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Chromatographic and Spectroscopic Data of Cannabinoids from *Cannabis sativa* L.

Arno Hazekamp, Anja Peltenburg, and Rob Verpoorte

Division of Pharmacognosy, Institute of Biology, Leiden University,
Leiden, The Netherlands

Christian Giroud

Laboratoire de Toxicologie et de Chimie Forensiques, Institute
Universitaire de Médecine légale, Lausanne, Switzerland

Abstract: Chromatographic and spectroscopic data was determined for 16 different major cannabinoids from *Cannabis sativa* plant material as well as 2 human metabolites of Δ^9 -tetrahydrocannabinol. Spectroscopic analysis included UV absorbance, infrared-spectral analysis, (GC-) mass spectrometry, and spectrophotometric analysis. Also, the fluorescent properties of the cannabinoids are presented. Most of this data is available from literature but scattered over a large amount of scientific papers. In this case, analyses were carried out under standardised conditions for each tested cannabinoid so spectroscopic data can be directly compared. Different methods for the analysis of cannabis preparations were used and are discussed for their usefulness in the identification and determination of separate cannabinoids. Data on the retention of the cannabinoids in HPLC, GC, and TLC are presented.

Keywords: *Cannabis sativa*, Plant material, Cannabinoids, Metabolites, Chromatographic data, Spectroscopic data

INTRODUCTION

In recent years a lot of research on the medical applications of *Cannabis sativa* L. has been initiated, as several, mostly European countries, move towards a more

Address correspondence to Arno Hazekamp, Division of Pharmacognosy, Institute of Biology, Leiden University, Einsteinweg 55, 2300 RA, Leiden, The Netherlands.
E-mail: ahazekamp@rocketmail.com

liberal view on the use of Cannabis as a medicine.^[1] Although more than 400 compounds have been identified in the Cannabis plant,^[2] most studies have focused on the effects of the cannabinoids, in particular $(-)\text{-}\Delta^9\text{-}(trans)\text{-tetrahydrocannabinol}$ ($\Delta^9\text{-THC}$). One reason is that the main pharmacological and psychoactive effects of Cannabis have been attributed to $\Delta^9\text{-THC}$. For instance, synthetic $\Delta^9\text{-THC}$ (dronabinol, MarinolTM) has been shown to possess anti-emetic properties useful in cancer therapy. However, in several medical studies the effect of $\Delta^9\text{-THC}$ or dronabinol alone could not match the effects of a total Cannabis preparation,^[3] indicating there might be other active compounds present.^[4] More than 60 cannabinoids^[2,5-7] have been identified in Cannabis, and occasionally new cannabinoids are being discovered.^[8] The chemical structures of the main cannabinoids from the Cannabis plant are shown in Figure 1, and their physical properties are listed in Table 1. Only a few of these cannabinoids have been studied in detail, although several of these have been shown to possess some biological activity (reviewed by^[9]).

To extend the knowledge of the therapeutic properties to cannabinoids other than $\Delta^9\text{-THC}$, large amounts of pure compounds must be available. Assessment of cannabinoids pharmacology is now almost restricted to the few that are commercially available (e.g., $\Delta^8\text{-THC}$, $\Delta^9\text{-THC}$, CBD, and CBN). Furthermore, pure cannabinoids must be available as reference compounds for their unequivocal identification and determination. For that purpose, chromatographic and spectroscopic methods and data are available from scientific literature. Although these data have been published for most known cannabinoids during isolation and identification experiments (see^[2] for an overview), they are scattered over a huge amount of scientific papers. Moreover, standardised data obtained under identical analytical conditions have not been reported yet. As far as we know, the fluorescent properties of the cannabinoids remain largely unknown.^[10]

This report lists the main chromatographic and spectroscopic data of 16 main cannabinoids and of two of their human metabolites obtained under identical analytical conditions. Methods were kept as straightforward, simple, and rapid as possible. The pros and cons of each method will also be discussed. All analyses were carried out for each cannabinoid as far as permitted by the amount available to us.

EXPERIMENTAL

Standards and Solvents

Reference compounds of $(-)\text{-}\Delta^9\text{-tetrahydrocannabinol}$ ($\Delta^9\text{-THC}$), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), $(-)\text{-}\Delta^9\text{-}(trans)\text{-tetrahydrocannabinolic acid A}$ (THCA), cannabidiolic acid (CBDA), and cannabigerolic acid (CBGA) were isolated from plant materials previously in our laboratory.^[11] A quantitative $^1\text{H-NMR}$ method was developed for their quanti-

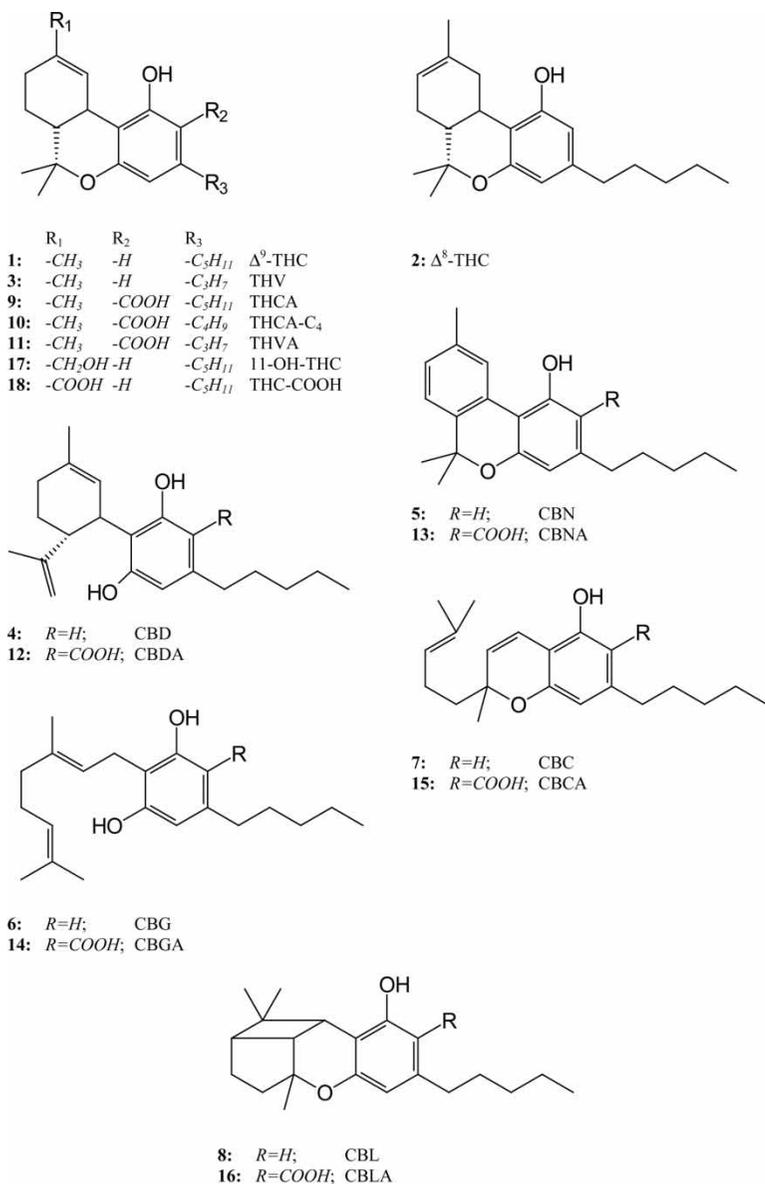


Figure 1. Structures of the cannabinoids.

