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Simple and sensitive determination of Δ^9-tetrahydrocannabinol, cannabidiol and cannabinol in hair by combined silylation, headspace solid phase microextraction and gas chromatography–mass spectrometry

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Abstract

A new method for determination of Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) in hair based on alkaline hair hydrolysis, extraction by iso-octane, combined derivatization with N,O-bis-(trimethylsilyl)-trifluoroacetamide and headspace solid phase microextraction of the extract residue, and gas chromatography–mass spectrometry was developed and evaluated. The limits of detection of the three compounds were 0.01–0.02 ng/mg. The method was routinely applied to more than 250 hair samples. In 77 positive samples, the concentrations ranged from LOD to 4.2 ng/mg for THC (mean 0.49 ng/mg), to 12.1 ng/mg for CBD (mean 0.37 ng/mg) and to 0.85 ng/mg for CBN (mean 0.12 ng/mg) using a sample amount of 30 mg. The frequently observed increase of the segmental drug concentrations from proximal to distal is explained by progressive accumulation in the hair shaft from sebum or side stream smoke.

Keywords: Cannabidiol in hair; Cannabinol in hair; Gas chromatography–mass spectrometry; Hair analysis; Headspace solid phase microextraction; Δ^9-Tetrahydrocannabinol in hair

1. Introduction

Considering the increasing abuse of marihuana or hashish in the last years, the detection of a long-term exposure by hair analysis becomes more and more important in the context of driving ability examination, workplace testing or other forensic matters as well as in psychiatric context. As a rule, the active cannabis constituent Δ^9-tetrahydrocannabinol (THC) is determined for this purpose, and the non-active substances cannabidiol (CBD) and cannabinol (CBN) are included for confirmation [1–11]. In the guidelines of the society of hair testing (SoHT) and the society of toxicologial and forensic chemistry (GTFCh) a cut-off for THC of 0.1 ng/mg was recommended [12], but it was shown in a recent paper by statistic comparison of hair results with the consumption frequency [13] that a cut-off of 0.05 ng/mg is more suitable to detect also occasional cannabis use besides regular consumption.

As, in principle, THC, CBD and CBN could be deposited in hair also directly from smoke in the surroundings of other cannabis users [14], the detection of these three compounds does not prove active consumption, but can be interpreted only in a way that the individual had contact with cannabis products. For unambiguous prove of cannabis consumption, the metabolite 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) was found to be suitable [4,10,15–19]. However, despite the much higher concentration and the long half-life of THC-COOH in blood, it is incorporated in hair only with a very low efficiency and MS-MS techniques with negative chemical ionisation and with a sensitivity of 0.2 pg/mg are necessary for its determination [12]. Therefore, it is common practice to confine the measurement to THC, CBD and CBN and offer the possibility of confirmation by additional determination of THC-COOH only in case of objection to the result.

Several methods for determination of cannabinoids in hair were described in the literature. For liberation of the analytes from the hair matrix, preferentially digestion with aqueous NaOH [1,2,4,5,7–9,11,15–20] and only exceptional extraction with methanol in ultrasonic bath [3,10] or extraction

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with chloroform/isopropanol after hydrolysis with beta-glucuronidase/arylsulfatase [6] were applied. The extraction of the hair hydrolysates was performed by liquid–liquid extraction, e.g. with hexane/ethylacetate (9:1 v/v) [1,2,4,6,11,15,18] or 1-chlorobutane [21] or by solid phase extraction [16,19].

THC, CBD and CBN can be detected by GC–MS-EI without derivatization [2,5]. However, silylation with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) or *N*-methyl-*N*-(tert-butyldimethylsilyl)-trifluoroacetamide (MBDSTFA) [7–10,18] as well as acylation with trifluoroacetanhydride [4] or propionic anhydride [3] lead to improved sensitivity. A mixed derivatization with pentafluoroacetanhydride (PFPA) and heptafluoropropanol (HFP) or hexafluoroisopropanol (HFIP) was generally performed, if THC-COOH was included and GC–MS–NCI was used [1,2,6,15–17,19,20]. A relatively simple alternative is also the methylation with trimethylsulfonium hydroxide [21]. Furthermore, also the ion pair extractive methylation with a mixture of dimethylsulphoxide, tetrabutylammoniumhydroxide (TBAH) and CH_3I in analogy to serum samples [22,23] was successfully adapted to THC in hair [13].

Gas chromatography–mass spectrometry with electron impact ionisation (GC–MS–EI) in selected ion monitoring mode (SIM) is the instrumental technique most frequently used for THC, CBD and CBN [1–3,5–7,9,11]. However, the very low concentrations of THC-COOH in hair can only be measured by using negative chemical ionisation (GC–MS–NCI) of the perfluorinated derivatives [4,15,17,19] or by GC–MS–MS techniques [8,10,16,18,20].

Solid phase microextraction (SPME) was first applied to hair analysis for cannabinoids by Strano-Rossi et al. [5] by dipping a 30 μm polydimethylsiloxane (PDMS) fibre directly into the solution obtained by hydrolysis of 50 mg hair in 200 μl 1 NaOH and subsequent neutralization to pH 7.5. The detection limits were between 0.1 and 0.2 ng/mg for THC, CBD and CBN and the same fibre could be used for 30 samplings. First experiments to use headspace solid phase microextraction (HS-SPME) from the neutralized NaOH hair hydrolysate (pH 4–7) showed that, in principle, the three substances can be detected but the sensitivity was still too low [24]. Better results were obtained by Mußhoff, Lachenmeier et al. [7–9] by combination of HS-SPME or headspace solid phase dynamic extraction (HS-SPDE) with subsequent headspace on-fibre or on-coating silylation. The extraction was performed directly from the headspace above the hair digestion by 1 ml 1 M NaOH + 1 g Na_2CO_3. Using EI–GC–MS, detection limits between 0.05 and 0.14 ng/mg were attained for THC, CBD and CBN. As a disadvantage of these fully automated methods, for every sample a new vial with derivatization reagent was necessary in order to avoid carry over between the samples. The LOD's of the three substances could be decreased to 0.04–0.05 ng/mg by performance of HS-SPDE in combination with GC–MS–MS [8].

