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Subcritical fluid extraction of opiates in hair of drug addicts

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Abstract

Hair analysis is a useful complement to blood and urine analysis in forensic science, but current procedures are tedious and time consuming. Owing to the difficulty of having a sufficient amount of hair in order to perform the optimization of the extraction method, standard hair was made and the procedure is described. The spiking method is reproducible and linearity can be obtained. Subcritical fluid extraction of opiates from standard and drug addicts' hair was optimized (extraction phase composition, extraction time, etc.) and compared with other extraction techniques (basic and acid hydrolysis, organic solvent and enzymatic digestion) followed by solid-phase extraction. Recovery, coefficient of variation, linearity, detection limits and quantification limits for the subcritical fluid extraction of opiates in hair are described.

1. Introduction

Hair has become a very useful diagnostic tool to complement blood and urine analysis for drugs of abuse in forensic science [1–4]. It is easily collected without trauma on the part of the donor, falsification of the sample is more difficult, it can be stored without deterioration and is not infectious like blood. Hair analysis is also very useful when no body fluids are available in post-mortem cases. In addition, hair analysis provides information about the degree of consumption of drugs of abuse over a large period of time and makes is possible to differentiate chronic from sporadic intake. Drugs are accumulated in hair from plasma lymph, sweat and sebum. They are incorporated in the matrix at the same time as the other components of the hair and are fixed in the hair shaft [5,6]. Since hair grows at a relatively constant rate (about 1 cm/month), depending on anatomical region, sex, age and race, drug residues in hair may represent a historical record of exposure over the period of growth of the hair sampled by cutting and analysing several segments from the root to the tip of hair.

Numerous extraction methods for opiates in hair have been described, e.g., liquid extraction with an organic solvent [7], basic or acid hydrolysis [8–10], and enzymatic digestion [11]. These extraction techniques provide good results, but are long and tedious. Further, 6-mono-

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acetylmorphine (6-MAM), which is the principal heroin marker and makes it possible to differentiate between heroin and codeine consumption, it is easily hydrolysed in strongly acidic or basic conditions, which means that important information is lost during the process. Enzymatic digestion gives excellent results without 6-MAM loss, but using thiols in a laboratory is often unpleasant.

These inconveniences can be avoided by using supercritical (or subcritical) fluid extraction (SFE). First, SFE is easily performed, it is fast (generally requiring 30–60 min) and it is milder than hydrolysis. Second, variations in the extraction conditions can be simply introduced by way of changes in pressure, temperature or nature of the extractant phase (CO_2 + polar modifiers). Fractionation can be used in order to avoid some washing steps. Third, SFE can be coupled on-line with all the chromatographic techniques [12], which can lessen the risk of contamination and allow better detection. The application of SFE to morphinic alkaloids in forensic sciences has been described previously [13,14].

This paper describes the subcritical fluid extraction of opiates in the hair of drug addicts. Standard hair was produced and its SFE behaviour was compared with the hair of eight drug addicts. Recoveries, calibration graphs, repeatabilities, detection limits and cut-off limits are discussed. SFE was compared with other extraction techniques. Finally, some applications of hair analysis are described.

2. Experimental

2.1. Materials

Drug abusers' hair were obtained from the Institute of Forensic Medicine of Geneva. Morphine and codeine used as standards were obtained from Siegfried (Zofingen, Switzerland). Ethylmorphine, 6-MAM standards and nalorphine were kindly provided by the Institute of Forensic Medicine of Geneva. ^{125}I-labelled morphine was obtained from the RIA kit Coat-A-Count for morphine in urine purchased from DPC (Los Angeles, CA, USA).

For supercritical fluid extraction, 99.99% purity CO_2 (Polygaz, Geneva, Switzerland) contained in a cylinder with an eductor tube was used. A charcoal-packed column was incorporated before the pump to prevent possible sample contamination by hydrocarbons. Bond Elut Certify cartridges are purchased from Varian (Harbor City, CA, USA). HPLC-grade quality methanol (99.9% purity) was purchased from Maechler (Basle, Switzerland) and triethylamine (99.5% purity) from Fluka (Buchs, Switzerland). Propionic anhydride (>99% grade) from Aldrich (Gillingham, UK) and pyridine (99.5% grade) from Merck (Darmstadt, Germany) were used as derivatization reagents prior to GC analyses.

Reagents used for enzymatic digestion were guanidine hydrochloride (99.5% grade) and 2-mercaptoethanol (99% grade) from Merck. Glusulase, containing 90% glucuronidase and 10% sulphatase, was purchased from DuPont (Wilmington, DE, USA).

2.2. Hair preparation

A previous washing procedure is very important, for two reasons: first, to eliminate the drugs that were not strongly incorporated into the hair matrix, such as those which are only adsorbed on the hair surface; such external contamination must be avoided in order to give a correct interpretation; and second, to clean the sample, mainly by eliminating the fat. The hair was briefly washed by percolation with 10 ml of water, then 10 ml of 0.1 *M* HCl and finally with 20 ml of methanol.

After this decontamination, blank and drug abusers' hair were pulverized for 10 min with a ball-mill purchased from Retsch (Schieritz, Hauenstein, Switzerland). A microphotograph of the pulverized hair was taken by electron microscopy with a JSM 6400 scanning electron microscope. Using Polaroid 665 film (Fig. 1). The particle size is small, about 30 μm for the largest ones, and homogeneous. Comparison with other pulverized hair samples showed similar results.