fication.^[12] (-)-Δ⁸-tetrahydrocannabinol (Δ⁸-THC) was obtained from Sigma. The main human metabolites of Δ⁹-THC, i.e., 11-hydroxy-THC (11-OH-THC) and 11-carboxy-THC (THC-COOH) were purchased from Cambridge isotope laboratories (CIL, Innerberg, Switzerland) and from

Table 1. Physicochemical properties of the cannabinoids

#	Cannabinoid	Full name (description)	MW (calc.)	Molecular formula		
				C	H	O
Neutral cannabinoids						
1	d9-THC	<i>trans</i> -(–)-delta-9-tetrahydrocannabinol	314.472	21	30	2
2	d8-THC	<i>trans</i> -(–)-delta-8-tetrahydrocannabinol	314.472	21	30	2
3	THV	<i>trans</i> -(–)-delta-9-tetrahydrocannabivarin (C3-isomer of THC)	286.418	19	26	2
4	CBD	cannabidiol	314.472	21	30	2
5	CBN	cannabinol	310.440	21	26	2
6	CBG	cannabigerol	316.488	21	32	2
7	CBC	cannabichromene	314.472	21	30	2
8	CBL	cannabicyclol	314.472	21	30	2
Acidic cannabinoids						
9	THCA	<i>trans</i> -(–)-delta-9-tetrahydrocannabinolic acid A	358.482	22	30	4
10	THCA-C4	<i>trans</i> -(–)-delta-9-tetrahydrocannabinolic acid-C4 (C4-isomer of THCA)	344.455	21	28	4
11	THVA	<i>trans</i> -(–)-delta-9-tetrahydrocannabivarinic acid (C3-isomer of THCA)	330.428	20	26	4
12	CBDA	cannabidiolic acid	358.482	22	30	4
13	CBNA	cannabinolic acid	354.450	22	26	4
14	CBGA	cannabigerolic acid	360.498	22	32	4
15	CBCA	cannabichromenic acid	358.482	22	30	4
16	CBLA	cannabicyclolic acid	358.482	22	30	4
Human metabolites						
17	11-OH-THC	11-hydroxy-tetrahydrocannabinol (metabolite of THC)	330.471	21	30	3
18	THC-COOH	11-carboxy-tetrahydrocannabinol (metabolite of THC)	344.455	21	28	4

Lipomed (Arllesheim, Switzerland), respectively. All these cannabinoids were available as certified and calibrated reference standards. The other cannabinoids used for this study were obtained by preparative HPLC on extracts of *Cannabis sativa* plant materials and identified by comparing their chromatographic and spectroscopic data with literature,^[13–15] and by a search in

UV^[16] and mass spectra databases.^[17,18] All organic solvents (analytical or HPLC reagent grade) were purchased from J.T. Baker (Deventer, The Netherlands) or from Fluka Chemie (Buchs, Switzerland).

Thin Layer Chromatography (TLC)

Samples in ethanol were spotted on 10 × 20 cm silica plates. Two different TLC systems were used. For the non-polar system, reversed phase (C₁₈) silicagel plates F254 No. 105559 (Merck, Darmstadt, Germany) were used with methanol/5% acetic acid 19:1 (v/v) as the eluent. For the polar system, normal phase silicagel plates F254 No. 105554 (Merck, Darmstadt, Germany) were used with chloroform/methanol 19:1 (v/v) as the eluent.

Plates were developed in saturated normal chambers (saturation time 15 minutes). Absorption of chromatographic spots was evaluated under UV 254 nm. General visualisation of compounds was done by spraying with modified anisaldehyde-sulphuric acid spray reagent.^[19] For selective visualisation of cannabinoids, the TLC plate was sprayed with 0.5% fast blue B salt (*o*-dianisidine-*bis*-(diazotized)-zinc double salt) (Sigma) in water, followed by 0.1 M NaOH.^[20]

Gas Chromatography-Mass Spectrometry (GC-MS)

To obtain GC retention times, molecular weights, and fragmentation spectra of cannabinoids, GC-MS analyses were performed on a Varian 3800 gas chromatograph, coupled to a Varian Saturn 2000 GC/MS apparatus. The system was controlled with Varian Saturn GC/MS workstation version 5.2 software. The GC was fitted with two different types of columns; a Durabond fused silica capillary column (30 m × 0.25 mm inner diameter) coated with DB-1 at a film thickness of 0.1 μm, and a similar column, coated with HP-50+ at a film thickness of 0.15 μm (J&W Scientific Inc., Rancho Cordova, CA). The oven temperature was programmed from 100°C to 280°C at a rate of 10°C/min. The oven was then kept at 280°C until the end of a 30 min run time. The injector and detector port temperatures were maintained at 280°C and 290°C, respectively. Helium was used as the carrier gas at a pressure of 65 kPa. The injection split ratio was 1/50. Elution time of Δ⁹-THC was used as internal reference to determine the relative retention times of all cannabinoids.

High-Performance Liquid Chromatography (HPLC) with Diode-Array and Fluorescence Detection

The HPLC profiles were acquired on an Agilent 1100 series HPLC, consisting of a G1322A solvent degasser, a G1311A quaternary solvent pump, and a

G1313A autosampler. The column was kept at constant temperature by using a G1316A column oven. The analytical column was a Waters XTerra MS C₁₈ (2.1 × 150 mm, 3.5 μm) fitted with a XTerra MS C₁₈ (2.1 × 10 mm, 3.5 μm) guard column. Light absorption and emission were detected by a G1315B UV-diode array detector (DAD) and a G1321A fluorescence detector (FLD). The system was controlled through a Vectra VL 420 DT computer equipped with Agilent A09.01 software. UV-spectra were measured on-line by DAD in the range of 195–400 nm with a slit of 2 nm. Fluorescence (FL) spectra were recorded on-line by the FLD in the range of 280–650 nm with a step of 5 nm after excitation at 222 nm. Retention times were expressed as relative to Δ⁹-THC.

DAD and FLD data of cannabinoids were recorded under acidic conditions, with a mobile phase consisting of a mixture of methanol-water containing 25 mM of formic acid (pH ± 3). The proportion of methanol was linearly increased from 65 to 100% over 25 minutes, and then kept constant for 3 minutes. Analysis under basic conditions was obtained with a mobile phase consisting of a mixture of acetonitrile-phosphate buffer (10 mM, pH 7.5). The acetonitrile concentration was increased from 40 to 100% in 25 minutes, and then kept constant for 3 minutes. After each run the column was re-equilibrated under initial conditions for 10 minutes. The flow rate was 0.3 mL/min and the total run time was 38 minutes. All determinations were carried out at 30°C.

Spectrophotometric Analysis (Extinction Coefficients)

Cannabinoids that were available as calibrated certified standards were diluted to a concentration of 0.01 mg/mL in ethanol to determine molar extinction coefficients in the range of 200 to 400 nm. A blank measurement was obtained with ethanol. UV-spectra were recorded using a Varian Cary 1 Bio UV-Visible spectrophotometer controlled by Cary 1/3E system software, version 3.02. A sample cell of 10 mm was used for all measurements.

