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ORIGINAL ARTICLE

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Concentrations of Δ^9-tetrahydrocannabinol, cocaine and 6-monoacetylmorphine in hair of drug abusers

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Abstract Hair samples taken from 850 individuals with presumed drug abuse were tested simultaneously for Δ^9-tetrahydrocannabinol (THC), cocaine, heroin, the primary heroin metabolite 6-monoacetylmorphine (6-MAM) and morphine. The drugs were extracted with methanol under sonication. Compared to other extraction procedures this solvent extraction technique provides high extraction yields and less experimental effort. The analyses were carried out using gas chromatography – mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode. This procedure allows the simultaneous detection of amphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA) and methylenedioxyethylamphetamine (MDE). THC was found in 104 (12.2%), cocaine in 230 (27%) and 6-MAM in 141 (16.6%) samples. In addition to 6-MAM, morphine was detected in 87 (10.2%) and heroin in 38 samples (4.5%). The concentrations found were in a range 0.009–16.7 ng/mg for THC, 0.037–129.68 ng/mg for cocaine, 0.028–79.82 ng/mg for 6-MAM, 0.045–53.14 ng/mg for heroin and 0.011–7.800 ng/mg for morphine. The statistical distribution of the drug concentrations compared with the self-reported consumption behaviour of the users may possibly lead to a better understanding of the relationship between drug dosage and corresponding concentrations in hair.

Key words GC-MS analysis · Drugs in hair · Simultaneous detection · TCH · Cocaine · Heroin · 6-MAM · Morphine · Methanol sonication extraction · Consumption behaviour

Introduction

Most investigations dealing with correlations of drug dosage and concentrations in hair are based on studies with legally available drugs such as codeine or methadone [1, 2]. Because of legal and ethical limitations which prevent human experiments, the pharmacokinetics of illicit drugs, with special focus on the hairs, have not yet been systematically explored. One still has to rely on empirical findings by comparing drug concentrations in hair with the drug consumption behaviour. In recent years the analysis of hair for illicit drugs has attracted growing attention and a number of analytical procedures have been established. But different isolation techniques often lead to varying results both qualitative and quantitative. This is one reason which renders it difficult to correlate drug levels in hair to a particular consumption behaviour. There is a lack of reliable statistical data, but an increasing amount of data obtained from standardized hair analyses, which are correlated with information on drug consumption, may enable a better interpretation of drug concentrations in hair.

In the present paper the results of 850 hair analyses for Δ^9-tetrahydrocannabinol (THC), cocaine, heroin, 6-monoacetylmorphine (6-MAM) and morphine were evaluated. Since all data were obtained using the same analytical procedure, a comparison of the results is possible. The drugs were extracted with methanol under sonication. This analytical technique allows the simultaneous extraction of amphetamines (including the methylenedioxy-derivatives such as MDMA), cocaine, methadone, opiates (heroin, 6-MAM, morphine, codein and dihydrocodein) and THC. After derivatisation with propionic acid anhydride all compounds can be measured in one GC-MS run.

Materials and methods

Sample material

Hair samples from 850 individuals with presumed drug abuse were analysed. Most of these persons had been required to provide an expertise such as hair analysis for reissueing the confiscated driving licence. Some of the individuals were involved in crimes. The history of drug abuse was explored during the medical examination, however, the reliability of the self-reported drug consumption behaviour is questionable.

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A strand of hair about 5 mm in diameter was cut from the back of the head as close as possible to the skin. The hair was fixed with a string and enveloped in aluminium foil. The proximal and distal end of the hair were marked. The samples were stored under dry conditions at room temperature until analysis.

