted methanolic THC solutions, e.g., standard solutions used for calibration, kept at +5°C were stable for at least 1 month and at room temperature and darkness (autosampler) for at least 5 days.

According to our experience, THC in an acid environment was not stable. A decomposition of 25% could be already detected after 5 h at room temperature (23°C) in a solution of 4.6 μg/ml THC in methanol–1.4 *M* aqueous hydrochloric acid (1:1, v/v). On the other hand THC dissolved in basic solutions was more stable. No decomposition was detectable after 22 h at 45°C in a solution of 3.1 μg/ml THC in 0.62 *M* methanolic potassium hydroxide. A decomposition of 9% after 21 h at 45°C in a solution of 2.6 μg/ml THC in methanol–1.24 *M* aqueous potassium hydroxide solution (1:1, v/v) was observed.

3.1.2. THCA-A

Solutions of THCA-A were less stable. Stock solutions in methanol stored at −20°C were stable for at least 3 months. Diluted methanolic THCA-A solutions, kept at +5°C were stable for 2 weeks and at room temperature for about 4 days (decomposition of 5% occurred within 12 days in a solution). A decomposition of 36% of the THCA-A (1 μg/ml) dissolved in methanol–1.4 *M* aqueous hydrochloric acid (1:1, v/v) and kept at room temperature (23°C) could be already detected after 4 h. In basic solutions THCA-A (0.6 μg/ml) was more stable than in acid but less than in neutral [decomposition of 34% after 24 h at 45°C in 0.62 *M* methanolic potassium hydroxide solution; decomposition of 34% after 17 h at 45°C in methanol–1.24 *M* aqueous potassium hydroxide solution (1:1, v/v)].

3.2. Extraction and recovery

3.2.1. Hempseed oil

Due to the sufficient sensitivity of the method no extraction and concentrating process is necessary. The sample is simply diluted and therefore the recovery should be 100%. Spiking experiments with a mean recovery of 97% confirmed these assumptions (Table 1).

3.2.2. Hemp seeds

Because of the inhomogeneous distribution of THC on the seeds, 100 g is the minimum sample mass for reproducible results. This sample is mixed to a homogenous pulp and only an aliquot of it is extracted.

Probably the inner part of the seeds contains no THC at all, but the seeds are contaminated on the surface. Several seeds appeared like sugar-coated with resin. A triple extraction of ''whole'' seeds (washing) with extraction solution also using sonication gave 90% efficiency of the total extractable THC after homogenizing the seeds. This is not contradictory to the assumption, that the inner part of the seeds contain no THC, but it is also not sufficient to proof it wholly. At least it proves that most of the THC is on the surface of the seeds.

The usefulness of the extraction procedure with methanol–chloroform at room temperature, resulting in good extraction efficiency without changing the original cannabinoid pattern is well documented [15,23]. We replaced chloroform with dichloromethane, which is less toxic and more volatile.

Spiking experiments using several hundred grams of seeds are only possible by dissipating several milligrams of standard compound. Therefore consecutive extraction experiments of naturally ''con-

Table 1
Recovery data for THC in different matrices

Matrix	n[a]	THC content (mg/kg)						
		HPLC–UV			HPLC–FD			Recovery ±SD (%)
		Mean	SD	RSD (%)	Mean	SD	RSD (%)	
Hemp seed oil No. 1	6	23.3	0.7	3.1	–[b]	–	–	97±9
Hemp seed oil No. 1, 20 mg/kg THC added	4	42.7	1.7	3.9	–	–	–	
Hemp seeds	2	Single extraction			Single extraction			UV 89±2
		123	2.3	1.8	129	3.0	2.3	FD 89±3
	2	Fivefold extraction			Fivefold extraction			(single compared to fivefold extraction)
		138	1.3	1.0	146	3.4	2.4	
Biscuits No. 20	3	3.38	0.27	8.0	–	–	–	93±6
Biscuits No. 20, 5 mg/kg THC added	3	8.03	0.07	0.9	–	–	–	
Herbal hemp	4	Single extraction			Single extraction			UV 94±11
		382	33	8.5	396	33	8.3	FD 94±10
	4	Triple extraction			Triple extraction			(single compared to triple extraction)
		408	29	7.2	419	27	6.4	

[a] Number of repetitive determinations.
[b] Not determined.

taminated'' seeds were chosen to evaluate the extraction efficiency. A single extraction gave an efficiency of 89% compared to that of a fivefold extraction (Table 1).

