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ScienceDirect
Journal of Chromatography B, 850 (2007) 515–522
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Development of a LC/MS/MS method for the analysis of cannabinoids in human EDTA-plasma and urine after small doses of *Cannabis sativa* extracts☆

Sandra B. Grauwiler[a], André Scholer[a], Jürgen Drewe[b,*]

[a] *Clinical Chemical Laboratory, Toxicology Section, University Hospital, Petersgraben 4, 4031 Basel, Switzerland*
[b] *Division of Clinical Pharmacology & Toxicology, University Hospital Basel, Hebelstr. 2, CH 4031 Basel, Switzerland*

Received 13 October 2006; accepted 22 December 2006
Available online 11 January 2007

Abstract

A novel high-performance liquid chromatographic separation method with tandem-mass spectrometry detection was developed for the simultaneous determination of Δ^9-tetrahydrocannabinol (THC) and its major metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) as well as the components cannabidiol (CBD) and cannabinol (CBN) in human EDTA-plasma and urine. Run time was 25 min. Lower limit of quantification was 0.2 ng/ml. The coefficients of variation of all inter- and intra-assay determinations were between 1.3 and 15.5%. The method was successfully applied to the determination of cannabinoids in human plasma and human urine after administration of Δ^9-tetrahydrocannabinol or *Cannabis sativa* extracts.

Keywords: Δ^9-Tetrahydrocannabinol; 11-Hydroxy-Δ^9-tetrahydrocannabinol; 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid; Cannabidiol; Cannabinol; Cannabinoids; LC/MS/MS; APCI; Human EDTA-plasma; Human urine

1. Introduction

After Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) is the next most abundant cannabinoid substance that occurs in the blood and urine following ingestion of hashish, marijuana or cannabis. According to recent results [1,2], the carboxylated precursors used in the biosynthesis of CBD and THC are both directly derived from cannabigerolic acid. The existence of the postulated enzyme CBD-cyclase catalyzing the synthesis of THC via CBD has not been experimentally confirmed. The CBD/THC ratio is mainly dependent on the genetic background of the individual plant.

There is some evidence that CBD is devoid of psychotropic actions and may even antagonize the psychotropic effects of THC [3]. This is thought to occur by inhibition of the hydroxylation of THC to 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) [4]. Cannabinoids are currently under clinical investigations because of the potential or proven sedative [5], anti-epileptic [6], anti-emetic- [7] and anti-inflammatory effects [8]. They may reduce intraocular pressure [9] and may have anxiolytic effects [10]. Psychic symptoms (such as acute and chronic psychosis and depression) have been observed after cannabinoid consumption [11,12], especially after high doses. CBD does not bind to the known cannabinoid receptors, and its mechanism of action is unknown yet [13].

To characterize the metabolic patterns of THC and its metabolites resulting from ingestion of cannabinoids, we have developed a sensitive and specific analytical method using LC/MS/MS and atmospheric pressure chemical ionization (APCI). This method was used to characterize the metabolism of cannabinoids based on the source (extracts or pure substances) and route of administration (oral ingestion).

Electrospray ionization (ESI) methods often are more sensitive than APCI methods. However, for the actual method APCI was chosen because of less matrix effects. In addition, the limits of detection (LODs) and limits of quantification (LOQs) were low enough for the determination of THC and its metabolites in the provided study.

☆ This paper was presented at the 44th Scientific Meeting of The International Association of Forensic Toxicologists (TIAFT), Ljubljana, Slovenia, 27 August–1 September 2006.

* Corresponding author. Tel.: +41 61 265 38 48; fax: +42 61 265 85 81.
E-mail addresses: juergen.drewe@unibas.ch, drewej@uhbs.ch (J. Drewe).

