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Journal of Chromatography B, 673 (1995) 173–181

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Supercritical fluid extraction of drugs in drug addict hair

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First received 24 March 1995; revised manuscript received 6 June 1995; accepted 20 June 1995

Abstract

Opiates were extracted from sixteen hair samples of drug addicts using a supercritical fluid extraction method with supercritical carbon dioxide and a modifier solution of methanol–triethylamine–water (2:2:1, v/v). The concentrations, as determined by GC–MS, ranged from 1.22 to 21.73 (mean 7.60 ng/mg), 0.17 to 1.54 (mean 0.69 ng/mg) and 0.15 to 14.09 ng/mg hair (mean 3.78 ng/mg) for codeine, morphine and 6-monoacetylmorphine, respectively. The reproducibility of the total procedure had a relative standard deviation of 13%, 17% and 14% for codeine, morphine and 6-monoacetylmorphine, respectively. By this method, concentrations of 0.3, 0.2 and 0.1 ng/mg hair for codeine, morphine and 6-monoacetylmorphine, respectively, could be detected. Relative extraction recoveries were 61%, 53% and 96% for codeine, morphine and 6-monoacetylmorphine, respectively.

1. Introduction

In 1979, Baumgartner et al. [1] reported the first use of radioimmunoassay to detect morphine in hair of heroin addicts. Currently, the presence of various drugs in hair can be identified by a variety of well-established laboratory procedures.

The sample preparation techniques and extraction procedures are often time-consuming and can require the use of toxic solvents. For example, acid hydrolysis [2] has an incubation time of 18 h in HCl. The enzymatic method [3] requires an incubation in the presence of ß-glucuronidase and arylsulfatase, which are expensive. 6-Monoacetylmorphine (6-MAM), conclusive proof of heroin intake, is hydrolyzed to morphine (MOR) under alkaline and strong acid conditions [4], and it is therefore not possible to distinguish between drug abuse (heroin) or medical use of morphine. Direct methanolic extraction [5] requires a sonication time of 5 h in methanol.

Supercritical fluid extraction (SFE) was first developed in the 1980s [6] and was applied to many matrices and analytes [7], but little is known about drug extraction from hair by a supercritical fluid. Recently, SFE was proposed as a new technology for drug extraction from hair. Morisson and MacCrehan [8] investigated SFE as an alternative to the currently used wet chemical methods for the selective recovery of cocaine (COC) from hair. Subcritical fluid extraction was proposed by Edder et al. [9,10] as a method of choice to extract opiates from hair of drug addicts, and SFE was used for qualitative determination of opiates and heroin by Sachs and Uhl [11].

This paper reports the establishment of an

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0378-4347/95/$09.50
SSDI 0378-4347(95)00281-2

extraction method for opiates in hair of drug addicts by the Hewlett Packard 7680 T SFE module on the basis of previously reported procedures [8–10].

2. Experimental

2.1. Chemicals

Methylene chloride (CH_2Cl_2) and methanol (MeOH) were HPLC-grade; triethylamine (Et_3N) was GC-grade (Merck, Darmstadt, Germany). Deuterated internal standards (IS-d_3), codeine-(COD), MOR- and 6-MAM-d_3 were purchased from Radian (Austin, USA). N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) was purchased from Interchim (Montluçon, France). Carbon dioxide (CO_2, SFC purity) was obtained from Air Liquid (Illkirch, France).

2.2. Hair samples

Hair samples were obtained from sixteen subjects who died after a fatal heroin overdose. Hair was collected at the back of the head, on the vertex posterior, cut as close as possible to the skin and stored in a plastic tube at room temperature. Hair was not pulverized because this step is time-consuming and not necessary to increase recovery. Before supercritical fluid extraction, the samples were decontaminated twice in CH_2Cl_2 (for 2 min at room temperature) to remove external substances.

2.3. SFE procedure

Decontaminated hair (50 mg) was placed into a 7-ml thimble, between two filters (MN 2101 type, Macherey–Nagel, Düren, Germany), and the thimble was introduced into the extraction chamber of the Hewlett Packard 7680 T SFE module. The SFE procedure consisted of a clean-up step followed by the extraction step.