Standard hair was prepared by adding an

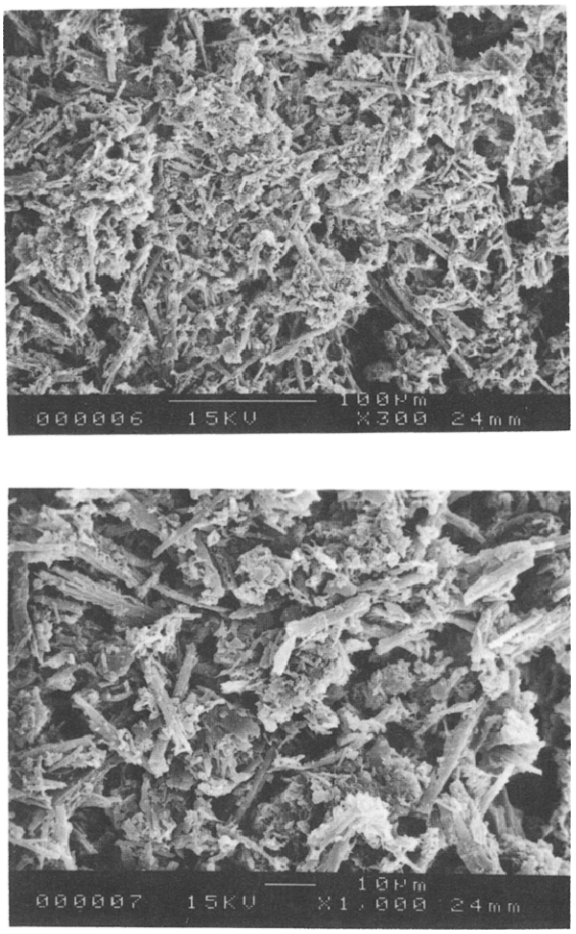

Fig. 1. Scanning electronic micrographs of pulverized hair.

aqueous standard opiate solution to the pulverized hair. The mixture was submitted to magnetic stirring for 5 h, then filtered and the hair was washed with 50 ml of water and 30 ml of methanol in order to retain only the most strongly bound opiate fraction.

The same procedure was performed with ^{125}I-labelled morphine for recovery studies; a COBAS γ-counter (Roche Diagnostica) was used for these experiments.

2.3. Supercritical fluid extraction technique

For supercritical fluid extraction, an extraction cell (volume 430 μl, dimensions 4.5 cm × 3.5 mm I.D.) was filled with pulverized hair (generally 50 mg) and placed in a supercritical extractor. The SFE apparatus was laboratory made and is shown in Fig. 2. Carlo Erba SFC300 syringe pumps (purchased from Brechbühler, Geneva, Switzerland) was used to deliver CO_2 and the modifier. A six-way Rheodyne switching valve was used to by-pass the extraction cell, which was placed in an oven held at 40°C. A block heater at 100°C was used to heat the restrictor in order to avoid dry-ice formation due to the decompression of the CO_2. In comparison with commercial systems, a major modification was made to the restrictor. With this restrictor, which has been described previously [13], the extrac-

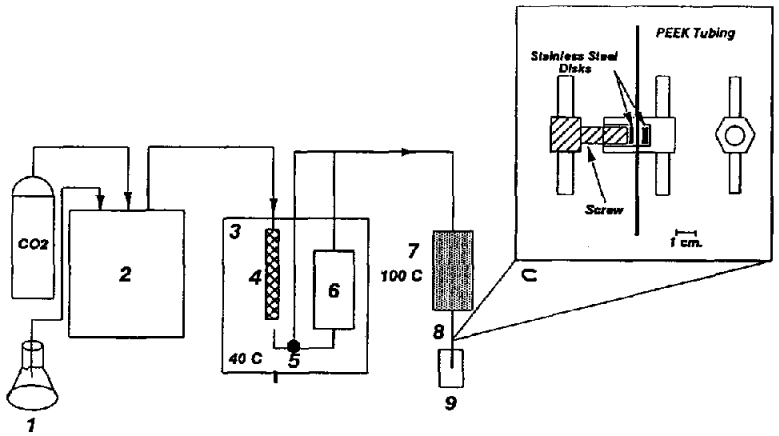

Fig. 2. Schematic diagram of the SFE system and details of the PEEK restrictor. 1 = Polar modifier; 2 = syringe pumps; 3 = oven; 4 = mixing column; 5 = switching valve; 6 = extraction cell; 7 = block heater; 8 = PEEK restrictor; 9 = collection vessel.

tion flow-rate can be accurately regulated at constant pressure during the extraction period without having to remove the collection vessel. A schematic diagram of the restriction system is shown in Fig. 2. PEEK tubing of 0.016 in. I.D. (Upchurch Scientific, Oak Harbor, WA, USA) was used as a restrictor. The end of the PEEK restrictor was immersed in methanol, where extracted drugs of abuse are collected.

2.4. Other extraction techniques

Basic hydrolysis was performed in a closed vial for 1 h at 100°C by adding 2 ml of 1 *M* NaOH to 50 mg of hair. The mixture was neutralized with 1 ml of 2 *M* HCl and buffered with 1 ml of 1/15 *M* phosphate buffer (pH 6.0). This solution was then extracted by solid-phase extraction (SPE) on Bond Elut Certify cartridges [15], activated beforehand with 3 ml of methanol and water. For the washing step 2 ml of water, 1 ml of 0.1 *M* acetate buffer (pH 4.0) and 2 ml of methanol were used. Elution of opiates was performed using 3 ml of dichloromethane–isopropanol (80:20, v/v) containing 2% of ammonia.