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doi:10.1016/j.jchromb.2006.12.045

Several methods are reported in the literature for the estimation of THC or its metabolites in plasma and urine by gas chromatography/mass spectrometry (GC/MS) after liquid/liquid or solid-phase extraction (SPE) and derivatization [14–17]. Major disadvantages of these methods are the elaborate sample preparation and the need to use various derivatization techniques for non-volatile and thermolabile compounds. Other reported chromatographic methods are high-performance liquid chromatography (HPLC) [14] with ultraviolet or electrochemical detection (UV, ED), and gas chromatography (GC) with electron capture, flame ionization or nitrogen–phosphorous detection (ECD, FID, NPD) [16]. Generally these methods lack either specificity or sensitivity. Recently, LC/MS or LC/MS/MS using electrospray ionization and atmospheric pressure chemical ionization were found to be suitable for the detection of drugs of abuse like THC and its main metabolites [18–27].

The following data show that our method has some distinct advantages, such as a high sensitivity (LOQ of 0.2 ng/ml in plasma) and the detection of five cannabinoids from two matrices (human plasma and urine), compared to the already reported methods.

The purpose of this paper is to describe a new, sensitive LC/MS/MS method for detection of Δ^9-tetrahydrocannabinol and its metabolites, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), as well as cannabidiol and cannabinol (CBN) from human EDTA-plasma and urine. This method was used to characterize metabolic patterns of the compounds resulting from pharmacokinetic transformations that occur in humans following ingestion of hashish, marijuana and *Cannabis sativa* extracts.

2. Experimental

2.1. Chemicals

THC and metabolite reference material was obtained from Lipomed (Arlesheim, Switzerland). All solvents (acetonitrile, isopropanol 20%, *ortho*-phosphoric acid 85%, ammonia solution 25%, diethylether, ethylacetate, acetic acid 100%, formic acid 98%) and chemicals (ammonium formate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate) in analytical grade were purchased from Merck (Darmstadt, Germany) and Aldrich (Sigma–Aldrich, Germany). Fetal bovine serum was obtained from biochroma (Berlin, Germany) and urine quality controls from Biorad (Reinach, Switzerland). Silanised glass vials (type I plus) were purchased from Schott AG (Muellheim-Huegelheim, Germany) and 6 ml glass test tubes from Gilson, Switzerland. Solid-phase extraction was carried out on Certify II extraction cartridges (Varian, Zug, Switzerland). De-ionized water was generated with a Milli-Q water purification system from Millipore (Kloten, Switzerland). β-Glucuronidase was obtained from Roche Diagnostics GmbH (Mannheim, Germany). The buffer (pH 6.0, 0.1 M) was prepared with a KH_2PO_4 solution (13.61 g/950 ml water), adjusted to the desired pH by appropriate addition of KOH 1 M and filled up with water to 1 l. The buffer (pH 9.1, 0.1 M) was prepared with a K_2HPO_4 solution (8.71 g/480 ml water), adjusted to the desired pH by appropriate addition of *ortho*-phosphoric acid 85% and filled up with water to 500 ml.

2.2. Specimens, standard solutions, calibration standards and quality controls

Stock THC and metabolite standard solutions were made in acetonitrile/H_2O (50/50, v/v). (The concentrations were THC, 11-OH-THC, CBD, CBN: 100 mg/l; THC-d3, THC-COOH-d3: 10 mg/l (v/v).) The standards were used to spike the matrix. Calibration standards for assay calibration and determination of the linear measuring range were prepared in bovine serum or human urine by spiking with the needed amount of the standard solutions to obtain the range of 0.2–100 ng/ml of THC and its metabolites. EDTA-plasma was taken from healthy volunteers. After drawing, the blood was gently inverted, centrifuged (10 min at 2000 × *g*) and the supernatant filled in a silanised glass vial. The tubes were sealed and stored at −70 °C.

In order to determine for quality control purposes, the intra- and inter-assay precision and the accuracy, blood quality controls were prepared in blank bovine serum by spiking with Δ^9-THC (3.0, 10.0 and 25.0 ng/ml). The four commercially available urine quality controls contained 0, 37.5, 65.0 and 125.0 ng/ml THC-COOH.