3.2.3. Biscuits

Due to the possible inhomogeneity of biscuits an appropriate amount has to be homogenized before the extraction step. The used procedure showed a extraction efficiency of 93% (Table 1).

3.2.4. Herbal hemp

Herbs sold for the preparation of tea or as spice were generally pulverized and represented a homogenous material. Other dried plant material was ground to a size of 1 mm (mesh size 1 mm) before extraction. For the extraction process, the same solvent mixture as for hemp seeds was used, but in a higher excess. Extraction efficiency was also evaluated using consecutive extraction experiments. In the case of herbal hemp single extraction showed an extraction efficiency of 94% compared to triple extraction (Table 1). Recovery experiments were executed only with 100 mg plant material otherwise the amount of THC standard needed would have been prohibitive. According to our experiments the recovery was between 90 and 100%.

3.2.5. Hemp tea (infusion)

Infusion is a kind of extraction using hot water as extraction solvent. In the case the THC content of an infusion (ready-to-drink) is of interest the following considerations are highly important. THC and THCA-A as lipophilic agents are water-soluble only in trace amounts. Therefore, in the decanted infusion a part of the cannabinoids may probably be bound (adsorbed) to small particles. Because of the hydrophobicity of the analytes the decanted infusion has to be converted into a ''stable solution'' by adding methanol before further treatment. Otherwise irreproducible losses of analyte on surfaces (such as vials, syringes, pipettes, filters) were observed in accordance with earlier data given in the literature [24]. A filtration of the methanolic solutions prepared for HPLC is possible but was usually not imperative. If a filtration step of a hemp tea is necessary, this procedure has to be realized after the

dilution with methanol because of the mentioned adsorption phenomena.

3.3. Chromatography

The presented chromatograms are typical for the respective sample matrices (Fig. 1). The UV traces of the hempseed extract and the hempseed oil showed an intense peak eluted shortly before THC not seen in sunflower or grapeseed oil. This peak had nearly the same retention time as cannabinol. But additional investigations using GC–MS revealed that it was none of the common cannabinoids.

The applied separation conditions showed a resolution of about 1 for the compounds Δ^9-THC and Δ^8-THC (Δ^9-THC eluting before Δ^8-THC). There were no interfering peaks in all studied matrices including 10 different baked cereal products containing hemp constituents. According to our experience a shortening of the gradient or working under isocratic conditions resulted in a loss of resolution or problems with interfering peaks, which adversely affected the determination of the THC. We therefore recommend to use gradient elution and to optimize the HPLC system for best resolution of the pair Δ^8- and Δ^9-THC. The fast and simple sample preparation, which includes no clean-up step, thereby requires a somewhat time consuming HPLC method. Additionally, to prevent a deterioration of the column due to ''dirty'' samples, flushing after each run with acetonitrile–water (9:1) was advantageous to remove possibly retained food constituents.

In contrast to the UV detection the fluorometric detection appeared to be very selective. As can be seen, in three of four cases the THC peak was the only signal within a time window of 5 min. Despite the lower sensitivity of the fluorometric detector the quantitation of THC in foods in the range of the Swiss legal limits can be correctly executed.

3.4. Detection and quantitation

The absorbance of THC was measured at 210 nm and not at the second maximum of about 280 nm because of the much better sensitivity. In contrast THCA-A was traced at 272 nm the second maximum in the acid HPLC eluent (bathochromic shift compared to neutral ethanol) as best compromise considering selectivity and sensitivity, but also 222 nm (first maximum) may be of interest.

As previously described by Bowd et al. [20], THC dissolved in ethanol fluoresced. Experiments revealed, that THCA-A exhibits similar fluorescence properties as THC. However in acid solutions, as in our HPLC eluent, only THC retained its fluorescence (Fig. 2). According to the UV spectra of THC in methanol a possible excitation wavelength around 210 nm (strong UV absorption) or 270 to 285 nm (2nd maximum) was expected. The excitation spectra in methanol confirmed these assumptions (strong maximum between 210 and 230 nm and second maximum around 275 nm). Because of the higher sensitivity achieved, the fluorescence detector was operated at the lower excitation wavelength. The optimum excitation wavelength using our HPLC parameters was around 230 nm (see Table 2).

