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Journal of Chromatography B, 772 (2002) 299–306

JOURNAL OF CHROMATOGRAPHY B

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Determination of cannabinoids by gas chromatography–mass spectrometry and large-volume programmed-temperature vaporiser injection using 25 μl of biological fluid

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Received 12 December 2001; received in revised form 15 February 2002; accepted 15 February 2002

Abstract

This paper presents a GC–MS confirmation method, based on large-volume programmed-temperature vaporisation (PTV) injection, for the determination of cannabinoids in plasma samples (or whole blood) with deuterium-labelled internal standards using only 25 μl of biological fluid. The analytes, Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), were enriched by means of solid-phase extraction cartridges containing octadecyl-bonded silica and were, subsequently, methylated. A 20 μl aliquot of an extract in hexane was injected into a PTV in solvent split mode. Method development and the results of the analyses of standard reference material and real samples are presented and discussed. This micro-method is precise and sensitive enough to assess relevant cannabinoid levels in human blood for forensic investigations as well as for clinical applications. © 2002 Published by Elsevier Science B.V.

Keywords: Large-volume programmed-temperature vaporiser injection; Cannabinoids

1. Introduction

In 1979, Vogt et al. [1,2] presented, for the first time, programmed-temperature vaporisation (PTV) in GC analysis. After a number of methodological investigations, systems capable of routine operation are now available commercially. By means of PTV injection systems, various targets can be realised: precise sample introduction under reduced thermal stress for the analytes, solvent elimination in front of the analytical column via a split outlet, especially for large-volume injection (LVI), or coupling of GC with sample preparation methods and other separation techniques.

Obviously, with regard to the technical possibilities provided by PTV injection, LVI has become of prime importance. There are a number of applications in environmental analysis or investigations of foodstuffs. Overviews of principles and applications are given in Refs. [3–5]. Although Vogt et al. recognised the potential of this injection technique and used it for the examination of samples in medicine [6], LVI does not currently play an im-

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1570-0232/02/$ – see front matter
PII: S1570-0232(02)00117-4

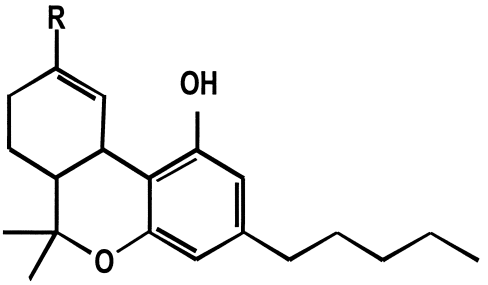
portant role in pharmacological and toxicological GC analysis. Only a few papers have been published using PTV and LVI in this field [7–13]. This can probably be traced back to the fact that analytical performance, as a rule, cannot really be improved by a simple increase in the injection volume if biological matrices (blood, serum, urine, etc.) are under investigation. In bioanalysis the signal-to-noise ratio decreases if the injection volume increases. However, PTV-LVI can be used to reduce consumption of the sample (blood) without a strong increase in the influence of the matrix or a deterioration in detection limits. Frequently, insufficient material is available in routine toxicological analysis of blood samples. For this purpose, LVI could be an interesting alternative without the need for substantial changes in sample preparation. The basic principle has already been demonstrated using pressure-pulsed splitless injection [14], although it is not the most capable technique (the maximum injection volume is limited to about 5 μl). The reduction in sample consumption was compensated by increasing the extract volume injected.

The objective of the current investigations was to develop and demonstrate a PTV-LVI-based confirmation method for the GC–MS analysis of cannabinoids (Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol) using only 25 μl of blood or serum.

2. Experimental

2.1. Chemicals and reference standards

All solvents and reagents were of analytical grade. One-hundred milligram solid-phase extraction columns (Bakerbond spe, Octadecyl) were obtained from Baker. The reagents for derivatisation (tetramethylammoniumhydroxide 25% in water, methyl sulfoxide and iodomethane, hydrochloric acid, acetic acid, isooctane) were purchased from Merck. The following cannabinoids (see Fig. 1) and their deuterated analogs (Promochem) were used for GC–MS analyses: Δ^9-tetrahydrocannabinol-D3 (100 μg/ml), 11-hydroxy-Δ^9-tetrahydrocannabinol-D3 (100 μg/ml), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-D3



THC	$R = CH_3$
11-OH-THC	$R = CH_2OH$
THC-COOH	R = COOH

Fig. 1. Chemical structure of the cannabinoids.