Acid hydrolysis was performed in a closed vial at 120°C for 30 min by adding 2 ml of 2 *M* HCl to 50 mg of hair. The mixture was neutralised with 10 *M* NaOH and buffered with 1 ml of 1/15 *M* phosphate buffer (pH 6.0) prior to extraction on Bond Elut Certify cartridges with the same procedure as described above.

Methanolic extraction was performed in a closed vial at 37°C for 18 h by adding 2 ml of methanol to 50 mg of washed and pulverized hair. The supernatant was collected and hair was rinsed with 2 ml of methanol. After evaporation of the solvent, the residue was dissolved in 2 ml of 1/15 *M* phosphate buffer (pH 6.0). Finally, the solution was extracted on Bond Elut Certify cartridges using the same procedure as above.

Enzymatic digestion was performed in a closed vial by adding 50 mg of guanidine hydrochloride, 50 μl of 2-mercaptoethanol, 25 μl of glucuronidase and 1 ml of 1/15 *M* phosphate buffer (pH 7.0) to 50 mg of hair. The mixture was incubated for 4 h at 45°C. The supernatant was collected and the hair was rinsed with 2 ml of 0.1 *M* phosphate buffer (pH 7.0). The solution was extracted on Bond Elut Certify cartridges as described above.

2.5. GC–MS procedure

The analysis was performed by GC–MS, as it is more powerful for identification and quantification [16]. The different metabolites that are necessary for forensic interpretation cannot be analysed simultaneously by radioimmunoassay (RIA).

Analyses were performed with an HP 5890 gas chromatograph coupled with an HP 5988 mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA) for detection. GC analysis was carried out after a derivatization step involving propionylation of the hydroxyl groups of morphinic alkaloids. Propionylation was preferred to acetylation in order to discriminate morphine from 6-MAM. A 100-μl volume of propionic anhydride and 100 μl pyridine were added to the residue obtained after evaporation of the solvent and heated at 60°C for 30 min. After evaporation of the derivatization reagents under nitrogen, the residue was dissolved in 100 μl of ethyl acetate. A 50-μl volume of nalorphine (2 ng/ml) was used as an internal standard.

Injection was performed in the splitless mode (270°C) and the injection volume was 3 μl. Helium was used as the carrier gas at a flow-rate of 1.1 ml/min. A 15 m × 0.25 mm I.D. capillary column of DB5, film thickness 0.25 μm (J&W Scientific, Folsom, CA, USA), was used. The following GC temperature programme was used: initial temperature 170°C, for 1 min, increased at 20°C/min to 256°C, held for 1 min, and then at 2°C/min to 270°C, with a final hold for 12.5 min. The detector temperature was 250°C. On the basis of the mass spectra of propionyl derivatives of opiates (Fig. 3), mass spectrometric detection was carried out by selected-ion monitoring (SIM) with the following ions: morphine, m/z = 397 and 341; codeine, m/z = 355 and 282; 6-MAM, m/z = 383 and 327; and ethylmorphine, m/z = 369 and 296.