Infrared Spectroscopy (IR)

Infrared spectra of cannabinoids that were available in sufficient amounts were measured using a Perkin Elmer paragon 1000PC FT-IR instrument, which was controlled by Perkin Elmer spectrum IR V2.00 software. Concentrated ethanolic solutions of the cannabinoids (25 μL) were mixed with finely ground KBr (Merck, IR-grade), and ethanol was evaporated under vacuum for 10 minutes. After proper calibration of the apparatus, IR-spectra were measured as an average of 4 scans in the wave number range of 500 to 4000 cm⁻¹. After acquisition, the spectra were smoothed by using the software.

RESULTS AND DISCUSSION

Spectroscopic and chromatographic data is shown for 18 different cannabinoids that were available to us. However, not all cannabinoids were available in large enough quantities to obtain exploitable data in all analyses that were carried out. Therefore, the presented data is not complete for all cannabinoids.

TLC

By using two TLC-systems (polar and apolar system) in combination with fast blue B spray reagent, it was possible to distinguish and detect all tested compounds. The R_f-values of the cannabinoids in both TLC-systems and their spot colour after spraying with fast blue B are shown in Table 2. The use of fast blue B as a selective detection reagent for cannabinoids^[20] results in differently coloured spots for some compounds. Unfortunately, these colours also depend on the concentration of the substance and on the presence of interfering compounds, the results must be, therefore, considered with caution. Nevertheless, we found that fast blue B was more sensitive for detection of cannabinoid spots than UV-detection under 254 nm. For example, the detection limit for Δ^9 -THC was 0.5 mg/mL (2 μ L spotted) with UV-detection under 254 nm, and around 0.002 mg/mL with fast blue B detection.

The main advantages of TLC are its ability to detect all spotted compounds, while analysing several samples simultaneously under identical conditions within a short timeframe. Lack in selectivity can sometimes be

Table 2. Relative retention (R_f) values of the cannabinoids in a polar (silica-gel) and non-polar (C₁₈) TLC-system. The colours of chromatographic spots after spraying with the cannabinoid-selective spray reagent fast blue B (FBB) are indicated

Color FBB	Nonpolar TLC system (RP-18)		Polar TLC system (silica)	
	Cannabinoid	R _f -value	Cannabinoid	R _f -value
Red	CBDA	0.68	Δ^9 -THC	0.65
Brown	CBGA	0.67	Δ^8 -THC	0.65
Orange-brown	CBG	0.59	CBD	0.64
red-brown	CBD	0.58	CBN	0.62
Purple	CBN	0.48	CBG	0.61
Red	Δ^9 -THC	0.44	CBC	0.58
Red	Δ^8 -THC	0.43	THCA	0.39
Red	THCA	0.40	CBDA	0.37
Purple	CBC	0.37	CBGA	0.31
Purple	CBCA	0.35	CBCA	0.25

overcome by the use of selective detection reagents. However, in the case of cannabinoids it seems impossible to obtain a good separation with positive identification of all cannabinoids when complex mixtures (e.g., plant extracts) are analysed. Several TLC systems are therefore needed for tentative identification. For instance, CBDA and CBGA or CBD and CBG, which were not separated in the non polar system, could be distinguished when using silica as stationary phase. On the other hand, Δ^8 -THC and Δ^9 -THC were found to co-elute on both systems (see Table 2). In conclusion, TLC is very useful to rapidly screen many samples for the presence of cannabinoids in crude plant extracts, or in eluting fractions collected after preparative chromatography. However, reproducibility of TLC depends on several parameters, e.g., relative humidity. Compared to other separation methods, TLC performances are also very low. Consequently, unequivocal identification of cannabinoids spots requires further methods.

GC/MS

Two different capillary column phases were used for GC analysis (HP-50+ and DB-1). The HP-50+ column was a medium-polar column, resulting in relatively longer retention times compared to the nonpolar DB-1 column. Simultaneous injection on both columns enables the distinction of all tested cannabinoids. Retention times (relative to Δ^9 -THC) of the analysed cannabinoids are shown in Table 3. All cannabinoids eluted well after other major cannabis components like terpenoids.

Because no derivatization was used in our case, the mass-spectra obtained by GC-MS (Figure 2) are similar for the acids and their corresponding neutral

Table 3. Relative retention time (RRT) of cannabinoids in GC using a non-polar (DB-1) and medium-polar (HP-50) column

	GC column type	
	DB-1	HP-50
Cannabinoid	RRT	RRT
THV	0.885	0.902
CBL	0.922	0.907
CBD	0.942	0.935
THC-C4	0.942	0.948
CBC	0.956	0.924
Δ^8 -THC	0.988	0.981
Δ^9 -THC	1	1
CBG	1.026	1.012
CBN	1.033	1.046

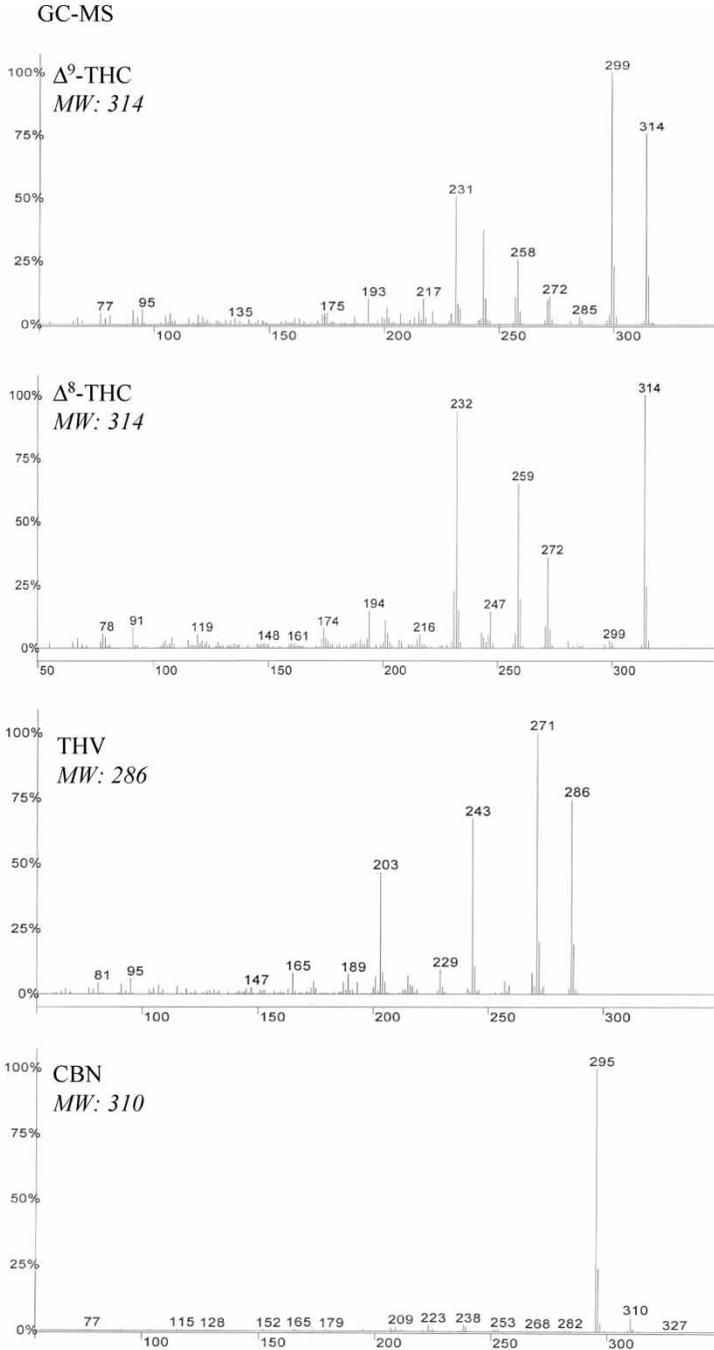


Figure 2. Mass-spectra in the range of M/Z 50-335 obtained by LC-MS.