(100 μg/ml), Δ^9-tetrahydrocannabinol (1 mg/ml), 11-hydroxy-Δ^9-tetrahydrocannabinol (100 μg/ml) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (100 μg/ml). The stock solutions were diluted with methanol.

A reference sample (serum control) was purchased from Medichem (41622 Medidrug BTMF 2/2000).

2.2. Apparatus

An HP 6890 Plus series gas chromatograph (Agilent Technologies) was operated using Chemstation B 02.05. The GC was equipped with a PTV injector including a CO_2 cooling system, a split/splitless injector, an HP 7683 automatic sampler and a mass spectrometer (HP 5973 MSD). Chromatographic separation was carried out on a MDN-5 fused-silica column (30 m×025 mm I.D., 0.25 μm film thickness, Supelco). Helium was used as carrier gas at a constant flow of 1 ml/min. The column temperature was initially 60 °C for 3.5 min, then raised by 50 °C/min to 255 °C, 5 °C/min to 320 °C, which was held for 3 min.

The mass spectrometer was operated in electron impact ionisation mode. The temperatures of the ion source and quadrupole were 230 and 150 °C, respectively. Selected ion monitoring was performed on the following molecular ions: *m/z* 313, 316, 328 and 331 for THC/THC-D3; *m/z* 313, 316, 358 and 361 for

11-OH-THC/11-OH-THC-D3; and *m/z* 313, 316, 357 and 360 for THC-COOH/THC-COOH-D3.

The parameters of sample injection into the GC–MS system are shown in Table 1.

2.3. Sample preparation

A method developed for the determination of cannabinoids [15,16] was used and slightly modified. After adding 25 μl of the deuterated standards to 0.5 ml acetic acid (1.5% in water), 0.5 ml of the blood sample (or plasma) was added for the standard procedure. In the case of the LVI method, 25 μl blood was added to 0.2 ml acetic acid (1.5% in water) spiked with 25 μl of the deuterated standards. The working concentrations of the deuterated standards were 10, 10 and 50 ng/ml for THD-D3, 11-OH-THC-D3 and THC-COOH-D3, respectively. After conditioning of the SPE cartridges using ethyl acetate, methanol and acetic acid (0.75% in water), the sample was extracted followed by a washing step. The elution step was performed using ethyl acetate (see Section 3.1.3). The eluate was evaporated to dryness. Tetramethylammoniumhydroxide (0.2 ml, 25% in water) and methyl sulfoxide (1.8 ml) were added followed by iodomethane. The samples were kept at room temperature for 15 min. Hydrochloric acid (200 μl, 0.2 *N*) was then added and the methylated derivatives were extracted into 1 ml isooctane. The octane phase was transferred into an autosampler vial, evaporated to dryness and redissolved in 50 μl hexane.

Table 1
Operating conditions for sample introduction

Standard procedure	
Injector	HP split/splitless
Injection mode	Splitless (for 1 min)
Temperature	280 °C
Injection volume	1 μl
LVI method	
Injector	HP PTV
Insert	Smooth PTV insert containing a plug of silanized glass wool
Injection mode	Solvent vent
Vent flow	300 ml/min
Vent time	2 min
Start temperature	60 °C (2 min)
Rate 1	720 °C/min to 250 °C for 3 min
Rate 2 (cleaning)	720 °C/min to 300 °C

3. Results and discussion

3.1. Method development

3.1.1. Operating conditions during sample introduction

PTV-LVI is based on separating the analytes from the solvent even during sample introduction. The solvent can be eliminated via the split outlet while the higher-boiling analytes are retained (at low injector temperature) and subsequently transferred to the column by heating the PTV. The operating conditions have to be carefully set to minimise losses of analytes during elimination of the solvent. Parameters such as temperature, carrier gas flow and the time of solvent elimination, as well as the conditions during the following desorption step, have to be considered. The optimisation is more complicated if the boiling point differences between solvent and analytes are small. (If necessary, packing materials and adsorbents, such as Tenax, should be used to retain the analytes during solvent elimination [17–19]. Unfortunately, the PTV-LVI technique is not suitable for analytes of very high volatility.)