First, the sample was submitted to a clean-up SFE step where pure CO_2 was employed to remove interfering endogenous compounds (Fig. 1). The chamber temperature was set at 100°C; density and flow-rate of the supercritical CO_2 were 0.68 g/ml and 2.0 ml/min, respectively. An equilibration time (static extraction) of 10 min and an extraction time (dynamic extraction) of 15 min were employed.

Cleanup step

EXTRACTION STEP 1

EXTRACTION CONDITIONS

density:	0.68	g/ml
pressure:	315	bar
chamber temperature:	100	C
flow rate:	2.0	ml/min
extraction fluid:	CO2	
equilibration time:	10.00	min
extraction time:	15.00	min
thimble volumes swept:	5.8	

EXTRACT TRAPPING CONDITIONS

nozzle temperature:	95	C
trap temperature:	25	C
trap packing:	Tenax	
void volume compensation:	1.0	ml

FRACTION OUTPUT

Rinse Substep	Solvent Name	Volume (ml)	Rate (ml/min)	Nozzle Temp	Trap Temp	Vial Number
1	Chloroform	1.8	1.0	30	30	1

Fig. 1. SFE parameters for the clean-up step by the HP 7680T module.

After this, 200 ng of IS-d_3 and 1 ml of modifier solution MeOH–Et_3N–H_2O (2:2:1, v/v) were introduced in the thimble, and the drugs were extracted from hair by the same method described in Fig. 1, but with two modifications. First, the dynamic extraction time was 20 min, and the trap temperature was set at 5°C instead of 25°C for the clean-up step, to ensure drug fixation. The TENAX trap used is a non-specific trap able to bind many compounds. The same trap was used throughout the entire study (lifetime of the trap 6 months). The compounds trapped by evaporation of the supercritical CO_2 were then eluted with 1.8 ml of chloroform.

2.4. GC–MS analysis

The chloroform was evaporated to dryness in a Speed Vac concentrator (Savant A 290), and the dry extract was derivatized with 30 μl of BSTFA + 1% TMCS for 20 min at 70°C. The derivatized extract was placed in the HP 7673 autosampler, and a 1-μl sample was injected onto the column (HP5-MS capillary column, 5% phenyl–95% methylsiloxane, 30 m × 0.25 mm × 0.25 μm film thickness) of a Hewlett Packard GC–MS system (5890 GC coupled with a 5971 MS).

Injector temperature was 270°C, and splitless injection was employed with a split-valve off-time of 0.75 min. The flow of carrier gas through the column was 1.0 ml/min (helium, purity grade N55). The column oven temperature was programmed to rise from an initial temperature of 60°C (kept for 1 min) to 295°C (kept for the final 10 min) at 30°C/min.

The detector was used in electronic impact mode at + 70 eV with an ion source temperature of 180°C. The detector was daily autotuned with perfluoroterbutylamine, and the electron multiplier voltage was set at + 400 V above the autotune voltage.

Qualitative and quantitative analyses were obtained in single ion monitoring (SIM) mode by comparison of retention times (t_R) and relative abundance of distinctive ions (m/z COD 371, MOR 429, 6-MAM 399) with those of IS-d_3 (m/z COD 374, MOR 432, 6-MAM 402).

3. Results and discussion

At the initial stage of this work, various analytical parameters were investigated to ensure maximal recoveries of the target drugs. Therefore, the influence of the chamber temperature and of the modifier solution volume was evaluated. All the following experiences were investigated on a unique, real hair sample obtained from a heroin addict, which deviates from procedures already tested as the other authors used soaked samples [8–10], but this more closely resembles the real situation.

The hair sample was cut in 0.5-cm pieces and homogenized by agitation for 1 h to eliminate drug concentration fluctuations along the hair strands. Hair samples submitted to the cleaning SFE procedure with pure supercritical CO_2 (Fig. 1) produced cleaner chromatograms with lower background and removed endogenous compounds which could interfere with codeine and morphine.

It was checked that this preliminary treatment did not affect drug concentrations in hair by comparing opiate concentrations before and after the clean-up SFE step.