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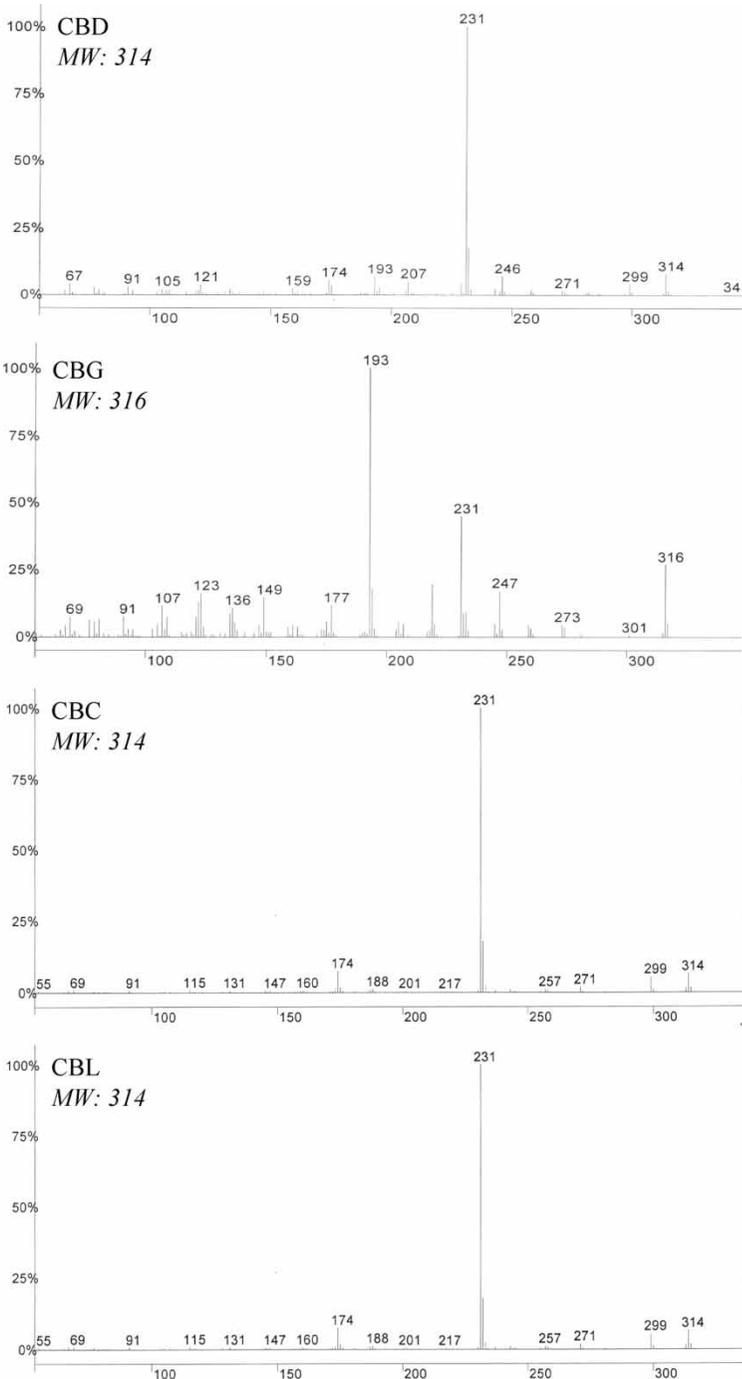


Figure 2. Continued.

cannabinoids (e.g., THCA and Δ^9 -THC). Although CBD is structurally quite distinct from CBC and CBL, these three cannabinoids nonetheless show similar MS spectra (compare spectra of Figure 2) with identical base peak ($m/z = 231$) and molecular ion ($m/z = 314$). Also their retention times in GC were quite similar (Table 3), but their separation is good enough to distinguish them. Cannabidiol differs from CBC and CBL with one significant fragment at $m/z = 246$. A retro-Diels-Alder reaction accounts for the formation of the minor ion at $m/z = 246$. Subsequent loss of a methyl fragment results in a contribution to the ion at $m/z = 231$.^[15] As can be seen in Figure 2, the base peak of all tested cannabinoids (except Δ^8 -THC) doesn't correspond to the molecular ion, but to a fragment, indicating that these cannabinoids are easily fragmented by GC-MS.

In the absence of derivatization, the high temperature that is applied in GC causes the decarboxylation of acidic cannabinoids to their corresponding neutral form.^[21] Since the cannabis plant mainly contains the (carboxylic-) acidic forms of cannabinoids,^[22] GC analysis is not the method of choice to establish the metabolic profile of a cannabinoid sample. To avoid decarboxylation, the acids must be derivatized, e.g., by silylation or formation of the alkylboronates.^[19] However, a 100% derivatization yield is difficult to obtain. Moreover, we believe that thermo-degradation (oxidation, isomerization) of cannabinoids in the injector port and column may also occur. In the case of Δ^9 -THC, a significant amount of Δ^8 -THC and CBN was detected in the GC-chromatogram, whereas other analyses (HPLC, NMR, TLC) did not show these compounds, which are known degradation products of Δ^9 -THC (data not shown). Despite these problems associated with GC, it remains a very useful method for the analysis of cannabinoids.^[21]

HPLC with UV/FLD Detection

With gradient-elution, most cannabinoids were base-line separated as sharp peaks with excellent peak purity level, yielding fully exploitable UV and fluorescence spectra. The retention times of cannabinoids (relative to Δ^9 -THC) are shown in Table 4. It is interesting to note that the relative elution time of the acidic cannabinoids can be influenced by changing the pH of the eluent, while the order of elution for the neutral cannabinoids remains the same.^[23] Notwithstanding these pH differences, the elution order of THCCOOH and THC was not modified. In this way, overlap between chromatographic peaks of acid and neutral cannabinoids can be decreased by changing the elution pH.