The cannabinoids (methylated derivatives), however, are uncomplicated and, using a carrier gas flow of 300 ml/min and a temperature of 60 °C during the solvent vent time of 2 min, no significant losses occur. A temperature of 230 °C and a splitless time of 1 min was found to be suitable to transfer the substances into the GC column.

3.1.2. Maximum volume of injection

The maximum volume of injection, with the whole volume entering the insert at the same time, was determined to be in the range 5 to 25 μl by injecting a standard solution of methylated THC. Fig. 2 shows, under the given conditions, an increase in the peak areas up to injection volumes of about 20 μl. Larger volumes of the liquid sample could not be accommodated in the glass-wool-packed insert and exceeded the liner capacity. To increase the quantity injected far in excess of 20 μl, other PTV-based sample introduction techniques, such as multiple

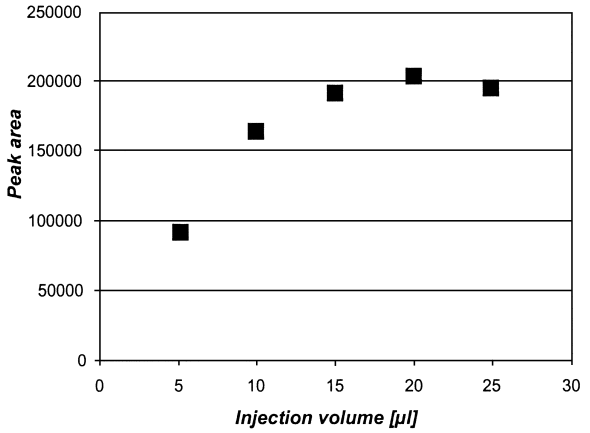


Fig. 2. Dependence of the peak areas on the injection volume determined by injecting methylated THC using the PTV in solvent split mode.

injection or speed-controlled injection, have to be utilised [20,21].

3.1.3. Sample preparation

As mentioned above, sample preparation was performed by means of a method developed earlier. Further optimisation concerned the elution step, to remove the analytes from the 100 mg SPE cartridges, and the elution profiles were determined using acetonitrile, methanol and ethyl acetate (see Fig. 3).

Acetonitrile proved to be unfavourable, especially for the removal of THC-COOH. The best results were obtained using ethyl acetate. Quantitative elution can be ensured by using 1 ml ethyl acetate.

3.1.4. Variation of sample and injection volumes

The basic principle of the approach was demonstrated by a stepwise increase of the injected extract volume, starting from 1 μl injection (using 500 μl serum) up to 20 μl injection (using 25 μl serum). As illustrated in Fig. 4, the reduction in serum volume can be compensated for by increasing the injection volume.

In the mass chromatograms of m/z 313, however, an increasing influence of matrix interferences is observed. In the case of THC, coelution seems to deteriorate the limit of detection. This peak is still seen in blank samples and increases if larger volumes of solvent are used. The quantification of THC, however, using the mass chromatogram of m/z 328 was unaffected by this interference.