3.1. Influence of the chamber temperature

The hair sample (50 mg) was extracted by increasing the chamber temperature. Modifier solution (400 μl) was added to the thimble, and the chamber temperature was set at 25, 40, 70 and 100°C in four separate extractions. Results are presented in Fig. 2.

The relative 100% recovery was determined by the higher 6-MAM concentration obtained. As the major drug of interest was 6-MAM, the percentage recovery was only evaluated for this analyte.

The higher 6-MAM extraction recovery was observed for a chamber temperature of 100°C. This temperature allowed efficient extraction of 6-MAM with minimal hydrolysis of 6-MAM to morphine. The potential hydrolysis to morphine was monitored via m/z 432 ion when only 6-MAM-d_3 was deposited into the thimble. Hy-

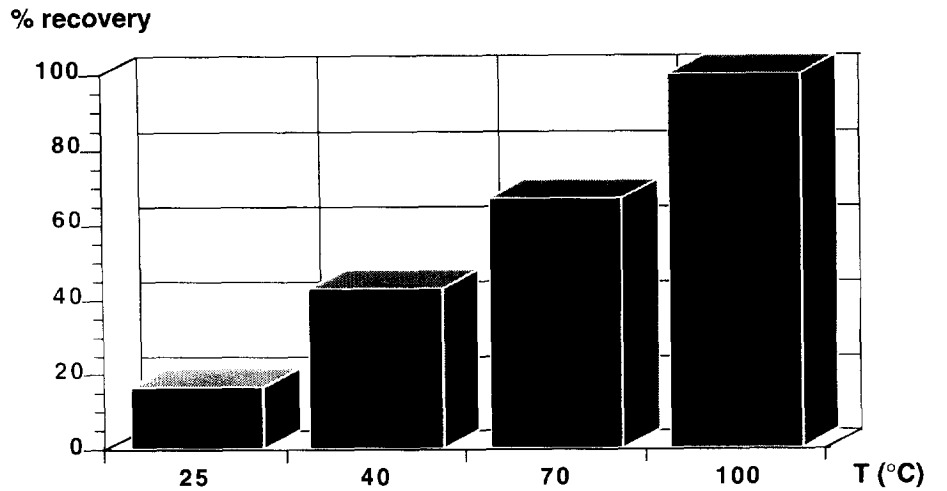

Fig. 2. Influence of the chamber temperature on 6-MAM extraction recovery.

drolysis of 6-MAM-d_3 produces morphine-d_3. Detector response for the m/z 432 ion was $<1\%$ of the response obtained with 6-MAM-d_3 (402).

To test a higher temperature by the 7680 T Hewlett Packard system, while maintaining a constant pressure, it is necessary to decrease the CO_2 density, and therefore, it would be impossible to attribute the potential modifications of the extraction recovery to the chamber temperature only. This is the reason why 100°C was the maximal temperature evaluated.

3.2. Influence of the modifier solution volume

The hair sample (50 mg) was extracted by increasing the volume of modifier solution in the thimble. The composition of the modifier solution as well as the ratio of the constituents were established previously by Edder et al. [9,10] and found suitable in this study. This ternary solvent was also used by Morisson and MacCrehan [8] for cocaine extraction, but in different proportions.