Although the UV-spectra of the analysed cannabinoids (Figure 3a) were left unchanged when the pH was changed from 3.0 to 7.5, the FL-spectra differ drastically (Figure 3b). Acidic cannabinoids completely lose their fluorescence under acidic conditions, while CBC has no fluorescence under

Table 4. Relative retention time (RRT) of cannabinoids in HPLC using a reversed phase column (C₁₈) and eluent with a basic (7.5) or acidic (3) pH

Acidic HPLC system		Basic HPLC system	
Cannabinoid	RRT	Cannabinoid	RRT
11-OH-THC	0.70	THC-COOH	0.26
THC-COOH	0.76	CBDA	0.34
CBD	0.76	THVA	0.36
THV	0.77	CBGA	0.40
CBG	0.78	THCA-C4	0.42
CBDA	0.82	CBNA	0.50
CBGA	0.92	THCA-A	0.51
CBN	0.93	CBLA	0.53
Δ^9 -THC	1.00	CBCA	0.61
Δ^8 -THC	1.03	CBD	0.83
THVA	1.04	CBG	0.83
CBC	1.12	CBN	0.95
THCA-C4	1.13	Δ^9 -THC	1.00
CBNA	1.21	Δ^8 -THC	1.01
THCA-A	1.25	CBC	1.08
CBLA	1.32	11-OH-THC	1.31
CBCA	1.34		

basic conditions, and CBN has no fluorescent properties at all. The fluorescent properties of the other analysed cannabinoids are not influenced by pH. The UV absorption and FL yield in Figure 3a and b cannot be compared because no standardised concentrations of the cannabinoids were used. Standardised UV-spectra were obtained using a spectrophotometer (see below and Figure 4).

In some cases, partially unresolved peaks could not be identified because their UV and fluorescence spectra were identical. This can be seen with Table 4 and in Figure 3a and b with CBD and CBG or Δ^8 -THC and Δ^9 -THC, which are characterised by very close retention times and identical UV and fluorescence spectra.

The chromophore of the cannabinoids corresponds to its substituted phenolic ring, as this is a common structural element among the tested cannabinoids. The UV spectrum of Δ^9 -THC is identical to that of olivetol, which shows the same phenolic ring structure and is the precursor of Δ^9 -THC and the other cannabinoids. The alkyl-side chain does not influence the UV-absorbance, as there is no difference between THCA (C₅-side chain) and THVA (C₃-side chain). The cyclization of the non-phenolic part of the cannabinoids also has no influence on the absorbance, except when another aromatic ring (CBN, CBNA) or a conjugated double bond (CBC, CBCA) is introduced.

(a) UV neutrals

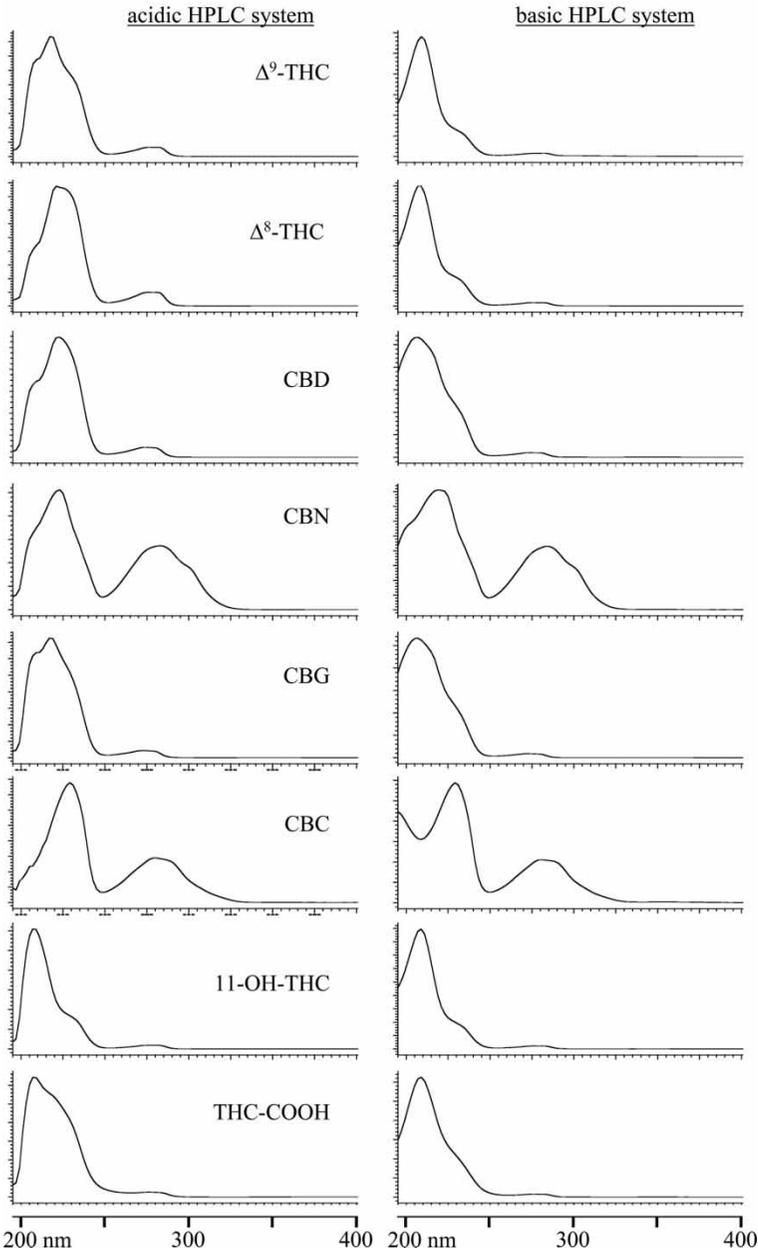
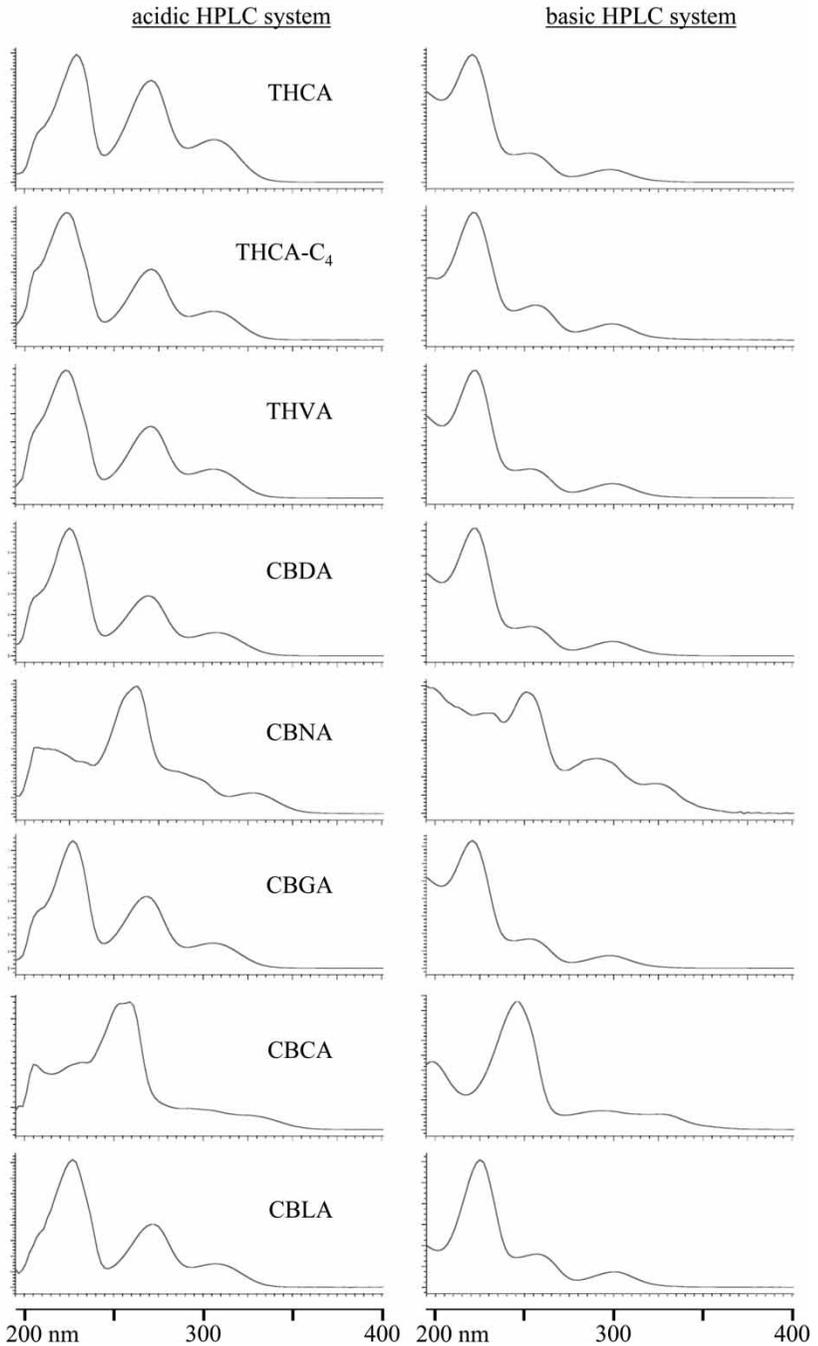