2.3. Extraction procedure

2.3.1. Human EDTA-plasma

The solid-phase extraction procedure was carried out on Varian Bond Elut Certify II cartridges (off-line). Certify II is a mixed mode sorbent with an anion exchange sorbent and retains acidic and neutral drugs (THC and metabolites). The cartridges were conditioned initially with 1 ml acetonitrile and followed by 1 ml 0.1 M phosphate buffer (pH 9.1). 1 ml EDTA-plasma-sample acidified with 20 μl *ortho*-phosphoric acid (85%) was loaded onto the cartridges. Cartridges were subsequently washed with 1 ml 40% acetonitrile and dried under vacuum at 45 kPa for 2 min. The compound of interest was eluted with 1 ml acetonitrile:ammonia (NH_4^+) = 98:2 (v/v) into a 6 ml glass test tube and aliquots were evaporated to dryness at 37 °C under nitrogen.

2.3.2. Human urine

First, a de-glucuronidation of THC-COOH-glucuronide to THC-COOH was done. To 2.5 ml urine 1 ml phosphate buffer (pH 6.0; 0.1 mmol/L) was added, mixed and supplemented with 50 μl β-glucuronidase. After vortexing, the sample was incubated at 50 °C for 3 h. The liquid–liquid extraction (LLE) procedure for urine was carried out in glass tubes (off-line). One millilitre phosphate buffer (0.1 M, pH 6.0) and 1 ml of acetic acid 0.1 M were added to 2 ml of the de-glucuronised urine. After vortexing, 4 ml organic phase (diethylether:ethylacetate 50% (v/v)) was added. After mechanical shaking (10 min) and centrifugation (10 min at 2000 × *g*), 3 ml of the organic phase were transferred to a 6 ml glass test tube and then evaporated to dryness at 37 °C under nitrogen.

The extracts were reconstituted in 60 μl of mobile phase (40% mobile phase A, 60% mobile phase B), 10 μl internal standard THC-d3 (EDTA-plasma), respectively, THC-COOH-d3 for urine analysis (10 mg/l in acetonitrile 50% (v/v)) was added, mixed and 50 μl injected into the LC/MS/MS system (see Section 2.4.1).

The internal standard was added in order to overcome possible variations of the concentrations in the LC/detection system. Possible deviations of the extraction rates were overcome by treating the standards in both matrices (EDTA-plasma and urine) in the same way as samples and by calibration each series of analysis.

2.4. LC/MS/MS

2.4.1. Chromatographic separation and mass spectral detection conditions

The chromatographic system consisted of a Rheos 2000 Micro HPLC pump (Thermo Finnigan, Allschwil, Switzerland) and a Midas Symbiosis Autosampler from Spark (Emmen, Netherlands) equipped with a 100 μl loop. A four-channel degasser was integrated into the Rheos CPS LC system. The LC/MS/MS apparatus was a LCQ Advantage MAX from Thermo Finnigan (Basel, Switzerland) equipped with an APCI device operating in the positive detection mode. The chromatographic separation was performed on a Synergi MAX-RP 80A C_{12} column (length 2 mm × 75 mm, i.d. 4 μm) from Brechbuehler (Zuerich, Switzerland). The mobile phase was delivered at a flow rate of 400 μl/min. Each chromatographic run was performed with a binary, linear A/B gradient (Solvent A was 10 mM ammonium formate, pH 3.0. Solvent B was 90% acetonitrile, 10% 10 mM ammonium formate, pH 3.0). The program was as follows: 0 min: 50% B; 1–12.0 min: 50–79% B; 12.01–12.50 min: 79–95% B; 12.51–15.00 min: 95% B; 15.01–25.00 min: B linear from 95 to 50%. All solvents were degassed before usage.

The following APCI inlet conditions were applied. The heated vaporizer was kept at 465 °C. Both the sheath gas and the auxiliary gas were nitrogen set at 60 and 15 relative units, respectively. The capillary entrance to the ion trap was at an offset of 10 V in the positive mode and was maintained at 220 °C. The corona current was 5 μA. For quantification, the selected ion monitoring mode was used. Table 1 lists the precursor and product ions and the relative collision energy for each analyte.