In the present study, a new method with improved sensitivity for determination of THC, CBD and CBN using alkaline hair digestion, subsequent liquid extraction, automated HS-SPME with in-sample silylation and GC–MS–SIM was developed, validated and routinely applied in context of driving ability examination.

2. Materials and methods

2.1. Hair samples

Hair samples were obtained from individuals who were summoned to hair analysis for illicit drugs after offences concerning possession of cannabis products or driving under the influence of this drug. Two samples of at least 100 mg hair (one for analysis and the second for deposition in case of objection) were cut as close as possible above the skin and independently stored in paper envelopes at room temperature until analysis. Since according to the guidelines only the proximal hair segment 0–6 cm was investigated, samples longer than 6 cm were fixed with a string in order to enable the separation of this segment. Samples with a lengths up to 6 cm were collected without fixation.

For internal quality control, a hair pool was prepared from cannabis positive samples by cutting the hair to pieces of 1–2 mm length and thoroughly mixing.

2.2. Instruments

The GC–MS analyses were performed with a gas chromatograph HP 6890N plus equipped with a capillary column HP5-MS inert, a mass selective detector HP 5973 MSD (Agilent GmbH, Waldbronn, Germany) and a multi-purpose sampler MPS 2 (Gerstel GmbH, Mühlheim/Ruhr, Germany). The SPME fibres were purchased from Supelco GmbH (Steinheim, Germany). The GC–MS instrument was operated with the software ChemStation (version D.00.01, Agilent GmbH, Waldbronn, Germany). For the automatic performance of the HS-SPME measurements at the MPS 2 the software CycleComposer (version 1.5.2, Chromtech GmbH, Idstein, Germany) was applied.

The hair samples were washed in an ultrasonic bath Sonorex Bandelin (Berlin, Germany). For liquid/liquid extraction a thermomixer Comfort (Eppendorf AG, Hamburg, Germany) was used. The evaporation of the solvents was carried out with a Liebisch evaporator (Liebisch GmbH, Bielefeld, Germany).

2.3. Reference substances and reagents

All solvents used in this study were purchased in proanalysis quality from Merck Eurolab GmbH (Darmstadt, Germany). The silylation reagents N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) and *N*-methyl-*N*-(tert-butyl-dimethylsilyl)-trifluoroacetamide (MBDSTFA) were obtained from Sigma–Aldrich GmbH (Steinheim, Germany). Trimethylsulfoniumhydroxide (TMSH, 0.2 mol/l in methanol) was purchased from Macherey–Nagel GmbH (Düren, Germany). Tetra-*n*-butylammoniumhydroxide (TBAH, 20% in H_2O) and methyl iodide were purchased from Merck (Darmstadt, Germany).

For calibration and quantification, standard solutions of cannabidiol and cannabinol (CBD and CBN, each 1 mg/ml

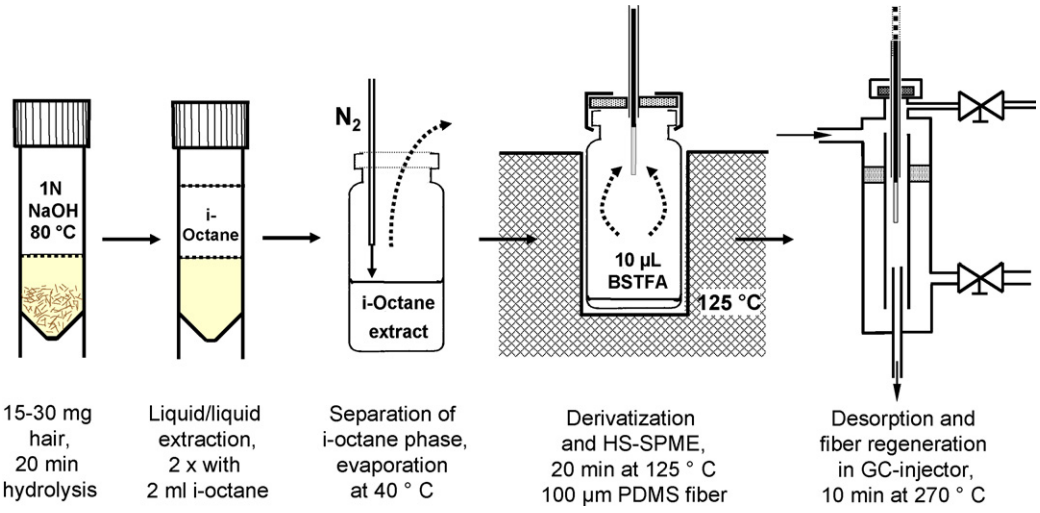

Fig. 1. Steps of the method for determination of THC, CBD and CBN from hair.

in methanol) from Sigma–Aldrich GmbH (Steinheim, Germany) and Δ^9-tetrahydrocannabinol as well as D_3-Δ^9-tetrahydrocannabinol (D_3-THC, 0.1 mg/ml in methanol) from LGC Promochem (Wesel, Germany) were used.