Figure 3. (a) UV-spectra in the range of 190–400 nm obtained in two HPLC-systems with acidic and basic pH. (b) Fluorescence spectra in the range of 280–650 nm obtained in two HPLC-systems with acidic and basic pH.

(continued)

UV acids

*Figure 3.* Continued.

(b) FLD neutrals

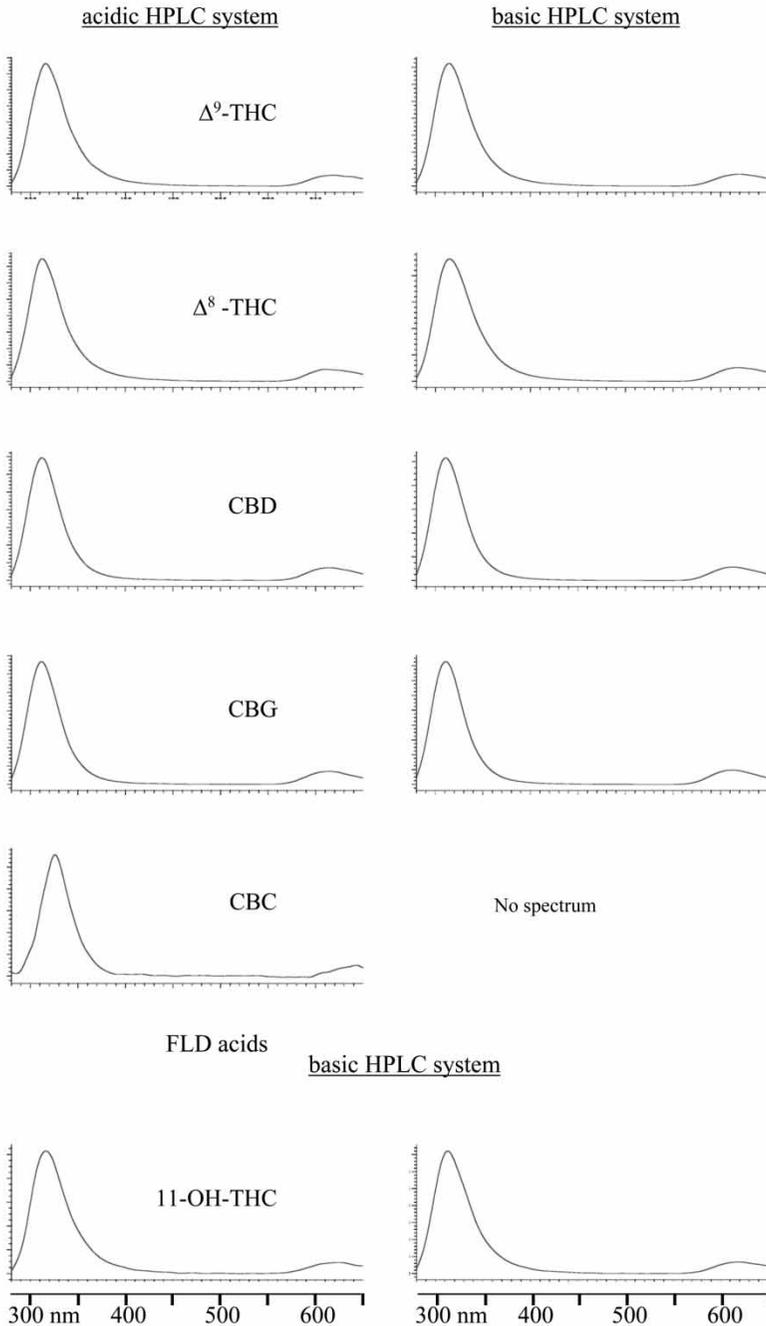


Figure 3. Continued.

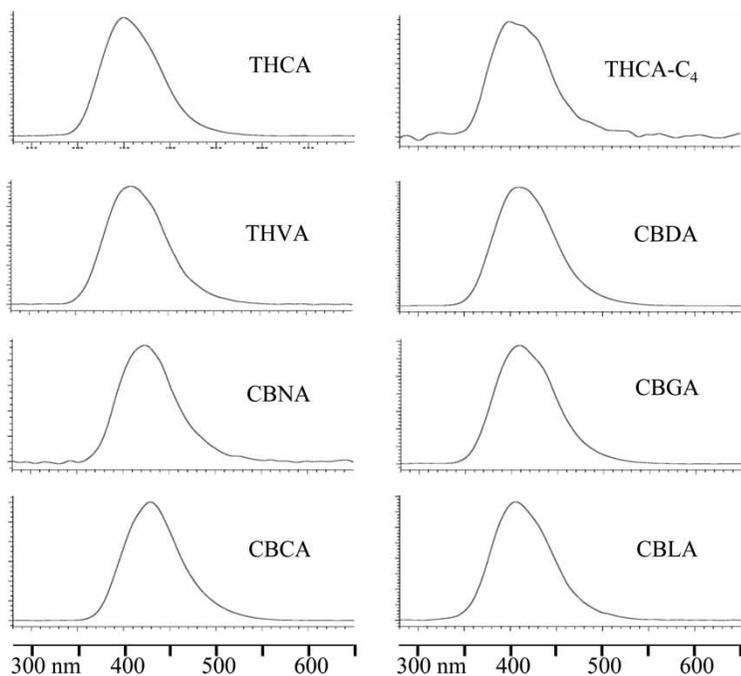


Figure 3. Continued.