Table 1
Precursor and product ions and the relative collision energy for each analyte

Substance	Mass APCI $[M-H]^+$ (m/z)	Product ions $[M-H]^+$ (m/z)	Collision energy (%)
CBN	311.2	293.2	34
CBD	315.1	259.2	34
THC-COOH	345.2	327.1	30
11-OH-THC	331.0	313.2	34
THC	315.2	259.1	38

2.5. LC/MS/MS assay validation

2.5.1. Linear measuring range, quantification, limit of quantification (LOQ), limit of detection (LOD), quality controls, precision, accuracy and recovery

2.5.1.1. Linear measuring range. The *linearity* of the measuring range was assessed with standard curves ranging from 0.2 to 100 ng/ml (0.2; 0.3; 0.5; 1.0; 2.0; 3.0; 5.0; 10.0; 25.0; 50.0; 100.0 ng/ml) in bovine serum and human urine and analyzed using the described LC/MS/MS method. Standard response curves were generated using a weighted ($1/x$) linear regression model.

2.5.1.2. Quantification. The concentrations of the analytes were calculated by comparing the peak area (%) of an analyte with the corresponding area (%) on the standard curve. System variations were adjusted by comparing the area (%) of the internal standards. The internal standards were THC-d3 for EDTA-plasma and THC-COOH-d3 for urine.

2.5.1.3. The limit of quantification (LOQ) and detection (LOD). Bovine serum and human urine samples spiked with decreasing concentrations of the analytes were analyzed in order to determine LOQs and LODs. Samples of each concentration were extracted and analyzed five times. The LOD was set at the lowest concentration where the signal of the compound was three standard deviations higher than the background noise. The LOQs were determined by measuring five samples per concentration for all substances. The LOQ was defined as the lowest concentration of THC and its metabolites which can still be determined with a precision <10% (CV).

2.5.1.4. Precision. Intra-assay precision was determined by replicate ($n=4$) analysis of the QC samples in one run for both fluids.

Inter-assay precision was determined by replicate analysis of the QC samples in several experiments performed at different days. Eight urine- and five EDTA-plasma-samples were measured. A comparison with the nominal concentrations of the QC samples was used to assess the accuracy (bias) of the method.

2.5.1.5. Accuracy and recovery. Accuracy was committed not to be >±20% of the nominal concentration.

Recovery was estimated in both matrices by comparing the response of a blank human urine or a blank bovine serum spiked with 100 ng/ml analyte before the extraction step ($n=4$) with the response obtained when the analyte was added to the corresponding matrix after the extraction procedure ($n=4$). The deuterated internal standard was added in all samples after the extraction step under both conditions.

2.5.2. Sample stability

The stability of the cannabinoids during storage was monitored in human EDTA-plasma (THC) and human urine samples spiked with the initial concentration of 100 ng/ml for all measured substances. The concentrations in the samples

were determined immediately after spiking (control samples, $n=3$). Aliquots of the same samples were filled into silanised glass vials, closed with chlorbutyl gum stoppers and aluminium seals. The tubes were frozen at −70 °C. The urine samples were thawed and measured ($n=3$) after 62 and 133 days, the EDTA-plasma-samples after 21 and 41 days.

For an evaluation of freeze/thaw stability, the spiked sample (same sample material as above) was analyzed before (control samples, $n=3$) and after three freeze/thaw cycles (stability samples, $n=3$). For each freeze/thaw cycle, the sample was frozen at −70 °C for 24 h, thawed, extracted and measured.

2.5.3. Assessment of matrix effects

To assess any possible suppression or enhancement of ionization due to the sample matrix, four types of experiments were performed.

In the first experiment blank samples from the test persons used as negative controls were analyzed.

The second test was to analyze blank bovine serum.

The third trial included the evaluation of the matrix as described by Matuszewski et al. [27]. This assessment includes the recovery and process efficiency.

For this test three sets of samples are necessary. Set A consists of aqueous standard solutions (100 ng/ml). For set B blank matrices are supplemented (after extraction) with the same amount of standards as used for set A. Set C consists of extracts of different blank matrices, supplemented with the same amount of standards but added before extraction.

Recovery was calculated (for the calculation the resulting peak areas are needed) with the following formula: RE% = C/B × 100. Matrix effects were calculated with the formula: ME% = B/A × 100. Process efficiency was calculated with the formula: PE% = C/A × 100. The difference in analyte concentration not more than 50% was acceptable for all three tests.