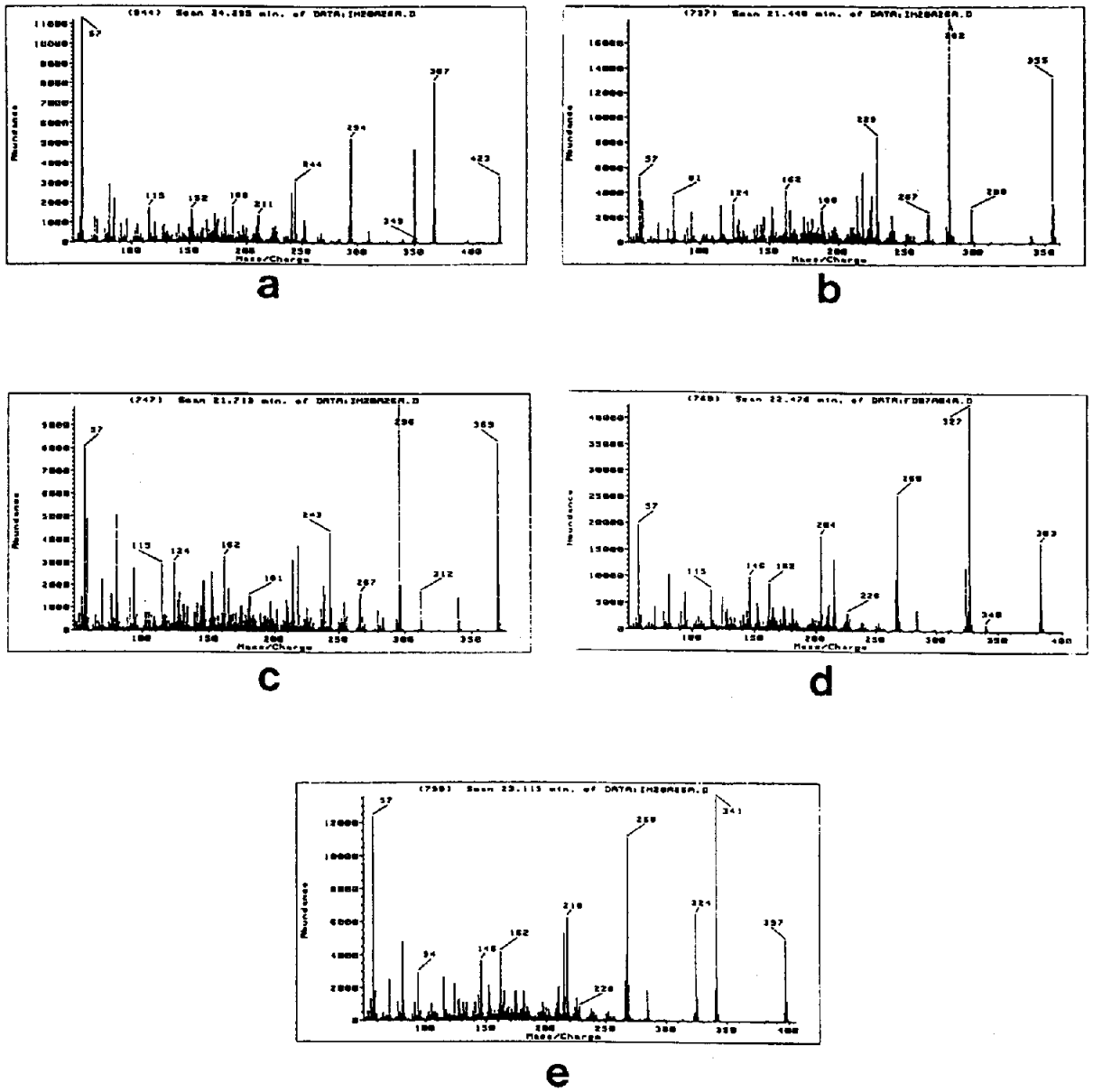

Fig. 3. Mass spectra of propionyl derivatives of opiates. (a) Nalorphine (internal standard); (b) codeine; (c) ethylmorphine; (d) 6-MAM; (e) morphine.

3. Results and discussion

The hair of eight drug addicts (obtained post-mortem in overdose cases) was washed, pulverized, extracted by SFE and analysed using GC–MS. The extraction behaviour was studied by varying the polarity of the extractant phase, which was composed of CO_2 and a polar modifier. The polar modifier was methanol–triethylamine (Et_3N)–H_2O (2:2:1, v/v/v), already used successfully for the SFE of opiates in urine after adsorption on a solid matrix [14]. The same hair

specimen was extracted successively with increasing concentrations (0, 2, 5, 8, 12, 15 and 20%) of polar modifier in CO_2. The SFE conditions were pressure 25 MPa, temperature 40°C, flow-rate 0.7 ml/min and extraction time 30 min. GC–MS analysis was performed on each fraction. The results obtained for morphine are given in Fig. 4 and show that, below 5% of polar modifier, only small amounts of morphine are extracted (less than 10%). It is therefore possible to add a second washing step by performing a 15-min extraction using CO_2–modifier (98:2, v/v), which can be used if necessary. Moreover, in seven cases, 15% of polar modifier was sufficient to extract morphine and increasing the polarity did not yield more morphine. Therefore, CO_2–modifier (85:15, v/v) was chosen in order for the extraction of opiates from hair.

With this mixture and at a temperature of 40°C, the fluid is not in the supercritical but in the subcritical state [17]. Hence the term subcritical instead of supercritical is used in the rest of this paper.

Fig. 5 shows chromatograms obtained from three real cases of samples from drug addicts. All opiates are well resolved and can be easily quantified. Further, only a few ghost peaks are present. The opiate content in hair (Table 1)

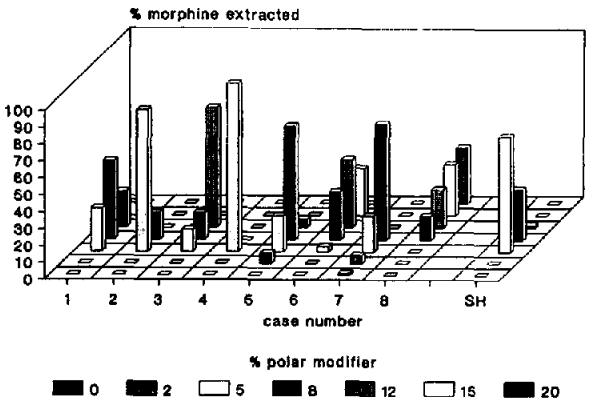

Fig. 4. Extracted morphine in hair of eight heroin abusers as a function of the polarity of the super- or subcritical fluid extractant phase. Variation of the percentage of the polar modifier [MeOH–Et_3N–H_2O (2:2:1, v/v/v)] in CO_2. Pressure = 25 MPa; temperature, 40°C; flow-rate, 0.7 ml/min; time, 30 min. SH = standard hair.

makes it clear that hair is a very interesting matrix and reveals toxicomania. In fact, it has been shown that hair analyses give fewer false negatives than urine or blood analyses [1–4], as 6-MAM is always obtained in the hair of drug abusers, in contrast to urine and blood analyses. In most biological matrices heroin decomposes into 6-MAM and then morphine. The detection of 6-MAM establishes heroin exposure, but this metabolite has an extremely short half-life and is detectable in urine only for ca. 8 h after the intake of a single dose [18,19]. Hence the detection of 6-MAM in hair might serve as an acceptable alternative to determining heroin exposure. The fact that the 6-MAM/morphine ratio is <1 in six of the eight cases must not be taken as a rule and is rather particular to these chosen cases.

3.1. Hair standards

It is difficult to obtain drug abusers' hair in amounts large enough to optimize the method, which often requires many experiments. Hence in many cases, comparison of data obtained with several hair samples is very ambiguous. Therefore, the preparation of a reference material that reproduces as closely as possible real drug addicts' hair could be very useful. A 50-mg amount of hair (200 mg spiked with 20 ml of 5 μg/ml aqueous morphine for 5 h) was extracted using the method described above. Fig. 4 shows that spiked morphine is relatively strongly bound to the hair matrix and reacts similarly to genuine drug addicts' hair, hence the standard hair can be used to optimize the method.