In the case of HPLC peak overlap, the use of MS-detection coupled to HPLC (LC-MS) and furthermore, LC-MS-MS can provide better clues about cannabinoid structure and identity. In the acid system (pH = 3), formic acid was used to make the eluent compatible with mass spectrometry. In contrast to HPLC-DAD or FI, which are carried out at room temperature, LC-MS with ionspray ionisation at a relatively high temperature (e.g., 500°C) may result in partial thermal decomposition of acid cannabinoids. An example of a LC-MS separation of a large array of THC metabolites in body fluids at a concentration of 50 ng/mL is shown in Figure 6. For separation, we used a Waters XTerra C₈ microbore column. In contrast to GC-MS operating in the EI mode, the mass spectra are very simple with one prominent $[MH]^+$ or $[M-H]^-$ pseudo-molecular ion and very little fragmentation. For better sensitivity, the data were recorded in the Selected Ion Monitoring (SIM) mode. Except THC ($[MH]^+ = 315.2$), all cannabinoids were measured in the negative ionisation mode. The monohydroxylated (8 β -OH- and 11-OH-THC) and dihydroxylated 8 β -11-diOH THC metabolites were well resolved from the acid inactive metabolite (THCCOOH) and its conjugated derivative (THCCOOH-glucuronide).

Spectrophotometric Analysis (Extinction Coefficients)

Very few UV-absorption spectra of calibrated cannabinoids are given in the scientific literature.^[16] They are generally characterised by a few parameters

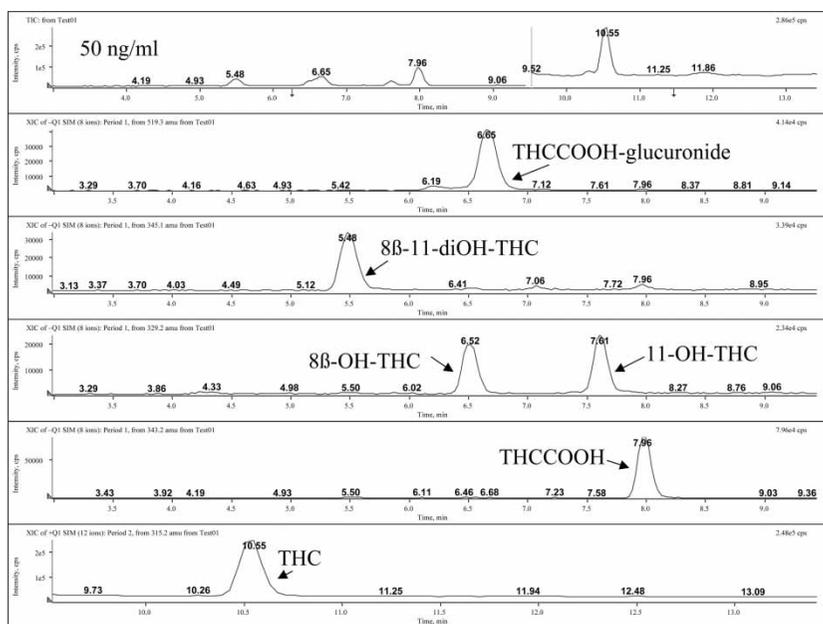


Figure 4. Chromatogram of a separation and identification of cannabinoid metabolites from human blood in a single chromatographic run, by using LC-MS. All cannabinoids can be identified because of the high selectivity of the mass-detector. The top chromatogram shows the total ion current (TIC).

(maxima and minima, shoulders of the UV spectra). The extinction coefficients are very seldom presented. Because most cannabinoids differ in their UV with several absorption peaks, many wavelengths can be selected for quantification. Figure 5 shows that absorption generally decreases with increasing wavelength. A better sensitivity can be obtained in the low 200–210 nm range, while selecting a higher wavelength will increase the selectivity by diminishing the risk of measuring interfering compounds. In order to get a rough estimate on the concentration of cannabinoids from any selected wavelength, a spectrum measured at 0.01 mg/mL between 200 and 400 nm is presented for 7 major cannabinoids (Figure 5). The extinction coefficients (ϵ) at 3 different maxima are also indicated.

Infrared Spectroscopy (IR)

Infrared spectroscopy has been a common tool for the identification and structure elucidation of cannabinoids and derivatives in isolation and synthesis experiments. As with UV-spectra, usually IR-spectra are reported by presenting a few maximum absorbance peaks only. Obviously, reported IR-spectra have been measured with a large variety of IR-spectrometers. In

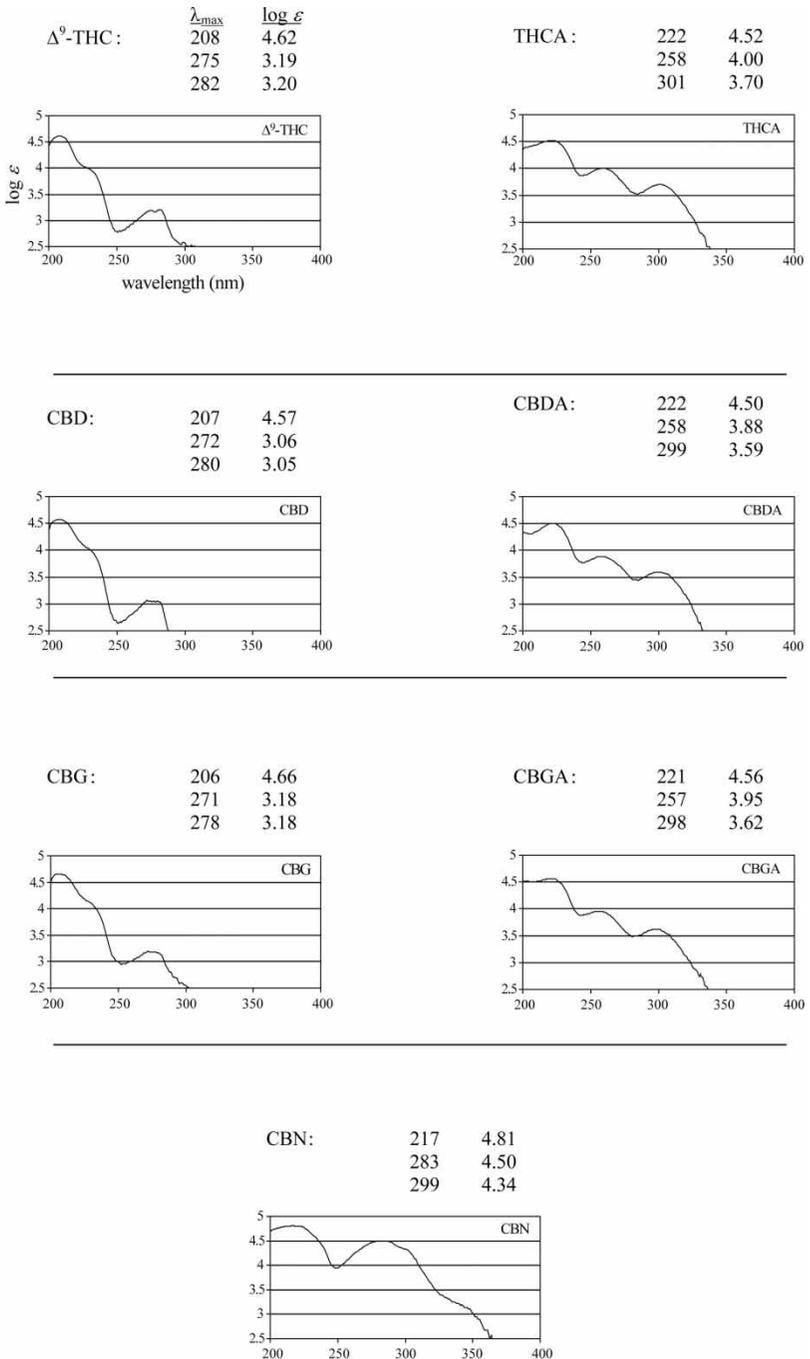


Figure 5. Extinction coefficients in the range of 200–400 nm at a concentration of 0.01 mg/mL in ethanol. Absorption values at maxima or shoulders are indicated.

this report (Figure 6) we present the full IR-spectra of 8 common natural cannabinoids measured on a single modern FT-IR-spectrometer.