The last test provided a continuous post-column infusion [28] of an analyte in a chromatographic run of an extract or a blank matrix. This procedure is based on the post-column infusion of an analyte in a chromatographic run of an extract or a blank matrix. This signal is compared to the signal obtained with the post-column infusion of the same model analyte in a chromatographic run with eluent only. This procedure indicates also critical areas in the chromatogram.

3. Results and discussion

An example of a typical chromatogram of 100 ng/ml of the analytes in bovine serum is shown in Fig. 1. The retention times were 13.80 ± 0.08 min for THC, 7.38 ± 0.08 min for 11-OH-THC, 7.75 ± 0.09 min for THC-COOH, 11.27 ± 0.08 min for CBD and 13.00 ± 0.15 min for CBN. The total run time for each sample was 25 min. Acceptable retention times were considered to be within a time window of ± 0.5 min.

3.1. Linearity

The linear measuring range was assessed with calibration curves ranging from 0.2 to 100 ng/ml in bovine serum and human urine. Fig. 2 shows an example of a standard response curve of each analyte in bovine serum generated using a weighted ($1/x$) linear regression model. The correlation coefficients (R^2) in the diagrams were ≥0.994 with one acceptable exception of 0.979 (Fig. 2).

3.2. The limit of quantification (LOQ) and detection (LOD)

The limit of quantification in EDTA-plasma was 0.2 ng/ml for CBN, THC, THC-COOH, CBD and 11-OH-THC, in urine 3 ng/ml for CBN, 1 ng/ml for CBD, THC, and THC-COOH and 2 ng/ml for 11-OH-THC.

The limit of detection in EDTA-plasma for all substances was 0.1 ng/ml. The LODs in urine were 0.5 ng/ml for THC, CBD and THC-COOH and 1 ng/ml for CBN and 11-OH-THC.

3.3. Accuracy and intra- and inter-assay precision

Results of the accuracy, intra- and inter-assay precision studies are presented in Tables 2 and 3. The deviation of accuracy of the intra-assay study in EDTA-plasma (THC) and urine (THC-COOH) did generally not exceed 18% (accepted 20%).

The deviation in the lowest concentrated urine control sample was 24% (intra) and 26% (inter) which exceeds the acceptable value (20%). This fact was ignored because of possible method differences in the choice of the nominal concentrations and a good precision (with coefficients of variation of 10.5% (intra-assay) and 15.5% (inter-assay)). In both matrices (EDTA-plasma and urine) the intra- and inter-assay precision displayed by the CV was much better than 20% (defined acceptable CV by the authors). CVs were in the range 1.3–10.5 (intra-assay) and 1.9–15.5 (inter-assay).

3.4. Stability

In urine, the substance stability tests at a temperature of −70 °C in silanised glass vials showed that the concentrations of each cannabinoid with the exception of THC did not decrease more than 20% after 5 months (mean of three measurements). THC concentration showed a decrease of 26%.

In EDTA-plasma, the THC concentration decreased steadily during the observation period with a decrease of 30% after 21 days and 43% after 41 days.

3.5. Assessment of matrix effects

Blood and urine samples from the test persons were taken immediately before the administration of the cannabis drug. The samples were used as negative controls and to compare the baseline chromatograms with those after the drug application. No peaks with measurable areas were found in the chromatograms (data not shown).