2.4. Procedure for determination of THC, CBD and CBN in hair

The hair samples were subsequently washed for 5 min with each 10 ml de-ionised water and 10 ml acetone in ultrasonic bath and dried. In case of longer samples, the segment 0–6 cm was separated. Shorter hair samples were investigated in full lengths. The hair was cut to pieces of about 1–2 mm length. The further steps of the optimized method are schematically shown in Fig. 1. 15–30 mg of the hair pieces were exactly weighed into a 10 ml sample tube. To this, 10 ng D_3-THC (internal standard for quantification of the three substances, 10 μl of 1 μg/ml solution in methanol) and 0.5 ml 1 M NaOH were added, the mixture heated to 80 °C for 20 min. After cooling to room temperature, the obtained solution was twice extracted with each 2 ml iso-octane. The organic phases were united in a 10 ml headspace vial and evaporated to dryness in a nitrogen stream at 40 °C. After addition of 10 μl BSTFA with 1% TMCS, the headspace vial was closed and placed on the sample rack of the sampler MPS 2. The HS-SPME was automatically performed after 5 min incubation at 125 °C with a 100 μm PDMS fibre at 125° C for 20 min. During preheating and headspace extraction, the heating station was agitated with 250 rpm, 60 s right, 15 s interval, 60 s left, 15 s interval. The injection into the GC–MS and the regeneration of the fibre was carried out at 270 °C for 10 min. The temperature program for the GC separation at the HP5-MS capillary column was 2 min at 130 °C followed by 20° per min up to 300 °C. The temperature of the interface, of the ion source and of the inlet were 300, 250 and 270 °C, respectively. The following *m/z* values were used for the detection of the TMS-derivatives of the three analytes and of the internal standard in the SIM mode with the quantifier ions printed bold: THC-TMS (Rt = 9.96 min) 303, 371, **386**; D_3-THC-TMS (Rt = 9.95 min) 306, 374, **389**; CBD-2TMS (Rt = 9.44 min) 351, **390**, 458; CBN-TMS (Rt = 10.33 min) 310, **367**, 382.

2.5. Determination of THC in hair by extractive derivatization with CH_3I

For comparison, eight hair samples were also analysed for THC by the method previously used by the authors. In this method, which was performed in analogy to the procedure described for serum by Möller et al. [22,23], the hair was digested by NaOH, extracted with *n*-hexane/ethylacetate (9:1, v/v), the solvent evaporated and the residue methylated under ion transfer conditions with a mixture of dimethylsulphoxide, tetrabutylammonium hydroxide and methyl iodide. Experimental details are described in [13].

3. Results and discussion

3.1. Optimisation of the analytical parameters

The main disadvantage of the HS-SPME methods for cannabinoid determination previously described [7,24] is the low extraction yield from the alkaline solution obtained from hair hydrolysis. In order to solve this problem, the concept of our method development was to separate at first the analytes from the alkaline solution by liquid/liquid extraction and to apply HS-SPME in combination with in-sample derivatization to the residue obtained by evaporation of this extract. Different from the applications of HS-SPME in hair analysis previously described [7,24–27], the analytes are not extracted from an aqueous phase.

Several series of experiments were carried out for optimisation of the different parameters of the procedure described in Section 2.4. All experiments were performed in triplicate. For the extraction of the analytes from the solution obtained by 1 M NaOH hair digestion, iso-octane, dichloromethane, 1-

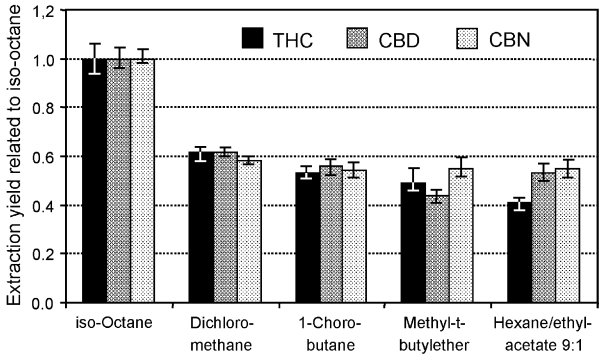

Fig. 2. Relative liquid/liquid extraction yields (mean and range of three measurements) of THC, CBD and CBN from the solution obtained by hair hydrolysis with 1 M NaOH with different solvents related to the most efficient solvent iso-octane.

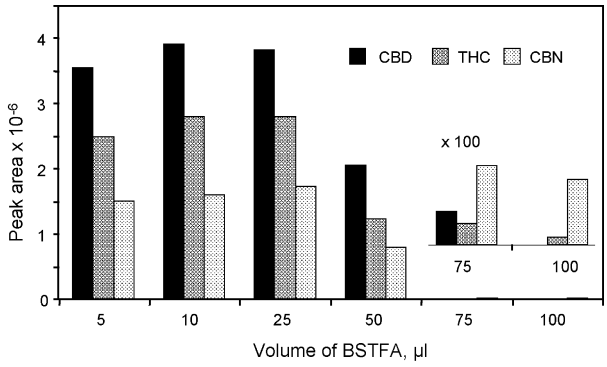

Fig. 4. Peak areas of THC, CBD and CBN obtained from 20 mg drug-free hair spiked with each 10 ng THC, CBD and CBN with different amounts of the silylation reagent BSTFA + 1% TMCS. The other parameters were chosen as described in Section 2.4.

chlorobutane, methyl-*t*-butyl ether and *n*-hexane/ethyl acetate (9:1 v/v) were tested. For this purpose, a drug-free hair sample was spiked with each 10 ng THC, CBD and CBN, hydrolysed with 0.5 ml 1 M NaOH and extracted with 2 × 2 ml of the solvents. The peak areas of the three compounds obtained by GC–MS–SIM after direct injection of the different extracts are shown in Fig. 2 in relation to iso-octane. It is seen that all three compounds are much more efficiently extracted with iso-octane as compared to the other solvents.