Extraction procedure

The total length of the hair samples was measured and special features such as colouring, bleaching etc. were noted. A segment of the hair (usually 6 cm if possible) was cut for analysis and washed for 5 min with water (5 mL), acetone (5 mL) and finally petroleumether (5 mL) in a polypropylene vial. After drying, the hair was cut into small pieces of about 1 mm and 50–200 mg were used for analysis. The hair was transferred to a polypropylene vial and 4 ml methanol and 200 ng methaqualone as internal standard (20 µL of a solution containing 10 ng/µL) were added. The closed vial was sonicated (120 W) for 5 at 50° C. The methanolic extract was then transferred to a silanized glass vial and the solvent was evaporated. For derivatisation 0.05 mL of propionic acid anhydride (PSA) was added and the mixture incubated at 100° C for 1 h. The excess propionic acid anhydride was evaporated. For GC-MS analysis the residue was dissolved in 0.05 mL ethyl acetate containing 5% PSA.

GC-MS analysis

For GC-MS analysis the following conditions were applied: a DB 1 capillary column (methyl silicone, 20 m × 0.25 mm i.d., 0.25 µm film thickness) was used. The carrier gas was helium with a flow rate of 1 mL/min. The injection temperature was 280° C and the transfer line temperature was 300° C. The temperature programme was started at 140° C, increased to 300° C at 20°/min and remained isothermal at 300° C for 8 min. The injection volume was 1 µL (splitless injection). EI (70 eV) was used for ionisation. The following ions were measured in the selected ion monitoring (SIM) mode:

THC (propionyl)	m/z = 297, 313, 370	(ret. time: 7.8 min)
Cocaine	m/z = 82, 182, 303	(ret. time: 8.3 min)
Heroin	m/z = 310, 327, 369	(ret. time: 8.2 min)
6-MAM (propionyl)	m/z = 268, 327, 383	(ret. time: 8.6 min)
Morphine (dipropionyl)	m/z = 268, 341, 397	(ret. time: 9.0 min)
Methaqualone (internal standard)	m/z = 235, 250	(ret. time: 6.2 min)

Quantification of THC, cocaine, 6-MAM and morphine was based on peak area ratios. A four-point calibration curve was established for each drug by measuring hair samples (200 mg) which were spiked with four different drug concentrations (0.1 ng/mg, 0.25 ng/mg, 0.5 ng/mg and 1 ng/mg). Heroin was quantified by using the 6-MAM calibration. In each series of hair analyses a spiked hair control sample and also a drug-free hair sample was assayed. The precision of the method is presented in Table 1. The detection limit for all compounds was approximately 0.1 ng/mg if at least 50 mg of hair was analysed.

Table 1 Drug concentrations found in multiple analyses of authentic hair samples

	THC [ng/mg]	Cocaine [ng/mg]	6-MAM [ng/mg]	Morphine [ng/mg]
Run 1	3.24	6.79	7.64	1.33
Run 2	3.10	6.67	7.99	1.29
Run 3	3.07	6.52	8.58	1.24
Run 4	3.18	6.58	8.44	1.24
Run 5		6.64	8.02	1.22
Mean	3.15	6.64	8.13	1.26
Standard deviation	0.08	0.10	0.38	0.05
Coefficient of variation	2.54	1.51	4.67	3.97

Instrumentation and reagents

For GC-MS, an HP 5890 gas chromatograph (Hewlett Packard) with an HP 5970 mass selective detector and HP 7673 A autosampler was used. For sonication the Bransonic 220 ultrasonic bath (1 phase, 120 W, frequency: 50 Kc) was used. Polypropylene vials (30 mL) with screw caps were purchased from Sarstaedt. The following reagents were used: methanol prep. solv., acetone p.a., petroleumether 40° C p.a., ethyl acetate p.a. were purchased from Merck. Propionic acid anhydride p.a. (PSA) from Fluka., cocaine-HCl, methaqualone, Δ^9-tetrahydrocannabinol, morphine-HCl, from Sigma. 6-monoacetylmorphine was synthesized according to Derks et al. [3].