3.1.5. Calibration, recovery, detection limits

Calibration curves were calculated from a series of spiked plasma samples, subsequent to the development of the method. The range of calibration was chosen with respect to general recommendations [22,23] and daily routine experience. A frequency distribution of cannabinoid concentrations, based on

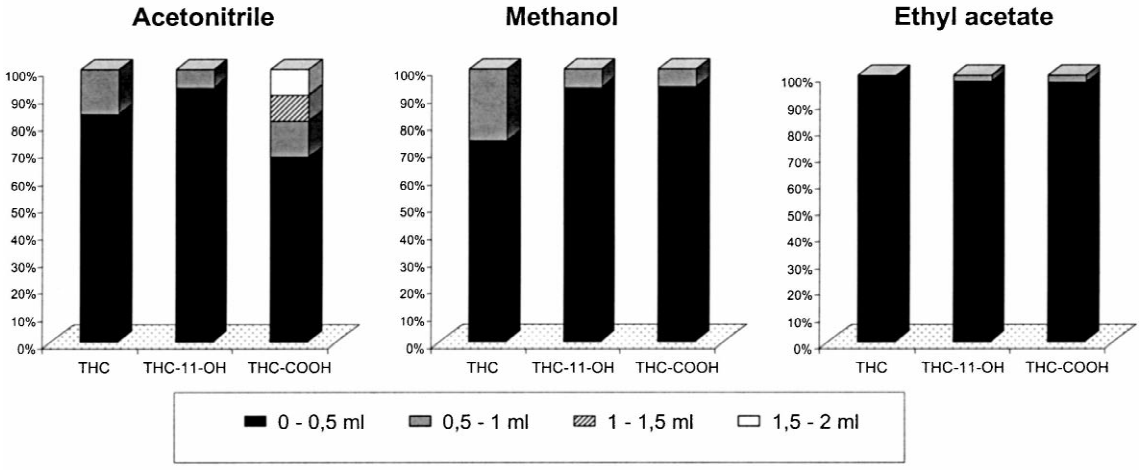


Fig. 3. Elution profiles for cannabinoids from the SPE cartridge using acetonitrile, methanol and ethyl acetate.

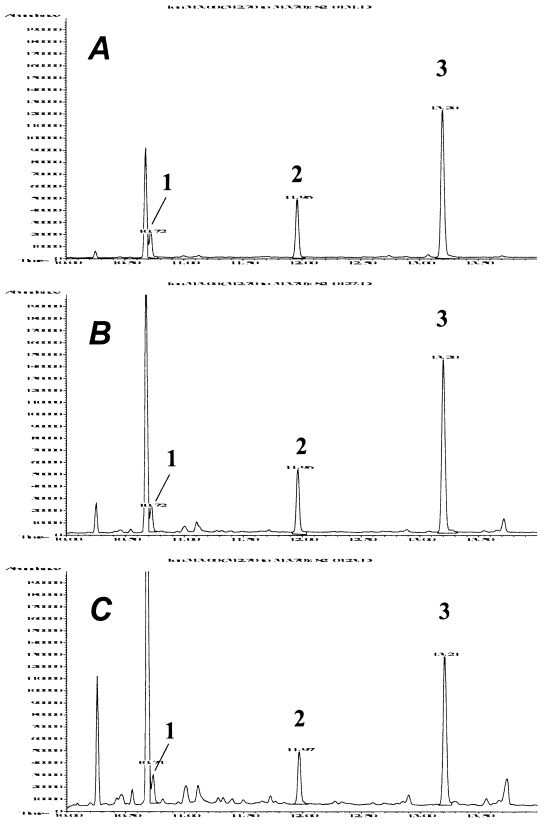


Fig. 4. Mass chromatograms (m/z 313) of cannabinoids in the extract of a spiked plasma sample: 1=THC (10 ng/ml), 2=11-OH-THC (10 ng/ml), 3=THC-COOH (50 ng/ml); injection volume: 1, 5 and 20 μl for (A), (B) and (C), respectively; serum consumption: 500, 100 and 25 μl for (A), (B) and (C), respectively.

investigations carried out during 2000, is given in Table 2.

On this basis 2, 5, 10, 20 or 30 ng/ml of THC and 11-OH-THC and 10, 25, 50, 100 or 150 ng/ml of THC-COOH were added to human serum. The response was found to be linear in the validated range, with correlation coefficients (r) >0.0997 (see Fig. 5).