For selection of the optimal SPME fibre, different fibres commercially available from Supelco were tested. Twenty milligrams drug-free hair were spiked with each 10 ng THC, CBD and CBN and treated according to the procedure described in Section 2.4. The peak areas obtained for THC are shown in Fig. 3. The non-polar 100 μm PDMS fibre appeared to be most efficient and, as expected, the relative extraction yield decreased with decreasing thickness and increasing polarity of the fibre coating. Similar results were also obtained for CBD and CBN.

The proper choice of the derivatization agent and of its optimal amount appeared to be decisive for the sensitivity of the method. The agent should react completely with all three compounds under the HS-SPME conditions, lead to products with a sufficient volatility and should not disturb the extraction process by retaining the analytes as a solvent in the sample phase or by replacing them on the fibre by competing for the adsorption or absorption sites. Experiments were carried out with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, b.p. 147 °C) with 1% trimethylchlorosilane (TMCS, b.p. 57 °C), *N*-methyl-*N*-(tert-butyldimethylsilyl)-trifluoroacetamide (MBDSTFA, b.p. 168–170 °C), trimethylsulfonium hydroxide (TMSH) in methanol and trifluoroacetanhydride (TFAA, b.p. 72 °C). Different from the other reagents, TFAA had to be removed completely by a nitrogen stream after incubation for 30 min at 80 °C before HS-SPME. The derivatized three substances were detectable with all reagents by HS-SPME and GC–MS but the best sensitivity was obtained with BSTFA/TMCS. For optimisation of the amount, the HS-SPME of spiked hair extracts was performed at 125 °C with 5, 10, 25, 50 and 75 μl of this reagent. The peak areas obtained for the three substances in one measurement series are shown in Fig. 4. Ten microlitres appeared to be sufficient for maximum peak areas of all three compounds. This amount was completely evaporated in the 10 ml headspace vial at 125 °C. Obviously the vapour pressure is sufficiently high although the boiling temperature of BSTFA (147 °C) is still above 125 °C. Therefore, the derivatization by the gaseous reagent occurs either in the thin film of the extract residue on the surface of the vial or in the gaseous phase with the evaporated analytes. The peak areas strongly decrease above 50 μl.

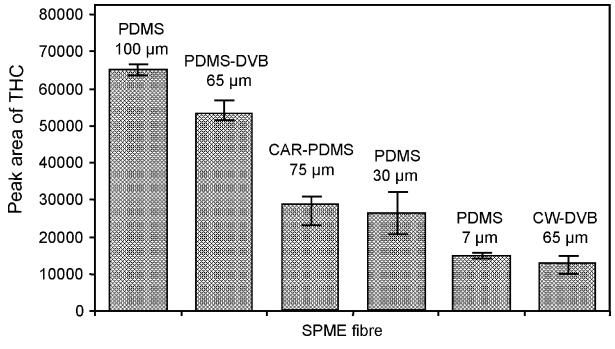

Fig. 3. Peak areas of THC obtained by HS-SPME and GC–MS with different SPME fibres (mean and range of three measurements) from 20 mg drug-free hair spiked with each 10 ng THC, CBD and CBN using the optimised conditions described in Section 2.4. PDMS, polydimethylsiloxane; DVB, divinylbenzene; CAR, carboxen; CW, carbowax.

Finally, also the effect of temperature (between 80 and 150 °C) and time (between 2 and 20 min) of the extraction was examined (Fig. 5). No equilibrium was attained within these limits. However, the load of disturbing matrix peaks increased strongly with increasing temperature. As a reasonable compromise, 125 °C were chosen. The time was limited to 20 min for reasons of instrumental capacity and enabled a sufficient sensitivity.

For the GC–MC–SIM measurement of the TMS-derivatives, the same experimental conditions were used as for the determination of cannabinoids from serum described in the previous paper [28]. A typical chromatogram from a positive hair sample is shown in Fig. 6.

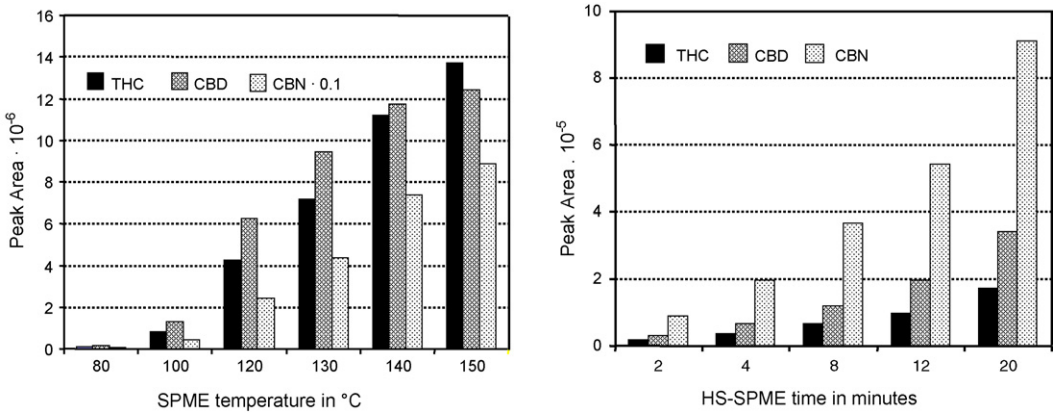

Fig. 5. Effect of extraction temperature and extraction time on the GC–MS peak areas obtained after HS-SPME from 20 mg drug-free hair samples spiked with each 10 ng THC, CBD and CBN. The remaining parameters were chosen as described in Section 2.4.