This spiking procedure is reproducible, the spiking yields for codeine, ethylmorphine, 6-MAM and morphine being 13.5, 14.5, 12 and 14.6% of the total drugs added, respectively, and the standard deviations for five replicate standard hair preparations were 1.4, 2.3, 1.7 and 1.4%.

In addition, the opiate content of hair is directly proportional to the opiate concentration of the spiking aqueous solution, making internal calibration possible and turning this method into a very attractive approach. Hair was extracted

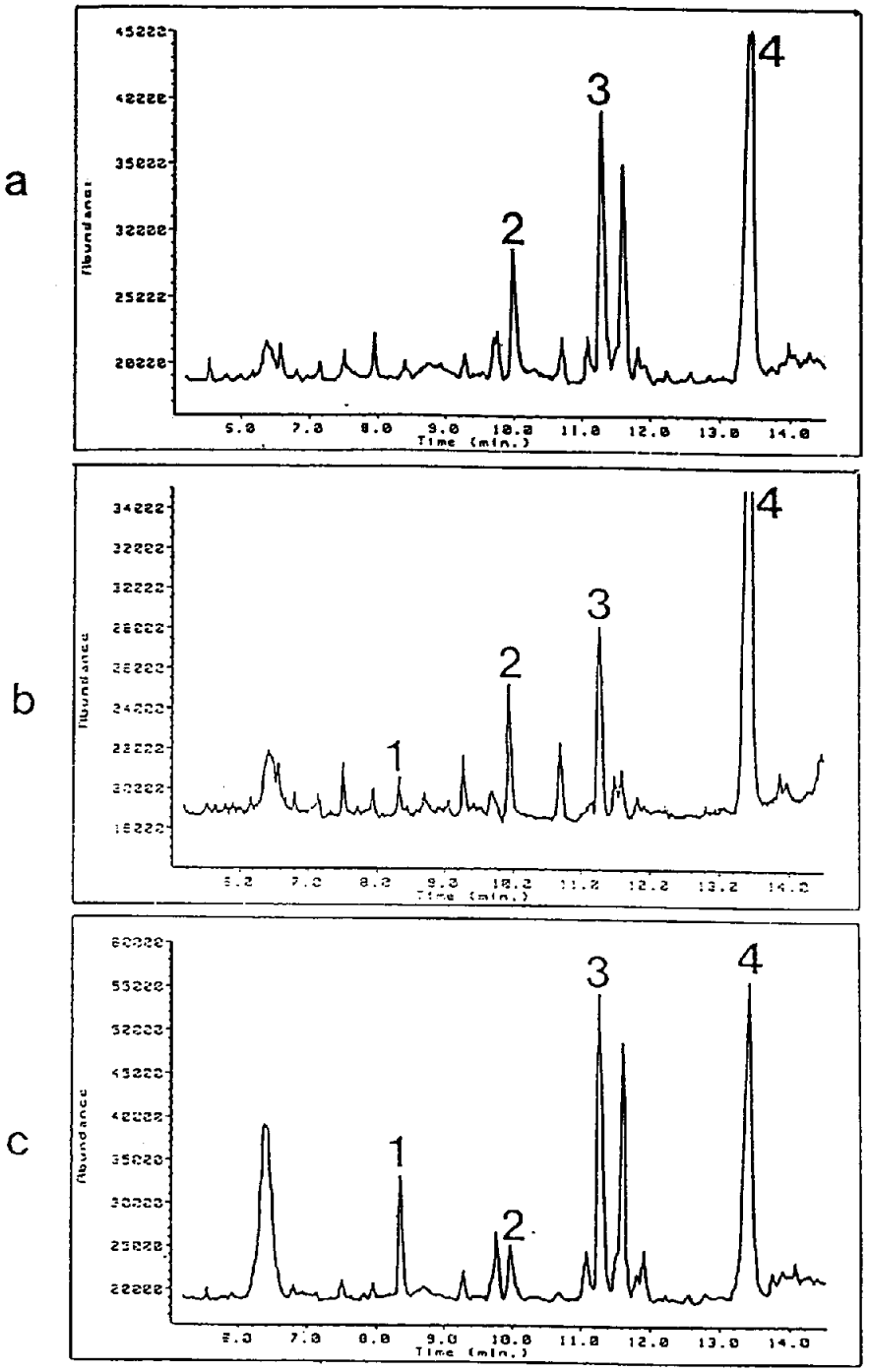

Fig. 5. Total ion current chromatograms of opiates in hair of drug addicts obtained by SFE. The following ions (propionyl derivatives) were detected in the same run: nalorphine (chromatographic standard), $m/z = 423$ and 367; morphine, $m/z = 397$ and 341; codeine, $m/z = 355$ and 282; 6-MAM, $m/z = 383$ and 327; ethylmorphine, $m/z = 369$ and 296. (a) Case 7, CO_2–MeOH–Et_3N–H_2O (92:3.2:3.2:1.6, v/v); (b) case 2, CO_2–MeOH–Et_3N–H_2O (92:3.2:3.2:1.6, v/v); (c) case 4, CO_2–MeOH–Et_3N–H_2O (95:2:2:1, v/v). Peaks: 1 = codeine; 2 = 6-MAM; 3 = morphine, 4 = nalorphine (internal standard).