Measured with the fluorometric detector and standard mixtures, the limit of detection was 0.1 ng for THC on the chromatographic system with a signal-to-noise ratio of 3.

With the UV detector a limit of 0.01 ng for THC and 0.05 ng for THCA-A was achieved under the same conditions. Further, the use of a sensitive diode array detector can be recommended in place of an UV detector.

Additionally, we also tested electrochemical detection [Coulochem II detector with amperometric analytical cell Model 5040 from ESA (Chelmsford, MA, USA), working potential +560 mV] as a further analytical tool. The limit of detection was 1 ng for THC with a signal-to-noise ratio of 3 at a similar selectivity like the fluorometric detector. In contrast to the fluorometric detector the electrochemical showed a drift of the sensitivity, and accordingly may be used only semiquantitatively. It is possible that these drawbacks could be overcome by further optimization of the procedure.

3.5. Accuracy

Further analytical results documenting the accuracy of the method are listed in Table 3. The UV and fluorescence results are in good accordance as well as those obtained by GC–MS. For all tested matrices the deviation of the results of the three different analytical methods was less than 15%. Consequently,

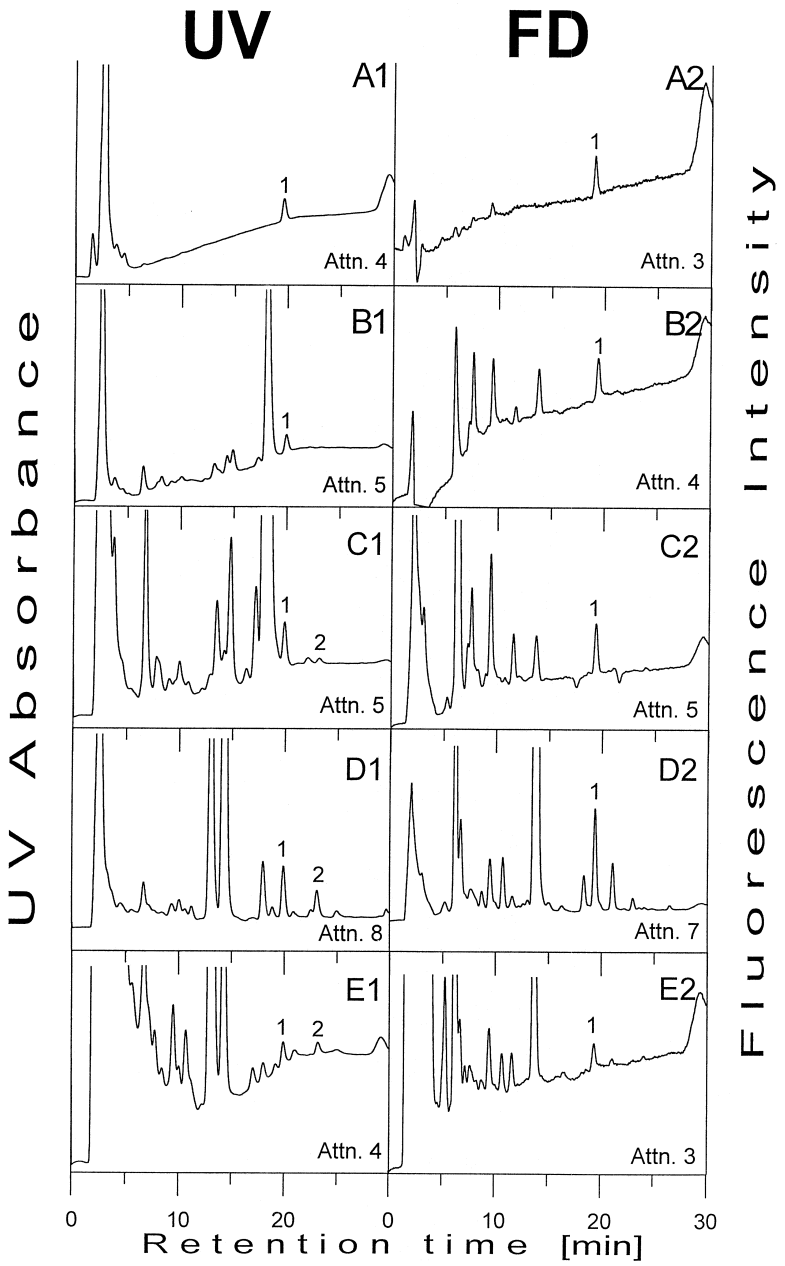