3.2. Calibration and validation of the method

The calibration and validation of the method was performed according to the guidelines of the society of toxicological and forensic chemistry (GTFCh) for quality assurance in forensic toxicological investigations [29] using the software "Valistat" (Arvecon GmbH, Walldorf, Germany) [30]. Each 30 mg of drug-free hair were spiked with six concentrations between 0.03 and 1.0 ng/mg of the three substances and analysed according to the procedure described in Section 2.4. Samples with each concentration were prepared and measured six times. From the data, the calibration curves and the limits of detection (LOD) and of quantification (LOQ) were calculated according to this software with a significance level of 99% (quantifier ions) and 90% (qualifier ions). The calculation is based on the confidence bands of the linear calibration and uses the standard deviation of the method, the number and concentrations of the calibration points and the number of repetitions for the determination of LOD and LOQ. The correlation coefficients R^2 of the linear calibration curves as well as LOD and LOQ are given in Table 1. In addition to that, LOD and LOQ were also estimated from the signal to noise ratios 3 and 10 of real samples with concentrations in this range leading to almost the same or even lower values.

Deviating from the guidelines of the GTFCh [29] only one concentration was studied with respect to accuracy and reproducibility of the method. For determination of the accuracy, a drug-free hair sample was spiked with 0.20 ng/mg of THC, CBD and CBN and aliquots of 30 mg analysed two times per day during 7 days (14 analyses). The results (bias −3.75 to +8.32%) show that all three substances are correctly determined. The intra-day and the inter-day reproducibility were determined by measuring a pool from cannabis positive hair samples (preparation see Section 2.1, concentrations 0.30 ng/mg THC, 0.11 ng/mg CBD and 0.15 ng/mg CBN) three times on 1

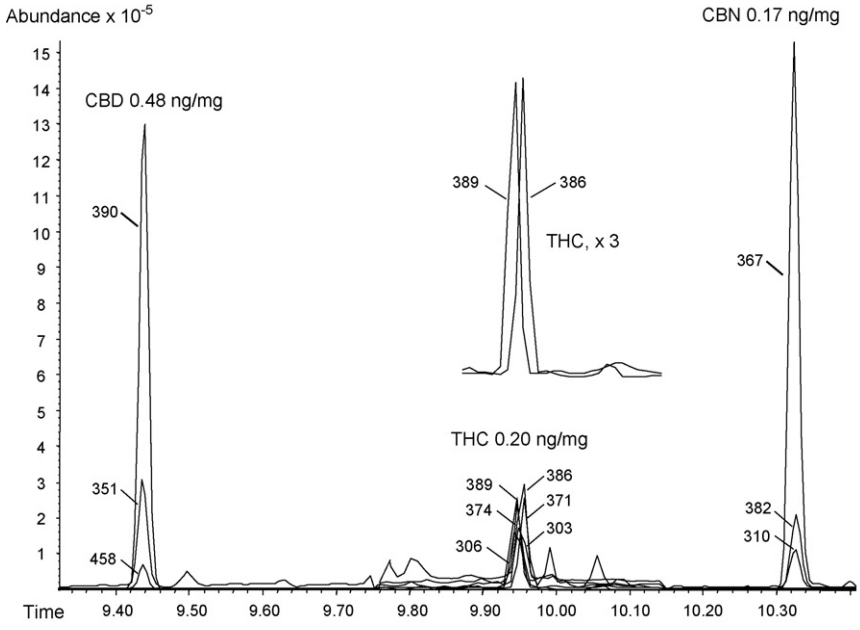

Fig. 6. Chromatogram obtained from the hair sample of a cannabis consumer (age 31, drug dealer) using the optimised procedure as described in Section 2.4.

Table 1
Results of the validation of the determination of THC, CBD and CBN from hair by HS-SPME and GC–MS and yields of the total sample preparation and of the HS-SPME step

Parameter	THC	CBD	CBN
R^2	0.9956	0.9948	0.991
LOD (ng/mg)	0.012	0.013	0.016
LOQ (ng/mg)	0.037	0.038	0.048
Accuracy, bias[a] (%)	−3.75	+8.3	+6.75
Intra-day reproducibility[b], R.S.D. (%)	5.9	10.1	6.5
Inter-day reproducibility[b], R.S.D. (%)	7.5	13.4	7.4
HS-SPME extraction yield (%)	29	66	14
Total yield of sample preparation (%)	8.9	59	6.4

[a] Concentrations of THC, CBD and CBN 0.2 ng/mg.
[b] Concentrations 0.30 ng/mg THC, 0.11 ng/mg CBD and 0.15 ng/mg CBN, R.S.D., relative standard deviation.

day for 7 days. The data are also given in Table 1 and show that the method works with a high reproducibility.