Table 1
Opiates concentrations (ng/mg) in hair from eight drug addicts: hair analysis by SFE and GC–MS ($n = 2$)

Subject	Concentration (ng/mg)					
	Codeine		Morphine		6-MAM	
1	0	0	0.88	0.71	0.45	0.58
2	0.15	0.25	1.13	1.25	1.38	1.58
3	1.3	0.95	1.86	1.54	0.70	0.47
4	1.0	1.2	0.55	0.68	0.53	0.45
5	0	0	1.86	1.94	0.23	0.40
6	0.42	0.56	2.71	3.44	3.33	2.7
7	0	0	2.52	2.34	0.78	0.70
8	1.72	2.07	3.31	3.88	0.48	0.34

using the enzymatic digestion method and was analysed by GC–MS. The hair concentration range obtained was between 2 and 10 ng/mg. When the opiate hair concentration observed was plotted as a function of the drug content of the spiking solution, linear graphs were obtained for codeine, ethylmorphine, 6-MAM and morphine. The correlation coefficients were 0.995, 0.996, 0.992 and 0.995, respectively. Spiked hair was used for 1 month after the preparation procedure without any decrease in opiate concentration.

3.2. *Influence of the triethylamine and water content of the extractant phase*

Starting with CO_2–methanol–Et_3N–water (85:6:6:3, v/v), the triethylamine and water contents were changed and their influence on SFE was studied. Standard hair (9 g), spiked with 100 ml of an aqueous solution containing 2 ng/mg of morphine, codeine, ethylmorphine and 6-MAM were prepared as described above. A 50-mg amount of the prepared hair was extracted by SFE after the two washing steps. The triethylamine content was changed in the range 1–6% with constant 6% MeOH and 3% H_2O and the water content was varied in the range of 0–3% with a constant 6% MeOH and 6% Et_3N. Fig. 6 shows that only about 25% of each drug was extracted with 1% Et_3N and only about 30%

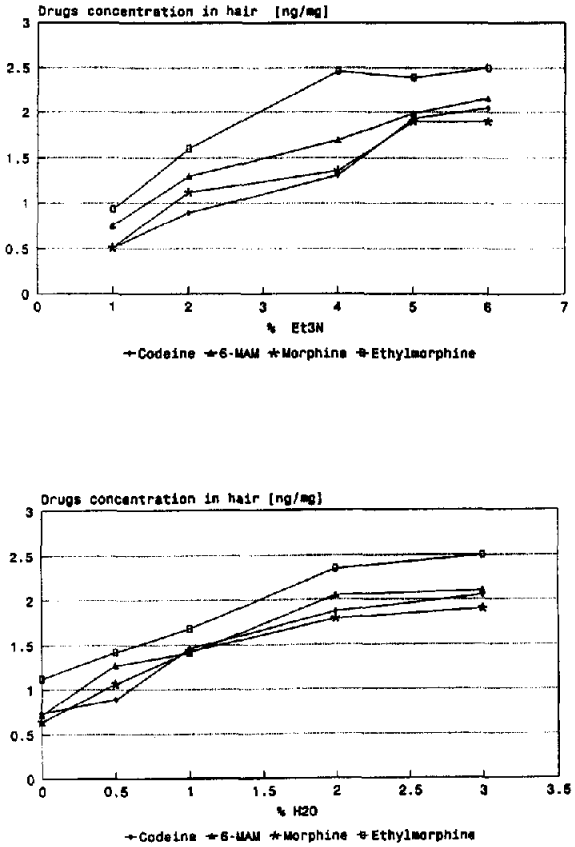

Fig. 6. Opiates concentrations (ng/mg) in spiked hair as a function of the triethylamine and water contents (%, v/v) in the subcritical fluid phase. ◆ = Codeine; ▲ = 6-MAM; * = morphine; □ = ethylmorphine.

without water. The maximum extracted concentrations were obtained with 6% Et_3N and 3% water. Hence CO_2–MeOH–Et_3N–H_2O (85:6:6:3, v/v) was the best solvent, but no evidence of total recovery could be obtained on the basis of these results.

3.3. Quantitative SFE of opiates in hair

A 20-ml volume of ^{125}I-labelled morphine (0.5 ml = 12 500 cpm) was used to spike blank hair (470 mg). Morphine was chosen as a model substance owing to its polarity. As morphine is quantitatively extracted, other opiates (less polar) are also extracted, as shown by the excellent correlation coefficients obtained in the range 0.1–2 ng/mg (see below). After the washing step, 50 mg of hair were counted and then extracted by SFE with CO_2–MeOH–Et_3N–H_2O (85:6:6:3, v/v), all the other conditions being the same as before. The hair and the collection solution were counted. The results were excellent and six replicate SFE steps gave a mean recovery of 93.5% with a coefficient of variation of 2.7%. With this extraction phase and a flow-rate of 0.7 ml/min at 25 MPa, optimization of the SFE extraction time was examined by collecting eluates after 2, 5, 10, 15, 20, 25, 30 and 40 min and analysing for morphine. The plot of morphine concentration vs. extraction time showed that no more morphine was collected after 20 min; therefore, to ensure that the total hair content was extracted, a 30-min extraction time was chosen.

SFE of opiates in hair is thus quantitative and the same extraction conditions were adopted for the remainder of this work. This approach is a very simple and rapid way of perfecting an extraction method. Moreover, for real cases, MS detection may be necessary in order to determine and identify (from a legal point of view) the different opiates, which allow the result to be interpreted.