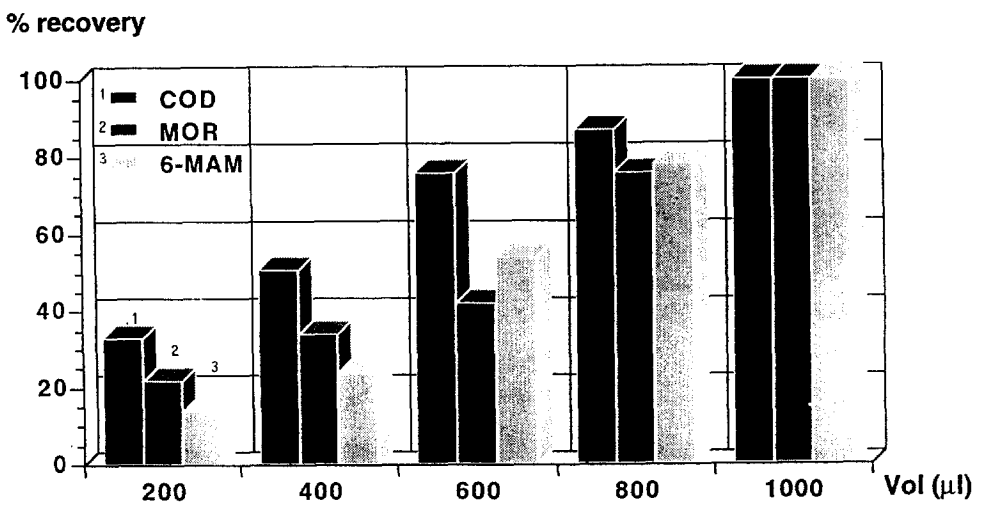

Fig. 3. Influence of the modifier solution volume on the opiate extraction recoveries.

Chamber temperature was set at 100°C, and 200, 400, 600, 800 and 1000 μl of solution were added in 5 separate extractions. Fig. 3 presents the extraction recoveries for COD, MOR and 6-MAM.

The relative 100% recovery was determined by the higher drug concentration obtained.

The optimal volume for COD, MOR and 6-MAM was 1 ml of MeOH–Et_3N–H_2O modifier solution (2:2:1, v/v). With higher volume, the organic phase obtained after SFE revealed the presence of aqueous solution that cannot be evaporated under adequate conditions. Static extraction times (5, 10 and 15 min) were also tested, but no significant higher extraction recovery was observed.

3.3. Reproducibility

Samples (50 mg) of hair obtained from the same patient were analyzed eight times through the entire procedure in one analysis day. The concentrations ranged from 9.1 to 13.3, 0.45 to 0.70 and 9.8 to 14.9 ng/mg hair for COD, MOR and 6-MAM, respectively. Results are presented in Table 1.

Standard deviations were 13%, 17% and 14% for COD, MOR and 6-MAM, respectively.

3.4. Limit of detection

The detection limits were evaluated for five different real hair samples by the signal-to-noise ratio calculated by the computer for a value of $S/N = 3$ ($n = 2$). By this method, concentrations of 0.3, 0.2 and 0.1 ng/mg hair of COD, MOR and 6-MAM, respectively, could be detected.

Table 1
Opiate concentration (ng/mg) and standard deviation (%) for opiates in a hair sample tested eight times by SFE

Drug	Range (ng/mg)	Mean (ng/mg)	S.D. (%)
COD	9.10–13.30	11.34	13.2
MOR	0.45–0.70	0.62	17.4
6-MAM	9.80–14.90	11.80	14.1

3.5. Extraction recovery

To determine the extraction recoveries, it was not possible to use hair of drug addicts due to the absence of reference and control material. In other studies in the literature, extraction recoveries were determined using spiked hair. A 50-mg sample of drug-free hair was spiked with 200 ng of the target drugs, corresponding to a final concentration of 4 ng/mg hair. Extraction recoveries were determined for COD, MOR and 6-MAM, in SIM mode, by comparing their representative peak areas after SFE versus those obtained with methanolic solution. Extraction recoveries were 61%, 53% and 96% for COD, MOR and 6-MAM, respectively.

3.6. Applications to real hair samples

Sixteen hair samples, previously analyzed by acid hydrolysis [2], were selected and extracted

Table 2
Opiate concentration (ng/mg) in hair of sixteen heroin addicts; each concentration is the mean of two separate experiments

Subject No.	COD	MOR	6-MAM
1	3.97	0.66	5.41
2	21.73	ND	0.78
3	1.32	0.56	1.03
4	ND	0.78	5.52
5	ND	0.70	6.59
6	12.97	1.34	0.37
7	14.91	0.44	1.29
8	2.33	1.45	10.11
9	ND	1.54	7.20
10	ND	0.18	0.93
11	ND	0.44	3.50
12	3.60	ND	1.65
13	1.50	0.17	0.71
14	1.22	0.25	0.15
15	1.76	0.24	0.27
16	18.20	0.84	14.90