The total yield of the sample preparation was obtained by comparing the peak areas of the three derivatized substances as obtained by the method from blank hair samples spiked with 10 ng of the three substances with that from direct injection of the same amounts after derivatization. The values (8.9, 59 and 6.4%, Table 1) are quite different for the THC, CBD and CBN. This is mainly caused by differences in the absolute yields of the HS-SPME step (29, 66 and 14%) that were independently determined by addition of the drugs not to the hair sample but to the iso-octane extract of a digested blank hair sample. These absolute HS-SPME yields are much higher than those described for the direct HS-SPME from alkaline hair hydrolysis solution (0.3–7.5% [7]) and are the main reason for the increase in sensitivity of the new method. HS-SPME yields are often lower than for other extraction procedures [24]. This does not essentially impede the sensitivity of the method since in contrast to other GC–MS-methods the total extract is injected and since the burden of disturbing matrix constituents is generally much lower in headspace sampling as compared to injection of an extract solution. The much higher yield of CBD is surprising. A possible explanation is that, different from THC and CBN, this compound is derivatized at two OH groups.

For comparison with the routine method previously used for THC determination in hair by the authors (Section 2.5), eight positive samples (0.18–4.5 ng/mg) were analysed with both methods on the same day. This was repeated on the next day once or twice leading to 20 concentration pairs of both methods. As a result, slightly higher concentrations were found by the HS-SPME method as compared to the extractive methylation method ($C_{\text{HS-SPME}}/C_{\text{EX-METH}} = 1.15 \pm 0.12$). The reason was not clear. However, considering the differences between the results from different methods in proficiency tests [31] and a certain inhomogeneity of hair samples, this is quite a satisfying agreement.

3.3. Practical applications

The method was regularly applied to hair samples in context of driving ability examination, of other forensic background and from death cases. Altogether more than 250 samples were

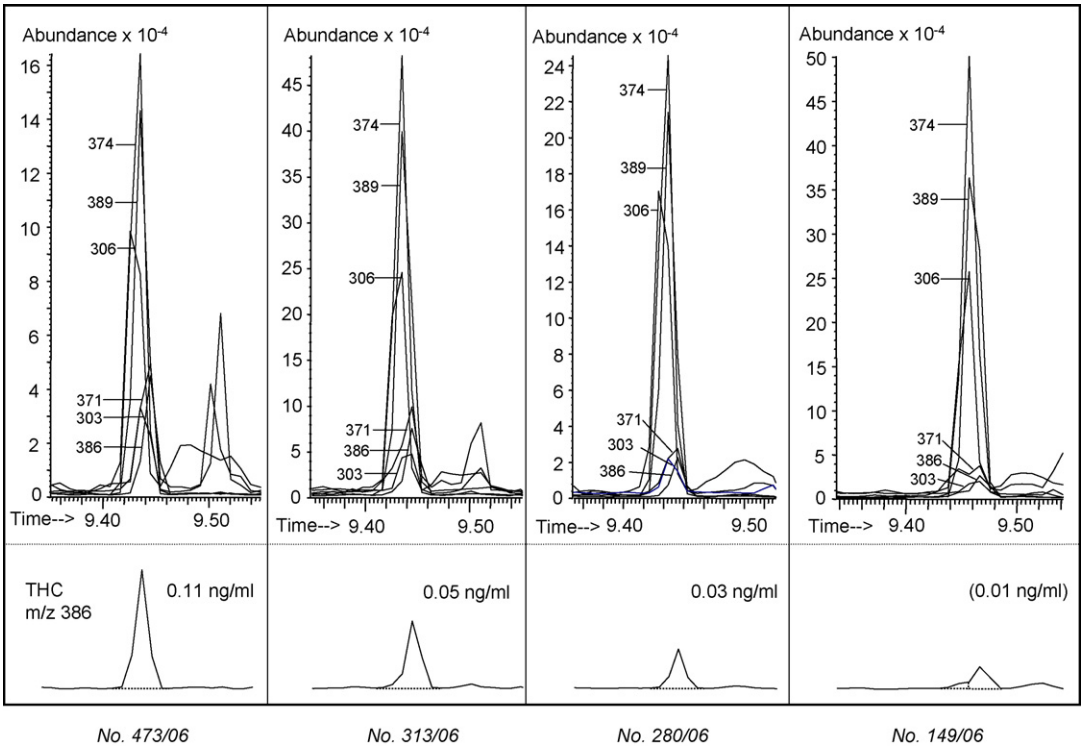

Fig. 7. Determination of THC in the low concentration range in hair samples of four driving ability examination cases. Analysed hair weight: 26.8, 36.1, 38.4 and 36.6 mg. Ten nanograms D_3-THC per sample. Different dates of measurement. The traces of the quantifier ion *m/z* 386 are separately shown in the lower part of the figure. Using a cut-off of 0.05 ng/mg, the cases 473/06 and 313/06 were judged to be positive whereas the cases 280/06 and 149/06 were judged to be negative.

Table 2
Distribution of the concentration ratios CBD/THC and CBN/THC in 77 cannabinoid positive hair samples

	Range of concentration ratio					
	0	0.01–0.10	0.11–0.50	0.51–1.00	1.01–5.00	>5.00
CBD/THC	8[a]	24[a]	23[a]	5[a]	12[a]	5[a]
CBN/THC	0[a]	9[a]	48[a]	12[a]	8[a]	0[a]

[a] Number of samples.

analysed. From these, THC was detected in 77 samples. The higher sensitivity of the method enables to lower the cut-off of THC in hair from 0.1 to 0.05 ng/mg. This is demonstrated in Fig. 7 at four examples from driving ability examination with the concentrations of 0.11, 0.05 ng/mg and in the range of LOD (0.03 and 0.01 ng/mg). There were no samples with a positive CBD or CBN result without being positive also for THC. The concentrations ranged for THC from LOD to 4.2 ng/mg (mean 0.49 ng/mg), for CBD from LOD to 12.1 ng/mg (mean 0.37 ng/mg) and for CBN from LOD to 0.85 ng/mg (mean