Calibration was carried out by SFE and GC–MS analysis of hair (50 mg) containing different amounts of spiked hair (50, 35, 20 and 10 mg). The plots were linear for the four opiates tested in the range of 0.5–2 ng/mg, which is the range generally observed with hair of drug addicts. The regression coefficients for codeine, ethylmorphine, 6-MAM and morphine were 0.995, 0.993, 0.997 and 0.996, respectively. The coefficients of variation ($n = 5$) were satisfactory (3% for morphine, 5.6% for ethylmorphine and about 10% for codeine and 6-MAM). The high coefficient of variation for codeine can be explained by the fact that the chosen ions used for detection were less selective than those for the other opiates.

Six 50-mg blank hair samples were extracted by SFE and the noise was integrated for the ion used for quantification ($m/z = 355$ for codeine, 369 for ethylmorphine, 383 for 6-MAM and 397 for morphine), in a retention time window of $t_r \pm 0.5$ min. The limits of detection (LOD) and quantification (LOQ) were determined ($n = 6$) using IUPAC methods [20]. For each substance the standard deviation of the blank value (S_B) was determined. The mean area converted from the noise was calculated as concentration equivalent based on a calibration graph. The LOD is defined as $3S_B$ and the LOQ as $10S_B$. The LOD and LOQ obtained for the four opiates tested are given in Table 2. With the analytical conditions described above, the LOD is 30 pg/mg for codeine, ethylmorphine and morphine and 50 pg/mg for 6-MAM. The LOQ in hair is 0.1 ng/mg for codeine, ethylmorphine and morphine and 0.2 ng/mg for 6-MAM. Further, blank hair spiked with different opiate concentrations showed good linearity in the range 1–0.1 ng/mg with correlation coefficients above 0.990. Fig. 7 shows SIM chromatograms of blank hair spiked with 0.1 ng/mg of opiates for the selected ions.

Table 2
Limit of detection (LOD) (pg/mg) and limit of quantification (LOQ) (ng/mg) of opiates in hair

Opiate	LOD (pg/mg)	LOQ (ng/mg)
Codeine	30	0.1
Ethylmorphine	30	0.1
6-MAM	50	0.2
Morphine	30	0.1

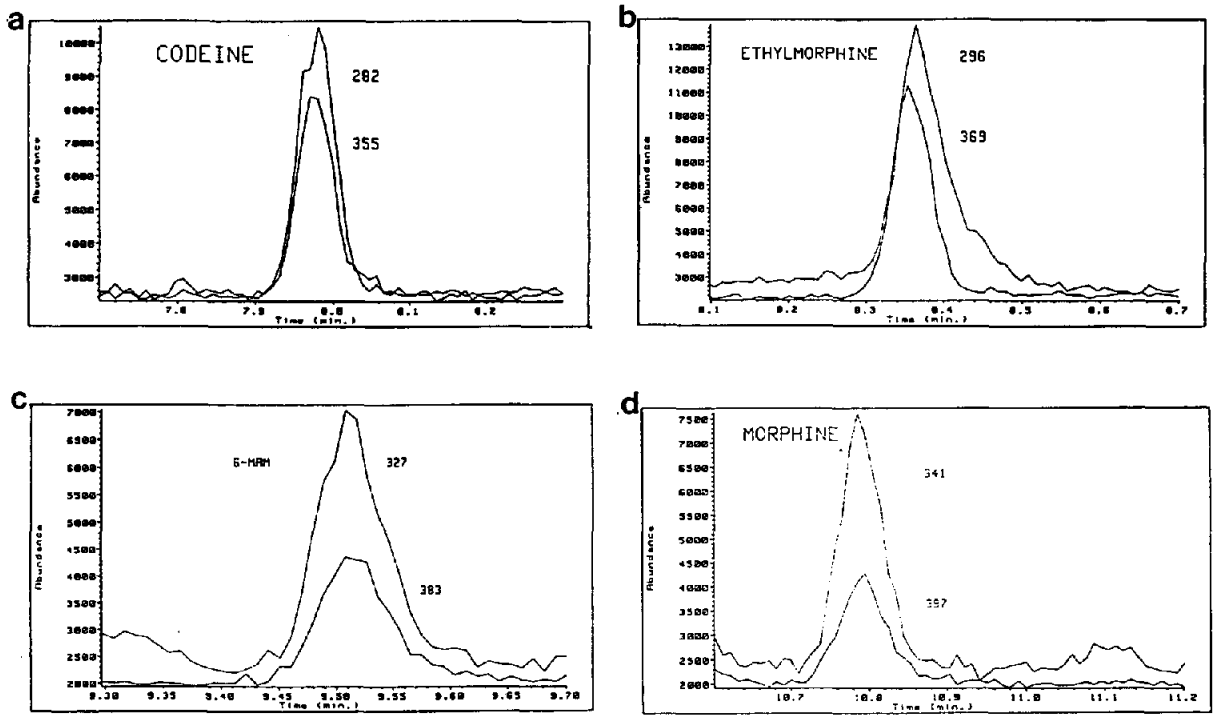

Fig. 7. Selected-ion chromatograms of blank hair extracted by SFE and spiked with 0.1 ng/mg of opiates. The following selected ions (propionyl derivatives) were detected and displayed in windows: (a) $m/z = 355$ and 282 for codeine; (b) $m/z = 369$ and 296 for ethylmorphine; (c) $m/z = 383$ and 327 for 6-MAM; (d) $m/z = 397$ and 341 for morphine.