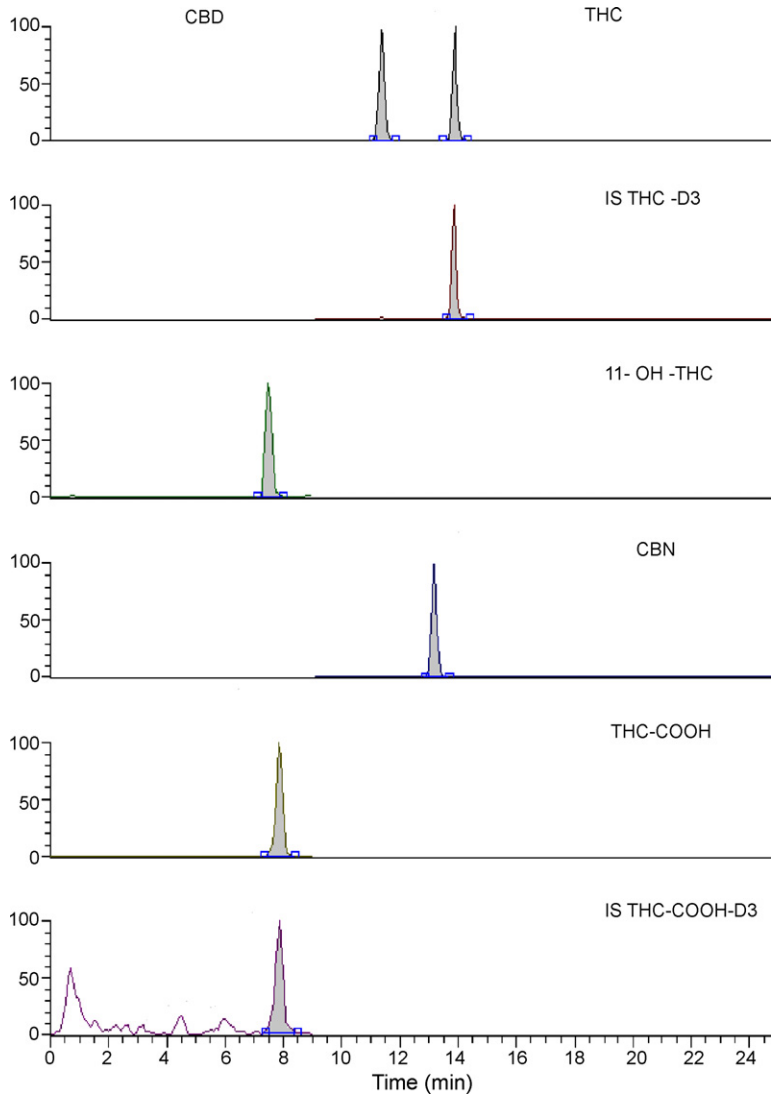

Fig. 1. LC/MS/MS chromatogram of 100 ng/ml of the analytes in bovine serum.

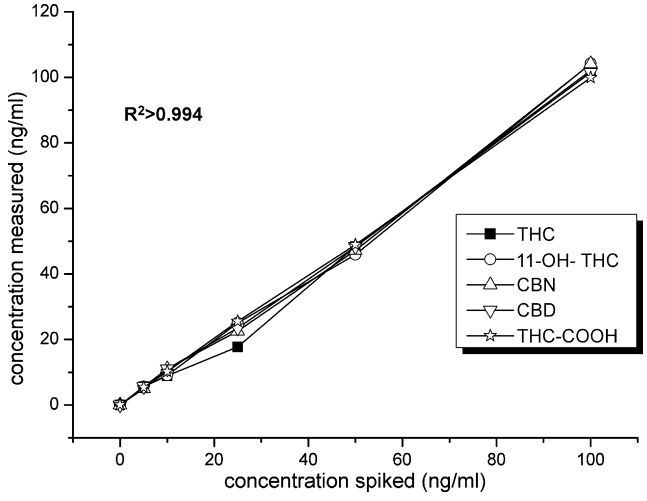

Fig. 2. Example of a standard response curve of each analyte in bovine serum.

3.6. Blank bovine serum used as negative controls

Blank bovine serum was measured to compare the chromatograms with the spiked bovine serum samples used for the standard curves. No peaks with measurable areas were found in the blank bovine serum chromatograms (data not shown).

Table 4 shows the recovery, matrix effects and process efficiency of the analytes.

3.7. Recovery

All studies show enough recovery (%) for each substance in both matrices (range 47.7–78.7) in relation to the sensitivity results (LOD/LOQ).

3.8. Matrix effects

This study showed the low influence on the qualitative and quantitative determinations, which was confirmed by the post-column infusion test. The range of this study was 73.4–283.8%

Table 2
Results of the intra-assay precision

	Intra-assay precision					
	Urine (THC-COOH)			EDTA-plasma (THC)		
Number of measurements (*n*)	4	4	4	4	4	4
Mean (ng/ml)	28.5	65.7	132.5	3.0	8.2	24.9
Nominal concentration (ng/ml)	37.5	65.0	125.0	3.0	10.0	25.0
S.D. (ng/ml)	3.0	0.9	1.7	0.2	0.4	1.9
CV (%)	10.5	1.4	1.3	8.8	5.2	7.9
Accuracy (%)	24.0	1.0	6.0	0.0	18.0	0.4

S.D.: Standard deviation; CV: coefficient of variation.