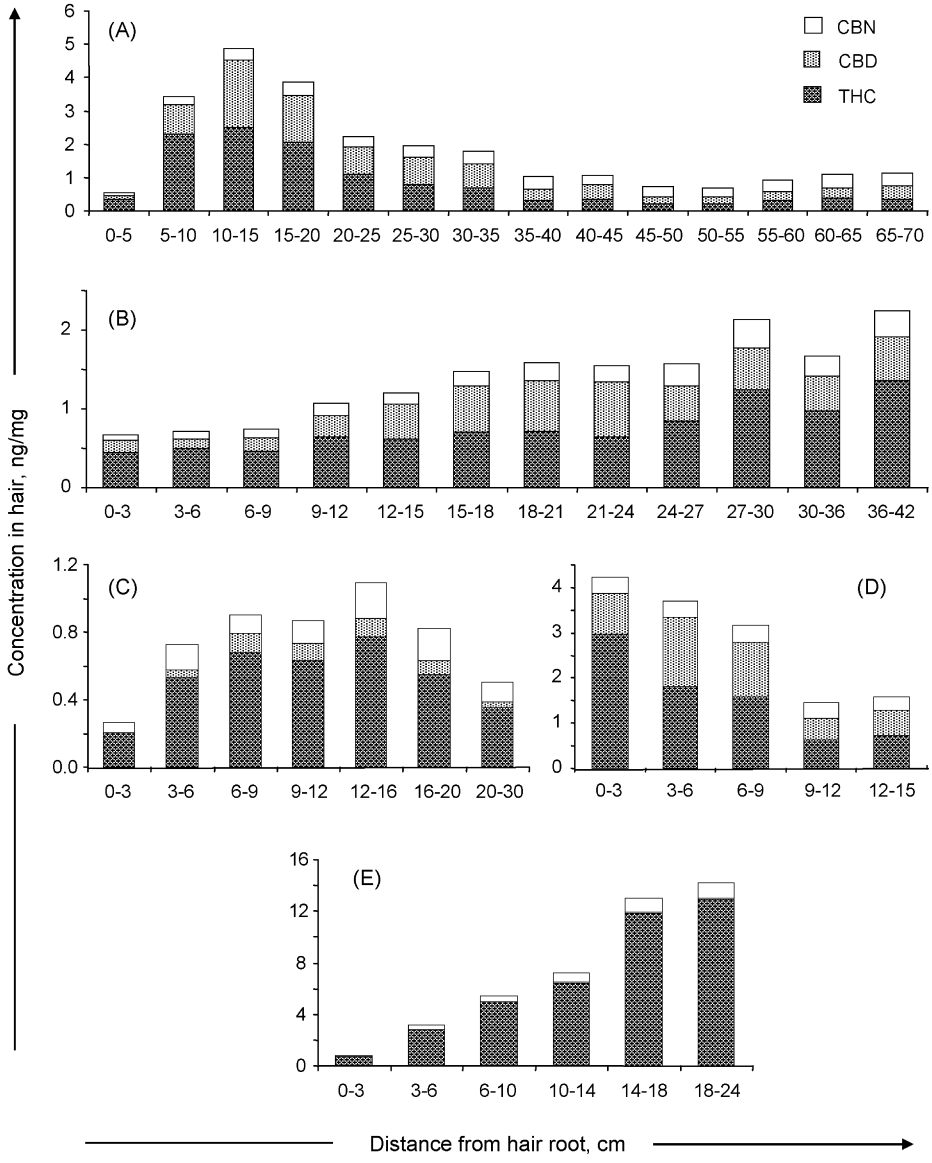

Fig. 8. Concentrations of THC, CBD and CBN in segments of long hair samples of cannabis consumers. A: volunteer, age 23, male, reported regular daily use, no change in last time. B: volunteer, age 32, male, reported 1–2 joints per day, no change in last time. C: sample from driving ability examination after driving under the influence of cannabis, age 26, male, no data about consumption habits. D: volunteer, age 28, female, red dyed hair, reported regular use four times per week 1–2 joints, no change in last time. E: sample from driving ability examination, age 19, male, admitted regular cannabis use, was summoned to hair analysis 6 weeks before sampling, no data about change of consumption habits in last time.

0.12 ng/mg). The data relate to the full hair lengths in case of short hair samples. In case of longer samples, the proximal segment 0–6 cm was measured. The distribution of the CBD/THC and of the CBN/THC ratios is shown in Table 2. It is seen that in the majority of the cases the concentrations of CBD and CBN are smaller than those of THC. However, the ratio CBD/THC varies strongly and there were 8 samples without detection of CBD and 5 samples with CBD more than fivefold higher than THC. On the other hand, CBN was detected in all THC positive cases and was in excess of THC only in eight of the 77 samples.

Six of the hair samples with lengths between 15 and 70 cm were analysed in 5 to 14 segments. Data about segmental hair concentrations of cannabinoids are rarely found in literature [31]. Therefore, the results of five of these cases are shown in Fig. 8 together with self-reported data about the consumption habits. It is noteworthy that for the first segments in almost all samples an increase of the concentrations from proximal to distal is found, even if no decrease of the consumption amount or consumption frequency in the time before sampling was reported. This can be regarded as an indication that an accumulation of the drugs in the completed hair outside of the hair root occurs, for example by incorporation from sebum or to a smaller extent also from side stream smoke. Until now, there is no real prove that this is the dominant incorporation mechanism of THC. However, the opposite course of the concentrations of THC and CBD on one hand (increase from proximal to distal) and THC-COOH on the other (decrease from proximal to distal) as described by Uhl [32] supports this hypothesis. In some of the samples, the concentrations decrease again in the more distal segments, probably caused by wash-out or degradation by hair care and hair cosmetics.