Results and discussion

Methanol sonication extraction procedure

The methanol sonication extraction of hair enables the simultaneous analysis of heroin, 6-monoacetylmorphine, morphine, cocaine and THC. Amphetamine and its derivatives, as well as dihydrocodeine and codeine, were also reliably detected (these results will be presented later). Compared to other extraction procedures this technique provides high yields for all these analytes and not produce artefacts of the parent drugs. Ion chromatograms of authentic hair samples containing heroin, 6-MAM and morphine as well as cocaine and THC are presented in Figs. 1 and 2. Unlike other hair extraction procedures using basic or acidic conditions, the methanol-sonication technique prevents hydrolysis of heroin and 6-MAM to morphine. Although heroin can be detected in hair by using other extraction techniques as reported by Goldberger et al. [4], Cone et al. [5] and Tagliaro et al. [6], the sonication extraction procedure with methanol seems to provide optimal recovery of heroin as well as its primary metabolite 6-MAM. Recent results from a comparison of different solvents used in a sonication hair extraction procedure indicated that methanol provides the best extraction yields for morphine, 6-MAM and heroin [7], which confirm our findings. Cocaine and the relatively non-polar THC were also readily extracted with the methanolsonication procedure.

The addition of even small amounts of acid to the solvent leads to an enhanced hydrolysis of heroin and 6-MAM to morphine and the extraction rate for THC was considerably decreased. This effect could be demonstrated by extracting a hair sample from a heroin addict with both pure methanol and methanol containing a small amount of HCl (see Fig. 3). The use of acidified methanol was established by Nakahara et al. [8] for the detection of amphetamines in hair and is very similar to our extraction procedure. However, using acidified methanol neither heroin nor acetylcodeine could be detected and most of 6-MAM was decomposed to morphine. The codeine de-

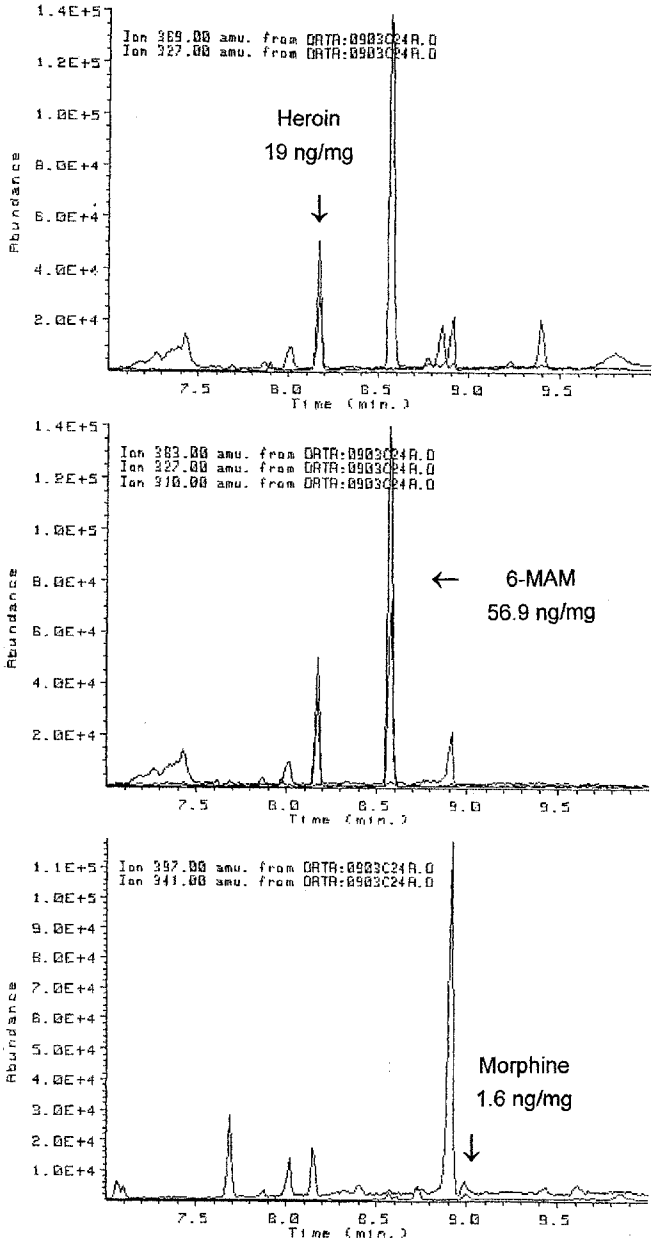

Fig. 1 Typical ion chromatograms of a hair extract (heroin user) with detection of heroin, 6-MAM (monopropionyl-derivative) and morphine (dipropionyl-derivative)