Table 3
Results of the inter-assay precision

	Inter-assay precision (ng/ml)					
	Urine (THC-COOH)			EDTA-plasma (THC)		
Number of measurements (*n*)	8	8	8	5	5	5
Mean (ng/ml)	27.8	64.7	132.6	2.9	8.8	27.0
Nominal concentration (ng/ml)	37.5	65.0	125.0	3.0	10.0	25.0
S.D. (ng/ml)	4.3	4.2	3.9	0.6	1.1	1.6
CV (%)	15.5	6.5	1.9	11.8	12.8	7.9
Accuracy (%)	26.0	0.4	5.6	3.3	12.0	8.0

S.D.: Standard deviation; CV: coefficient of variation.

and shows a common effect for several substances in different matrices on the extraction rate over 100% yield compared to aqueous standards.

3.9. Process efficiency

The sensitivity for the determination of the substances is high enough for the analysis in the samples of the provided trial. This is due to overall good process efficiency. One exception was CBN because of a low recovery (47.7%), bad process efficiency (34.7%) and a relatively high LOD.

3.10. Post-column infusion after Bonfiglio et al. [28]

The post-column infusion (100 μl/min) of the cannabinoids in a chromatographic run of eluent only, indicated no critical areas in the chromatograms compared with those of blank human urine and bovine serum. No critical area around the retention time of THC or the other analytes was detected (data not shown). No change in the ionization process (enhancement, suppression of the ionization) of an analyte due to a co-eluting compound was found.

3.11. An example of determination after administration of cannabinoids to human subjects

The described method was applied to determine the pharmacokinetics of THC and its metabolites after administration of either 20 mg synthetic THC (Marinol™) or an extract from *C. sativa* (capsule containing 20 mg of cannabinoids) to the same healthy male volunteer. Figs. 3 and 4 show that the metabolites are detectable for at least 24 h. Except after the intake of THC, no CBD was detectable in the EDTA-plasma.

The range of the measured cannabinoid concentrations is within 0–11.0 ng/ml. The consumption of THC (Marinol™) shows higher concentrations compared to those after the administration of the herbal extract.

3.12. Comparison with other methods

The analysis of plasma and urine samples for cannabinoids by GC/MS is daily routine in forensic toxicology laboratories. Normally, the investigations are limited to THC, 11-OH-THC and THC-COOH and detection limits of 1 ng/ml are sufficient. However, in the present study, CBD and CBN had to be included

Table 4
Recovery, matrix effect and process efficiency

	Recovery (%)		Matrix effects (%)		Process efficiency (%)	
	Bovine serum	Urine	Bovine serum	Urine	Bovine serum	Urine
THC	77.5	78.7	97.6	87.9	70.7	70.3
11-OH-THC	77.6	63.4	117.6	154.2	91.5	142.6
CBD	71.4	70.0	84.2	93.1	60.0	69.5
CBN	47.7	78.1	73.4	85.1	34.7	68.7
THC-COOH	50.0	61.4	172.9	283.8	87.5	173.9

Parameters were determined according to Matuszewski et al. [27].