Fig. 1. Ultraviolet (UV) and fluorescence (FD) chromatograms: detectors connected in series, order fluorescence/ultraviolet; injected sample amount always 10 μl; attenuation (Attn.) is comparable within the same detection method (UV: A1–E1 and FD: A2–E2); peaks: (1) THC; (2) THCA-A. (A) THC standard, 0.05 ng/μl. (B) Hempseed oil extract (equivalent to 13.7 μg oil); measured concentrations of THC: UV 0.069 ng/μl and FD 0.07 ng/μl; corresponding to 50 mg THC per kg oil. (C) Hempseed extract (equivalent to 50 μg seed); measured concentrations: UV 0.21 ng/μl THC and 0.08 ng/μl THCA-A, FD 0.18 ng/μl THC; corresponding to 42 mg THC and 16 mg THCA-A per kg seed. (D) Hemp herb extract (equivalent to 33.3 μg herb); measured concentrations: UV 1.95 ng/μl THC and 4.27 ng/μl THCA-A, FD 1.86 ng/μl THC; corresponding to 0.58 mg THC and 1.28 mg THCA-A per g herb. (E) Hemp tea (equivalent to 2 mg infusion); measured concentrations: UV 0.05 ng/μl THC and 0.09 ng/μl THCA-A, FD 0.04 ng/μl THC; corresponding to 0.25 mg THC and 0.45 mg THCA-A per kg infusion.

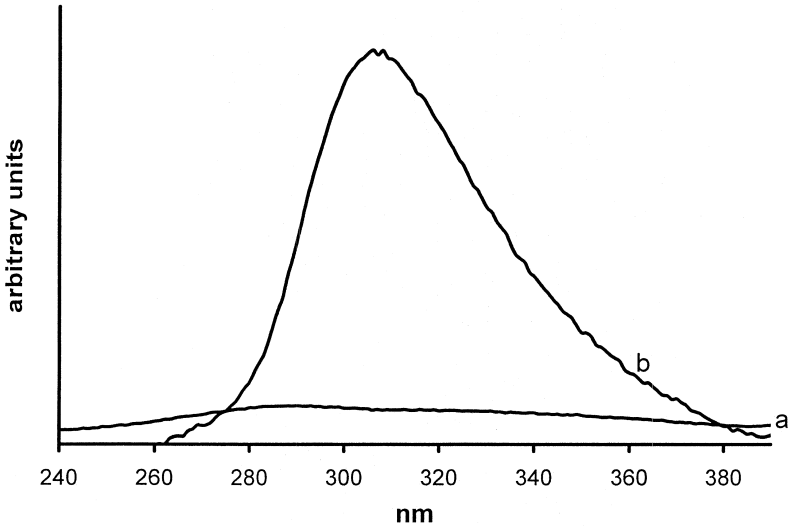

Fig. 2. Fluorescence spectra of THC; excitation wavelength 210 nm. (a) Mobile phase: acetonitrile–water with 8.6 g 85% phosphoric acid per liter (7:3, v/v). (b) THC dissolved in mobile phase (10 mg/l); flow: 100 μl/min, background corrected.

Table 2
Optimization of the THC signal at emission wavelength 305 nm[a]

Excitation (nm)	THC signal (arbitrary units)	Noise (arbitrary units)	Signal/noise
210	132	1	132
230	89	0.6	148
250	7.3	0.2	37
270	6.2	0.2	31

[a] Determination by HPLC, conditions as described in ''Chromatography''; injected amount 9.55 ng THC.

fluorescence detection represents a selective and sufficiently sensitive method to quantify THC amounts in several food products.