3.4. Comparison with other extraction techniques

The same hair spiked with codeine, morphine, 6-MAM and ethylmorphine was extracted using different methods and analysed by GC–MS. The opiate concentrations in hair obtained with methanolic extraction, basic and acidic hydrolysis and enzymatic digestion compared with the SFE method are given in Table 3. Methanolic extraction followed by SPE using Bond Elut Certify cartridges is surprisingly good, but the

Table 3
Comparison of SFE with other extraction techniques for opiates in hair: opiate concentrations (ng/mg) in hair and coefficients of variation (%) obtained with methanol extraction, basic and acidic hydrolyses, enzymatic digestion and SFE

Opiate	Methanol extraction ($n = 5$)		Enzymatic digestion ($n = 5$)		Acid hydrolysis ($n = 3$)		Basic hydrolysis ($n = 3$)		Subcritical fluid extraction ($n = 5$)	
	Concentration (ng/mg)	C.V. (%)	Concentration (ng/mg)	C.V. (%)	Concentration (ng/mg)	C.V. (%)	Concentration (ng/mg)	C.V. (%)	Concentration (ng/mg)	C.V. (%)
Codeine	1.98	18	1.92	17	1.22	15	1.47	10	1.99	12
Ethylmorphine	2.41	9.8	2.19	3.3	1.95	10	2.15	13.8	2.56	5.6
6-MAM	2.18	7.9	2.49	10	0	–	0	–	2.18	10
Morphine	1.21	7.5	1.73	13	2.39	14	2.46	13	1.93	3.0

recovery is poor for morphine, which is the most polar of the opiates tested, and is most strongly bound to the hair matrix. Statistical comparison using the Student *t*-test, showed that SFE and methanolic extraction are similar for codeine, ethylmorphine and 6-MAM (95% confidence interval). As expected, the methods are totally different concerning morphine. Further, this procedure is very time consuming, requiring ca. 18–24 h. Strongly basic or acidic hydrolysis is unsatisfactory because 6-MAM is lost during hydrolysis and partially transformed into morphine. As already indicated, 6-MAM is very useful for interpreting results and establishing heroin exposure. In addition, morphine determination in a sample containing 6-MAM gives a too high concentration of morphine.

Enzymatic digestion followed by SPE using Bond Elut Certify cartridges also provides good extraction as well. Statistical comparison using the Student *t*-test showed that SFE and enzymatic digestion are similar for codeine and ethylmorphine (95% confidence interval), but the methods are slightly different concerning morphine and 6-MAM. In fact, the higher morphine and lower 6-MAM concentrations observed with SFE compared with the enzymatic method can be explained by a small extent of hydrolysis of 6-MAM to morphine due to the water and triethylamine content of the subcritical fluid extracting phase. In addition, the chromatogram obtained is cleaner with enzymatic digestion–SPE than with SFE. However, this method also has some disadvantages, e.g., thiols are often unpleasant to use in a laboratory and must be handled with care, and hydrolysis coupled with SPE is more time consuming than SFE alone.

In order to demonstrate the applicability of the SFE procedure, a history of consumption by a drug addict was performed on the basis of hair analysis. As it provides on exposure to drugs over time, hair analysis may be useful in order to verify self-reported histories of methadone consumption (substitute for heroin) in treatment centres, to provide pre-employment and employee drug testing, or in any situation in which a history of drug abuse is desired. In addition, hair analysis can be useful when the history of drug use is difficult or impossible to obtain, e.g., from psychiatric patients or infants. The history of drug use is obtained by cutting hair in segments from the root to the tip and analysing each segment. Fig. 8 shows the history obtained for a drug addict and gives the time of heroin exposure as 1 year, with an increase in the level of consumption in recent months. The high concentration of 6-MAM clearly demonstrates heroin intake.

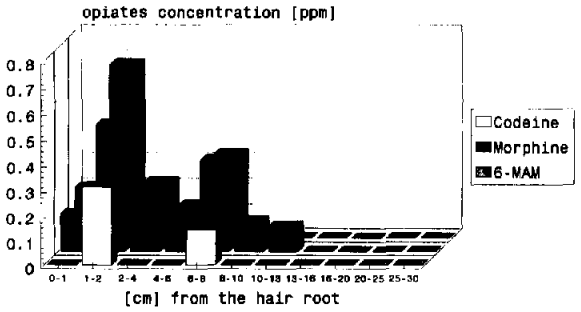

Fig. 8. History of heroin consumption by a drug addict based on hair analysis using SFE. Morphine, 6-MAM and codeine concentrations (ng/mg) in hair as a function of the hair length (cm) from the root to the tip.

4. Conclusions

Reference hair containing opiates showing almost the same extraction behaviour as real drug addicts' hair was prepared. In addition, the spiking method shows reasonable repeatability (about 10%) and calibration with this material is possible. Subcritical fluid extraction is a method of choice in order to extract opiates in the hair of drug addicts. SFE is easy to perform and very fast, 30 min being sufficient to extract opiates quantitatively from hair, whereas other techniques require several hours. The SFE method using CO_2–MeOH–Et_3N–H_2O (85:6:6:3, v/v) as an extractant phase is quantitative, does not destroy 6-MAM and provides acceptable sensitivity and repeatability.

Enzymatic digestion coupled with SPE gives excellent results, but is slightly more time consuming. Further, the possibility of coupling online SFE with chromatographic systems such as GC–MS makes SFE a very attractive method for

drug extraction from complex biological matrices such as hair, which, in the future, will become a very useful matrix in forensic toxicology.

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