Further investigations were carried out using spiked serum samples at a concentration of 5 ng/ml of each compound. The relative standard deviations ($n=5$) of the method were 5.9, 4.6 and 4.0% for THC, 11-OH-THC and THC-COOH, respectively, and the mean recoveries were >90% throughout. Signal-to-noise ratios were used to estimate the

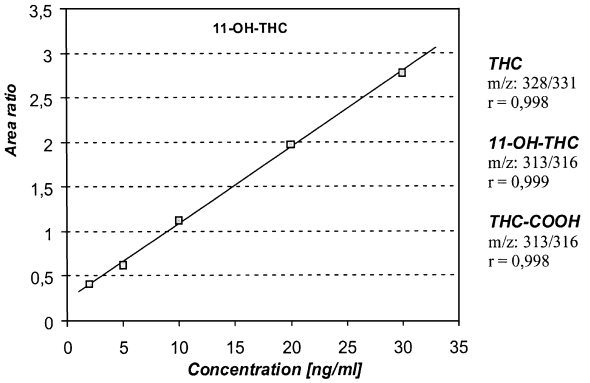


Fig. 5. Calibration curve for 11-OH-THC and calculated correlation coefficients for THC, 11-OH-THC and THC-COOH.

Table 2
Frequency distribution of cannabinoid concentrations determined at the Institute of Legal Medicine, Leipzig, during 2000

THC ($n=102$)		11-OH-THC ($n=101$)		THC-COOH ($n=121$)	
Range (ng/ml)	Quantity	Range (ng/ml)	Quantity	Range (ng/ml)	Quantity
0–2	21	0–1	16	0–20	10
2–4	18	1–2	29	20–40	25
4–6	20	2–3	17	40–60	24
6–8	11	3–4	15	60–80	10
8–10	8	4–5	3	80–100	19
10–12	6	5–6	0	100–120	8
12–14	2	6–7	4	120–140	2
14–16	3	7–8	4	140–160	5
16–18	4	8–9	2	160–180	3
18–20	2	9–10	4	180–200	4
20–30	5	10–12	3	200–220	3
30–40	2	12–14	4	220–240	8

limits of detection ($S/N = 3$) at about 0.7, 0.8 and 0.7 ng/ml for THC, 11-OH-THC and THC-COOH, respectively.

3.2. Application of the method

3.2.1. Standard reference material

The method was applied to investigate a serum control (41622 Medidrug BTMF 2/2000). Lyophilised serum was reconstituted, according to the recommendations, with distilled water. The reference values (detected values) and the confidence range in force were found to be within the bounds of external quality monitoring BTMF 2/98 by the GTFCH (Society of Toxicological and Forensic Chemistry) [24].

The cannabinoid concentrations, summarised in Table 3, were calculated based on the calibration curves obtained from spiked serum samples (see Section 3.1.5).

The detected cannabinoid levels were found to be in the confidence range. In the case of THC, better conformity could probably be achieved by using a current calibration.

3.2.2. Real samples

Fig. 6 shows the chromatogram of a sample obtained during a routine investigation at our institute. An 18-year-old male was required to give a blood sample by traffic control. As a result of a positive immunoassay (ABBOT, ADx) in urine, a GC–MS confirmation method was applied to obtain serum cannabinoid concentrations. The analyses were carried out using the standard procedure (500 μl serum and 1 μl injection) as well as the PTV-based LVI method. Table 4 shows a comparison of the results of both analyses.

A test series using further serum and urine samples showed no considerable differences or disadvantages of the new method.

4. Conclusions

The GC–MS confirmation method reported here was applied to the determination of cannabinoids in biological fluids (standard reference material and real samples). Using an aliquot of 25 μl serum, the PTV-LVI method meets the requirements of routine analysis. The performance is comparable to existing GC–MS and LC–MS methods [25–28], but sample consumption is much smaller.

In principle, it will be possible to extend the procedure of the micro-method to other substances in the field of drug analysis. However, to avoid losses of volatile analytes (such as amphetamines) during solvent elimination, the selection of appropriate PTV conditions, probably by using adsorbent-packed injector inserts, will be required. These aspects are currently under investigation.