CONCLUSION

A growing interest in Cannabis as a source of medicinal compounds has emerged during the last few years. Several crude preparations or synthetic

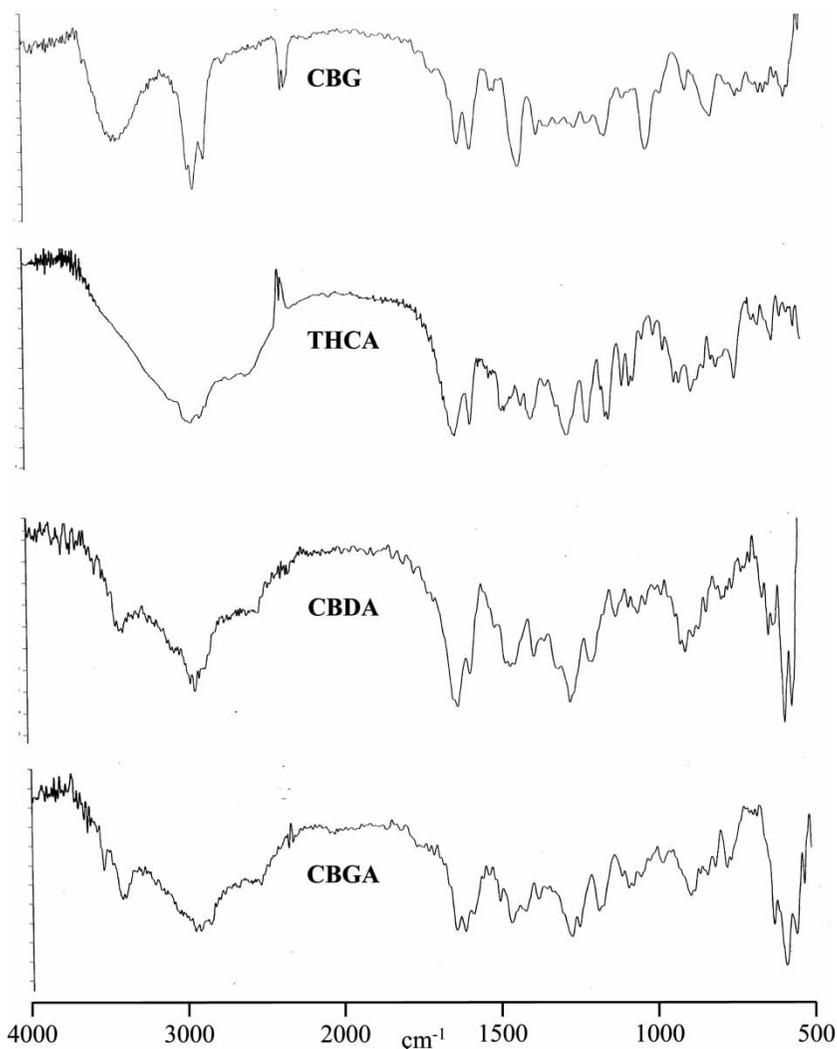


Figure 6. IR-spectra in the range of 500–4000 cm^{-1} obtained by Fourier-transform (FT)-IR spectrometry.

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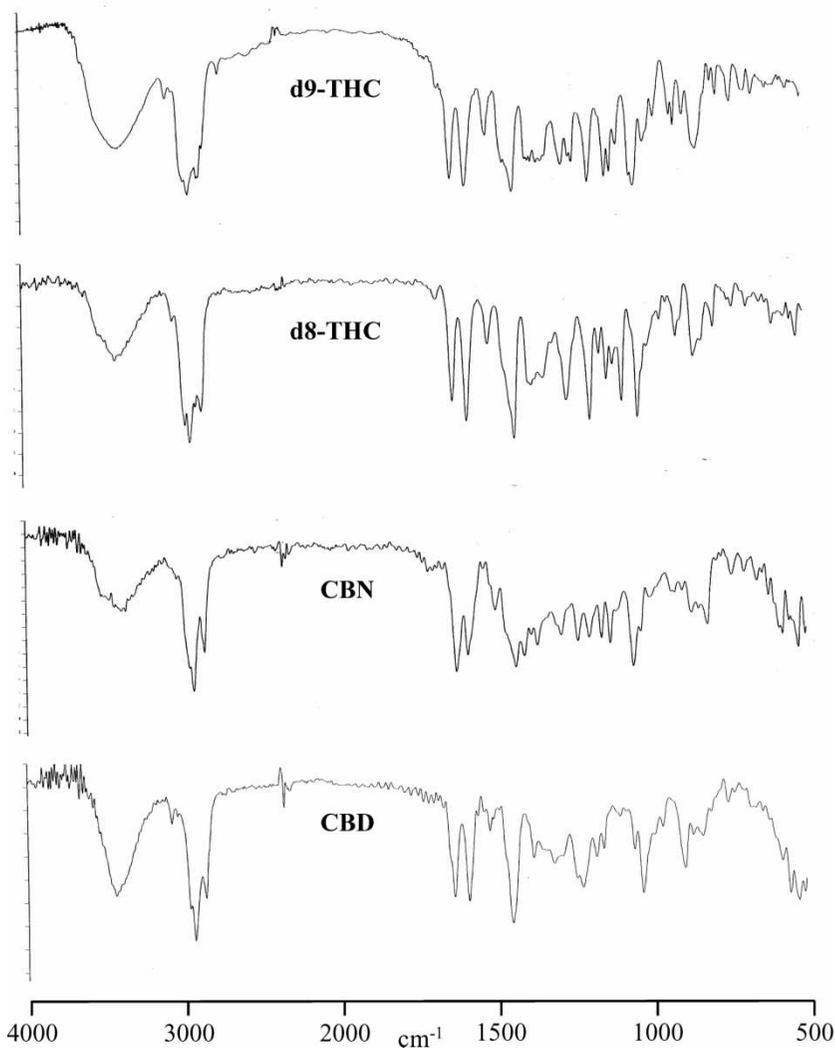


Figure 6. Continued.

drugs derived from Cannabis are under development, or in the clinical pipeline for distribution on the market. For carrying out all these investigations, pharmacologically pure cannabinoids must be available in large quantities. Reference compounds for analytical research must also be present. Chromatographic and spectroscopic data are, therefore, a prerequisite for their determination and identification.

The analytical data presented here makes it possible to positively identify the major cannabinoids found in the Cannabis plant. Presenting all analytical parameters measured under standardised conditions should facilitate the

identification of cannabinoids isolated from, or present in, Cannabis preparations. Unequivocal identification of cannabinoids cannot totally rely on only one of the tested methods because confusion of some common cannabinoids always remains possible.

Finally, we believe that the use of LC-MS, and especially LC-MS-MS, should make it possible to identify all tested cannabinoids in one single analysis, even in the low ng/mL concentration range.

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