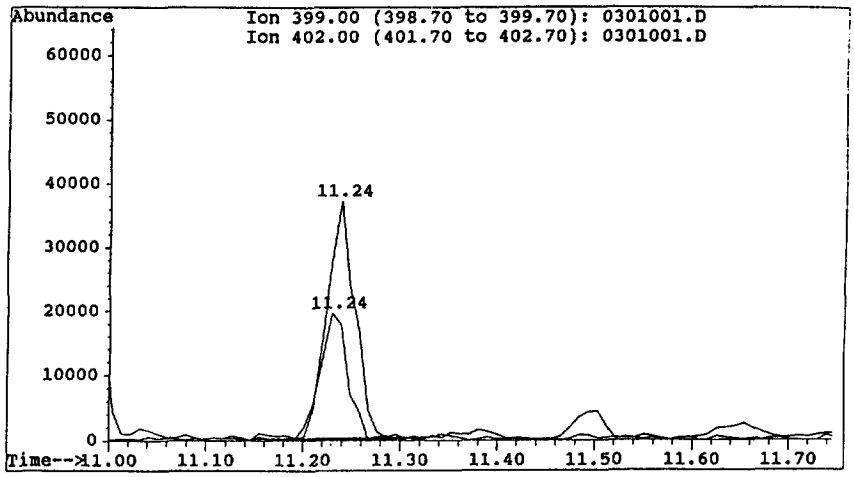

Fig. 4. SIM chromatogram of 6-MAM (m/z 399) and 6-MAM-d_3 (m/z 402) of an extract of hair obtained from a heroin addict. The concentration determined was 7.55 ng/mg. Time in min.

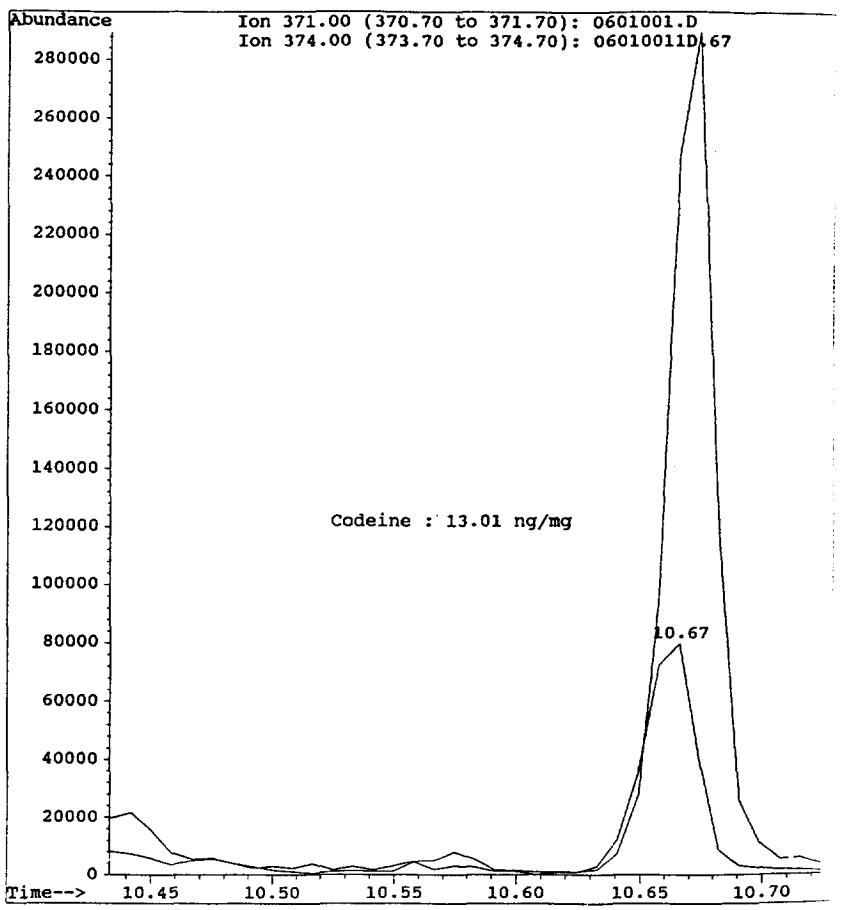

Fig. 5. SIM chromatogram of an extract of hair positive for COD (m/z 371). The concentration determined was 13.01 ng/mg. Time in min.