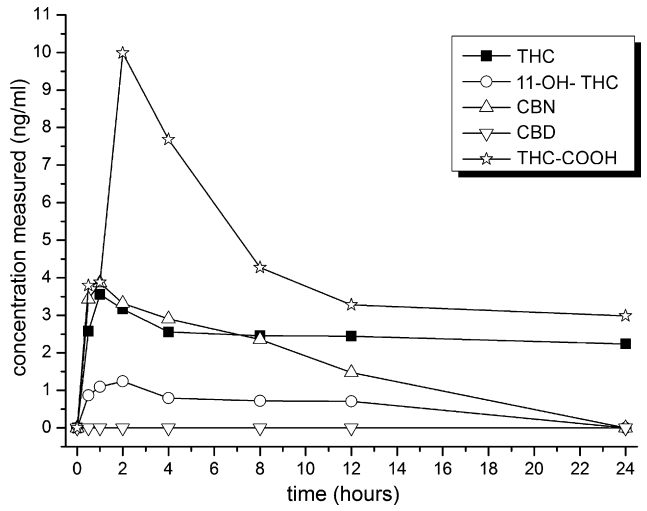

Fig. 3. Plasma concentration time curve of THC and its major metabolites as well as CBD and CBN after the intake of 20 mg synthetic THC (Marinol™) in one healthy male subject.

and a detection limit below 0.5 ng/ml was necessary in order to follow up the concentrations for a sufficiently long time after drug administration.

Only 2–2.5 ml plasma per sample were available. Therefore, the method, particularly the extraction, had to be adapted to these requirements before application to the study.

The described LC/MS/MS method allows the simultaneous analysis of five cannabinoids from urine or plasma in the same run over 25 min. There are several other methods published in the literature so far. Most reported methods detect only one or two metabolites from one matrix. Another important issue of cannabinoid analytics, especially for forensic purposes, is a high sensitivity. The described method has a LOQ of 0.2 ng/ml and is more sensitive than previously described analytical methods used in pharmacokinetic trials. Most of those reported methods show LOQs >5 ng/ml.

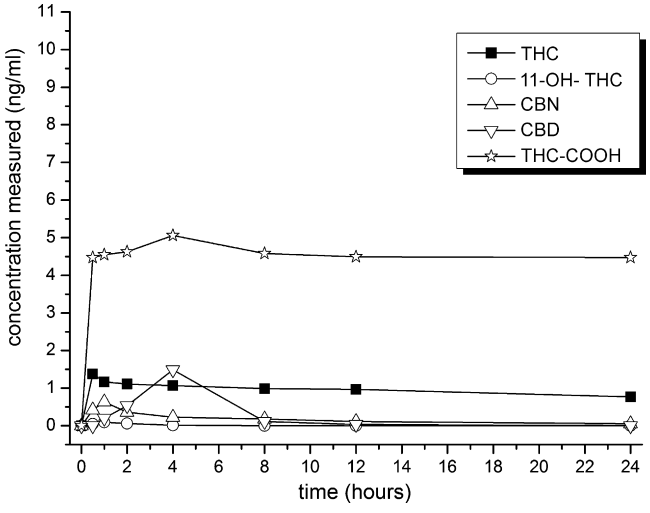

Fig. 4. Plasma concentration time curve of THC and its major metabolites as well as CBD and CBN after the intake of a *Cannabis sativa* extract (containing 20 mg of cannabinoids) in one healthy male subject.

Only one GC/MS method reported likewise the simultaneous analysis in plasma of THC, 11-OH-THC, CBN, CBD and THC-COOH. This method had a slightly lower sensitivity with a LOQ of 0.5–3.9 ng/ml and had a run time of 13 min [29]. However, this method is more elaborate than ours.

Compared to other reported GC/MS methods [14–17] for estimation of THC or its metabolites, definite advantage of our LC/MS/MS method is a gain of time. There is no need to do an elaborate sample preparation and to use various derivatization techniques for non-volatile and thermolabile compounds like in GC/MS.

4. Conclusions

We developed and validated a sensitive and selective method for the determination of THC, its major metabolites as well as CBD and CBN in human EDTA-plasma and urine, using high-performance liquid chromatographic separation with tandem-mass spectrometry detection which showed a satisfactory overall analytical performance well suited for applications in medical science. With a LOQ of 0.2 ng/ml (EDTA-plasma), pharmacokinetic profiles of the drugs and their concentrations could be determined for up to 24 h after a single oral administration of a *C. sativa* extract capsule of 20 mg THC_{tot} (Figs. 3 and 4). The stability tests showed a constant decrease of THC in urine and of all parameters in EDTA-plasma. This decrease does not exceed 10% if the samples are analyzed within a short time period. This method is very efficient because it permits the measurement of low concentrations and the simultaneous quantification of five analytes.

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