3.6. Repeatability

The relative standard deviation (RSD) under repeatability conditions for standard solutions of THC and THCA-A was 0.5% each ($n=6$, quantitation with data from UV detection) within the calibration range.

Table 3
Comparison of different methods

Matrix	THC content (mg/kg)		
	HPLC–UV	HPLC–FD	GC–MS
Hemp seed oil	880	–[a]	840 (methylated)[b]
	20.5	22.2	17.2 (silylated)[c]
	11.7	–	12.3 (silylated)
	4.1	4.2	3.9 (silylated)
Hemp seeds	5.2	5.6	5.4 (silylated)
	3.9	4.3	4.4 (silylated)
Herbal hemp	1480	1470	–
	1020	1040	1200 (silylated)
Hemp tea (infusion of herbal hemp)	1.00	1.01	–

[a] Not determined.
[b] Methylated with Methelute (Pierce No. 49301, 0.2 *M* trimethylanilinium hydroxide in methanol) in the injectorblock.
[c] Silylated with bis(trimethylsilyl)trifluoroacetamide (Fluka No. 15198), 30 min at 60°C.

Table 4
Repeatability data

Matrix	CN[a]	n[b]	THC content (mg/kg)					
			HPLC–UV			HPLC–FD		
			Mean	SD	RSD (%)	Mean	SD	RSD (%)
Hemp seed oil	1	6	23.3	0.7	3.1	–	–	–
	3	4	50.74	0.22	0.4	–	–	–
Hemp seeds	11	6	25.5	2.3	9.1	26.5	2.3	8.8
	12	6	2.24	0.25	11.1	2.75[c]	0.56	20.5
	13	6	3.17	0.12	3.6	–	–	–
Biscuits	20	3	3.38	0.27	8.0	–	–	–
Herbal hemp	30	4	382	33	8.5	396	33	8.3
	31	3	726	39	5.4	–	–	–
Hemp tea	40	2[d]	0.805	0.082	10.1	–	–	–
	41	2[d]	7.85	0.21	2.7	–	–	–

[a] Code number of sample.
[b] Number of repetitive determinations.
[c] At detection limit.
[d] Two separate infusions of the same herb.

For the oil dilutions (same test material but independent sample preparation; $n=6$) the RSD was 2%. The RSD for hemp seeds under repeatability conditions was in the range of 3–11% (Table 4). With different herbal hemp samples we calculated an average RSD of 7% under repeatability conditions (Table 4). The higher RSD with real samples appears to be rather the result of sample inhomogeneities than of a higher RSD of the analytical procedure. However, it appears that the proposed sample preparation procedures yield a sufficiently homogeneous material for food-control purposes.

3.7. Collaborative study

The proposed method with UV detection shall be published in the Swiss Food Manual and was applied in a collaborative study with 12 participants and four different hempseed oil samples. A stock solution of standard, a diluted test solution to estimate the resolution (mixture of Δ^9-THC and Δ^8-THC), a diluted THC solution with defined concentration, a hempseed oil sample with defined concentration and 12 unknowns (three identical samples each of four different hempseed oils in a blind test arrangement)

Table 5
Collaborative study, THC in hempseed oil[a]

Sample No.	$\bar{x}$[b]	s_r[c]	(RSD_r)[d] (%)	s_R[e]	(RSD_R)[f] (%)	r[g]	R[h]
1	23.3	2.32	(9.9)	2.54	(10.9)	6.5	7.1
2	31.5	1.43	(4.5)	3.00	(9.4)	4.0	8.3
3	49.6	2.41	(4.9)	3.44	(6.9)	6.7	9.6
4	58.3	1.73	(3.0)	5.35	(9.2)	4.8	15.0

[a] All calculations performed using robust statistics [26].
[b] Mean value, mg/kg.
[c] Repeatability standard deviation, mg/kg.
[d] Repeatability relative standard deviation.
[e] Reproducibility standard deviation, mg/kg.
[f] Reproducibility relative standard deviation.
[g] Repeatability limit ($r=2.8s_r$), mg/kg.
[h] Reproducibility limit ($R=2.8s_R$), mg/kg.

were distributed to the participants. A single determination of each unknown was required which corresponded to a triplicate determination of the four samples. The mean values and the respective repeatability and reproducibility data are presented in Table 5.

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