by the Hewlett Packard 7680 T SFE module. In Table 2 are listed the concentrations of COD, MOR and 6-MAM detected ($n = 2$). Concentrations ranged from 1.22 to 21.73 (mean 7.60 ng/mg), 0.17 to 1.54 (mean 0.69 ng/mg) and 0.15 to 14.09 ng/mg hair (mean 3.78 ng/mg) for COD, MOR and 6-MAM, respectively. These concentrations are in the range of those noted in the literature. No comparative study with other extractive procedures has been done. Fig. 4 illustrates the SIM chromatogram of 6-MAM ($t_R = 11.24$ min) in hair of a heroin addict. The concentration determined was 7.55 ng/mg hair and revealed chronic heroin exposure. In another sample, a concentration of 13.01 ng/mg hair of COD was detected at the $t_R = 10.67$ min of the SIM chromatogram (Fig. 5).

3.7. Detection of cocaine and cannabinoids by SFE and GC–MS

Two hair samples testing positive for cocaine (COC) [2] and cannabinoids [12], respectively, were extracted by the 7680 T SFE module using the same SFE procedure described previously for opiates.

A COC concentration of 13.10 ng/mg hair was detected in the positive cocaine sample. The SIM chromatogram is illustrated in Fig. 6 and revealed the presence of COC at $t_R = 9.60$ min.

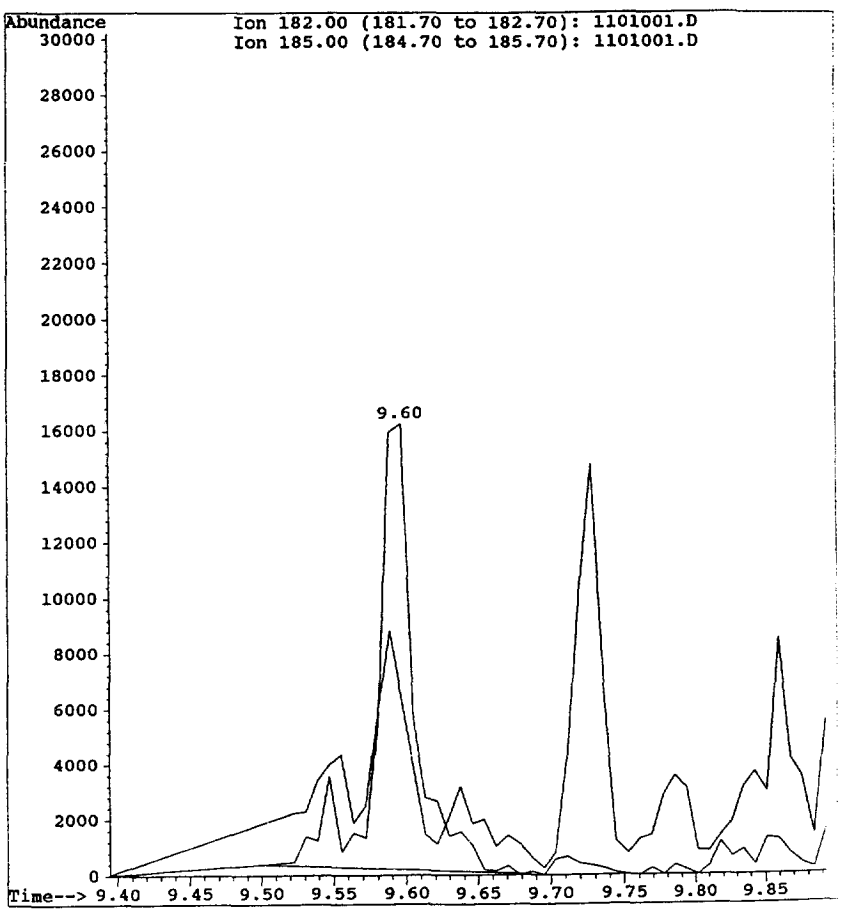

Fig. 6. SIM chromatogram illustrating the presence of COC in hair of a drug addict at the retention time 9.60 min. The concentration determined was 13.10 ng/mg of hair. Time in min.