By means of PTV-LVI the consumption of samples could be reduced drastically. Therefore, the quantity of material available is no longer a limiting factor. Even in the case of small sample volumes, several target analyses or repeated examinations can be carried out to verify the results. Furthermore, there are interesting possibilities with respect to miniaturisation of sample preparation and saving consumables, time and cost (for instance, using smaller SPE cartridges, less solvent and a shorter preparation

Table 3
Application of the PTV-based method to a certified serum control (41622 Medidrug BTMF 2/2000, Medichem)

	THC (ng/ml) m/z 328/331	11-OH-THC (ng/ml) m/z 313/316	THC-COOH (ng/ml) m/z 313/316
Sample 1	3.7	7.3	21.9
Sample 2	3.4	7.1	22.3
Sample 3	3.6	7.7	24.0
Average conc.	3.6	7.4	22.7
Detected value	5.2	7.4	24
Confidence range	3.3–7.1	4.9–12.4	17.2–37.6

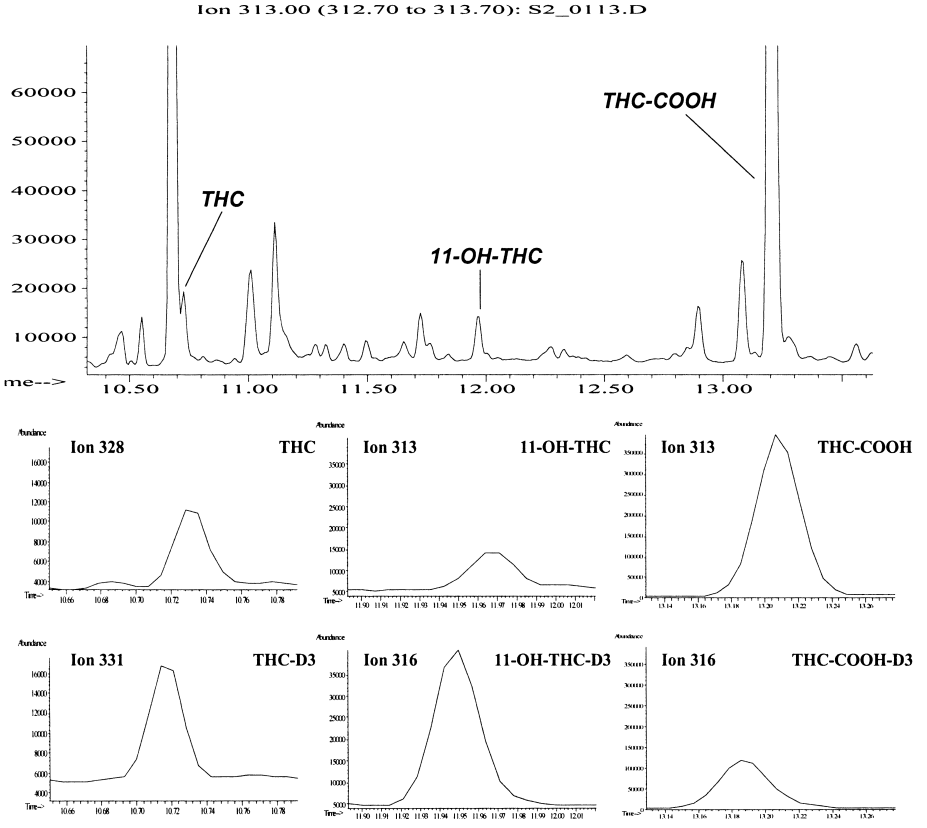


Fig. 6. Mass chromatogram obtained using 25 μl of a blood sample from a cannabis consumer (PTV-based method). For quantitative results, see Table 4.

time). Possibly, the application of multistage methods for screening analyses in plasma samples (or saliva), developed using PTV-LVI, could be combined with fast GC analysis and automated computer-based data analysis for further improvements of the toxicological analysis.

Table 4
Comparison of the cannabinoid levels in a serum sample determined using the standard procedure and the PTV-based method

	Standard procedure (ng/ml)	PTV-LVI (ng/ml)
THC	5.4	6.3
11-OH-THC	2.6	2.8
THC-COOH	122	138

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