An exception from this general course is sample D with decreasing concentrations from proximal to distal despite the reported regular drug use. This was a red dyed hair sample and it can be assumed that this different course is caused by repeated aggressive hair cosmetics.

4. Conclusion

It follows from the results that the combined application of derivatization and HS-SPME to the residue of hair extracts leads to an increase in sensitivity of the determination of THC, CBD and CBN as compared to the injection of the solution of the derivatized drugs. This advantage is made possible by a clean-up effect of the headspace enrichment on the fibre and by the injection of a higher portion of the sample in comparison to the usual liquid injection of the derivatized drug solution. The performance of HS-SPME from the "dry" extract residue in presence of a small amount of the derivatization agent enables the application of higher temperatures and, in this way, leads to higher extraction yields. In the practical test over 2 years, the method proved to be robust and reproducible. In comparison to the method previously used (Section 2.5) it does not require additional manual work if an automatic HS-SPME sampler is applied.

References

[1] V. Cirimele, P. Kintz, P. Mangin, Forensic Sci. Int. 70 (1995) 175.
[2] V. Cirimele, H. Sachs, P. Kintz, P. Mangin, J. Anal. Toxicol. 20 (1996) 13.
[3] G. Kauert, J. Röhrich, Int. J. Legal Med. 108 (1996) 294.
[4] D. Wilkins, H. Haughey, E. Cone, M. Huestis, R. Foltz, D. Rollins, J. Anal. Toxicol. 19 (1995) 483.
[5] S. Strano-Rossi, M. Chiarotti, J. Anal. Toxicol. 23 (1999) 7.
[6] M.J. Baptista, P.V. Monsanto, E.G. Pinho Marques, A. Bermejo, S. Avila, A.M. Castanheira, C. Margalho, M. Barroso, D.N. Vieira, Forensic Sci. Int. 128 (2002) 66.
[7] F. Musshoff, H.P. Junker, D.W. Lachenmeier, L. Kroener, B. Madea, J. Anal. Toxicol. 26 (2002) 554.
[8] D.W. Lachenmeier, L. Kroener, F. Musshoff, B. Madea, Rapid Commun. Mass Spectrom. 17 (2003) 472.
[9] F. Musshoff, D.W. Lachenmeier, L. Kroener, B. Madea, Forensic Sci. Int. 133 (2003) 32.
[10] M. Uhl, H. Sachs, Forensic Sci. Int. 145 (2004) 143.
[11] J.Y. Kim, S.I. Suh, M.K. In, K.J. Paeng, B. C. Arch. Pharm. Res. 28 (2005) 1086.
[12] Society of Hair Testing, Forensic Sci. Int. 145 (2004) 83.
[13] F. Pragst, T. Nadulski, Ann. Toxicol. Anal. 17 (2005) 237.
[14] J. Thorspecken, G. Skopp, L. Potsch, Clin. Chem. 50 (2004) 596.
[15] P. Kintz, V. Cirimele, P. Mangin, J. Forensic Sci. 40 (1995) 619.
[16] M. Uhl, Forensic Sci. Int. 107 (2000) 169.
[17] H. Sachs, U. Dressler, Forensic Sci. Int. 107 (2000) 239.
[18] M. Chiarotti, L. Costamagna, Forensic Sci. Int. 114 (2000) 1.
[19] C. Moore, F. Guzaldo, T. Donahue, J. Anal. Toxicol. 25 (2001) 55.
[20] R. Marsili, S. Martello, M. Felli, S. Fiorina, M. Chiarotti, Rapid Commun. Mass Spectrom. 19 (2005) 1566.
[21] F. Sporkert, D. Rentsch, in: F. Pragst, R. Aderjan (Eds.), Ausgewählte Aspekte der Forensischen Toxikologie, Verlag Dr. Dieter Helm, Heppenheim, 2004, p. 334.
[22] M.R. Moeller, G. Doerr, S. Warth, J. Forensic Sci. 37 (1992) 969.
[23] S. Steinmeyer, D. Bregel, S. Warth, T. Kraemer, M.R. Moeller, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 772 (2002) 239.
[24] F. Sporkert, F. Pragst, Forensic Sci. Int. 107 (2000) 129.
[25] F. Sporkert, F. Pragst, J. Anal. Toxicol. 24 (2000) 316.
[26] F. Sporkert, F. Pragst, J. Chromatogr. B Biomed. Sci. Appl. 746 (2000) 255.
[27] F. Pragst, V. Auwaerter, F. Sporkert, K. Spiegel, Forensic Sci. Int. 121 (2001) 76.
[28] T. Nadulski, F. Sporkert, M. Schnelle, A.M. Stadelmann, P. Roser, T. Schefter, F. Pragst, J. Anal. Toxicol. 29 (2005) 782.
[29] F. Peters, M. Hartung, M. Herbold, G. Schmidt, Toxichem. + Krimtech. 71 (2004) 146.
[30] G. Schmitt, M. Herbold, F. Peters, Valistat, Version 1.0. Methodenvalidierung nach den Methoden der GTFCh unter Berücksichtigung der ISO 5725. Software and Manual (ISBN 3-9808734-0-4), Arvecon GmbH, Walldorf, Germany, 2003.
[31] C. Jurado, H. Sachs, Forensic Sci. Int. 133 (2003) 175.
[32] M. Uhl, in: P. Kintz (Ed.) Analytical and Practical Aspects of Drug Testing in Hair. CRC Press, Boca Raton, 2006. p. 127.