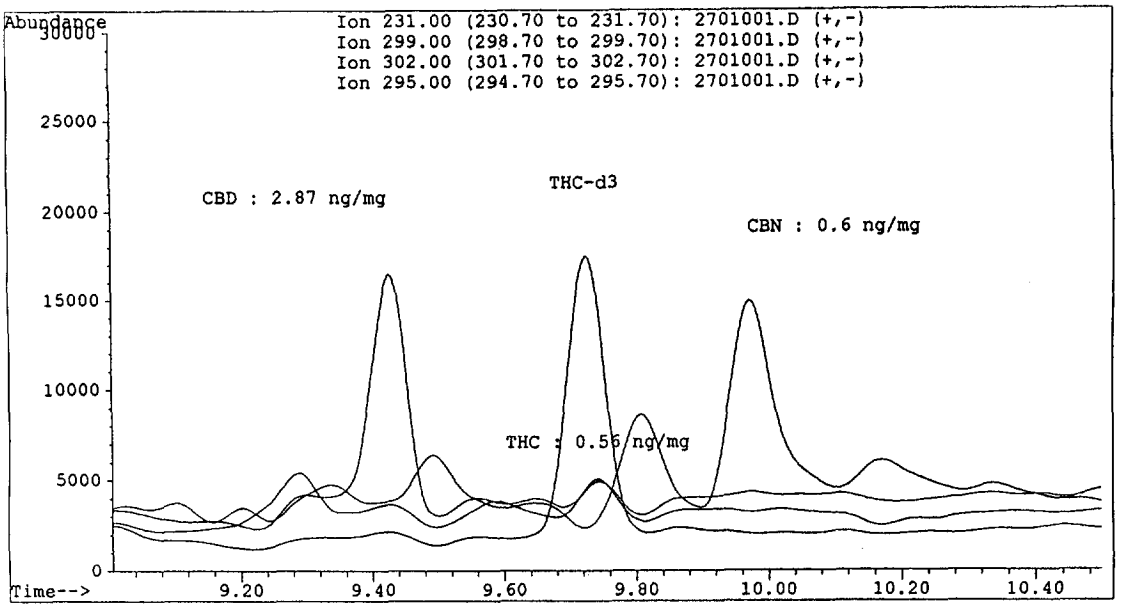

Fig. 7. SIM chromatogram of hair positive for cannabinoids extracted by SFE. CBD (2.87 ng/mg), THC (0.56 ng/mg) and CBN (0.60 ng/mg) were simultaneously detected and quantitated with the help of THC-d_3 (m/z 302). Time in min.

For hair samples positive for cannabinoids, SFE and GC–MS detection confirmed the presence of Δ^9-tetrahydrocannabinol (THC, m/z 299), cannabidiol (CBD, m/z 231) and cannabinol (CBN, m/z 295). The concentrations were 2.87, 0.56 and 0.60 ng/mg hair for CBD, THC and CBN, respectively (Fig. 7). This is the first report indicating that SFE is able to extract cannabinoids from hair.

4. Conclusions

This study confirmed that SFE represents a new technique for the extraction of drugs in human hair. We have demonstrated that opiates can be detected after extraction by the Hewlett Packard 7680 T SFE module using a rapid and easy-to-perform procedure, in contrast to other extraction methods [2–5]. One major disadvantage of our system was the non-selectivity of the trap elution. The elution of a more selective trap, like ODS (octadecylsilane), by solvents of different polarity would resolve this problem.

Many other advantages can be noted: SFE avoids the use of environmentally damaging solvents (only 1 ml of modifier solution and 1.8 ml of chloroform were used); COC and cannabinoids seem to be extracted during the same procedure described for opiates. So, SFE could be proposed as a new screening method; SFE can be automated (evaporation of the extract, derivatization) and coupled on-line with chromatographic systems such as GC–MS; SFE, in a daily routine, is clearly faster than other traditional methods of sample preparation.

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