tected in this hair sample must be derived primarily from acetylcodeine, which is a common ingredient of street heroin samples. Therefore, codeine and morphine concentrations in hairs determined after acidic or alkaline extraction should be carefully evaluated. Dihydrocodeine was found in a higher yield after acidification, cocaine in equal concentrations and THC in much lower concentrations compared to the method presented in this paper. This problem of achieving different results by using different analytical methods has already been pointed out by Sachs and Raff [9], who compared various methods for detecting opiates and cocaine in hair.

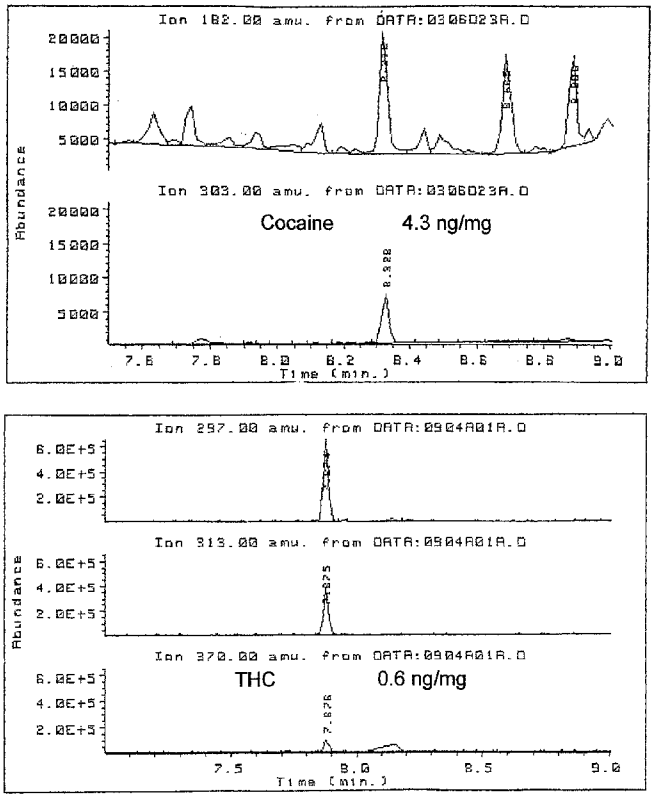

Fig. 2 Typical ion chromatograms of hair extracts containing cocaine and THC (propionyl-derivative)

Distribution of drugs and drug concentrations

From a total of 850 hair samples THC was found in 104 samples (12.2%), cocaine in 230 (27%), and 6-MAM in 141 (16.6%). Besides 6-MAM, morphine was detected in 87 (10.2%) and heroin in 38 samples (4.5%). The mean concentrations were 1.5 ng/mg for THC, 6.7 ng/mg for cocaine, 5.5 ng/mg for 6-MAM, 5.2 ng/mg for heroin and 0.9 ng/mg for morphine (Table 2). The detailed results are presented in Figs. 4–6.

Figure 4 shows the concentrations of 6-MAM in ascending order and the detection of heroin and morphine found in hair of heroin users. 6-MAM was found more frequently than morphine and heroin. In cases, where 6-MAM was present in a concentration of less than 1 ng/mg, morphine could be detected in only 31.7% and heroin in 5% of the samples. Above 1 ng/mg 6-MAM, morphine was detected in 90% and above 10 ng/mg 6-MAM, heroin was found in 76.5% and morphine in 100% of the samples. Heroin concentrations which exceeded those of 6-MAM more than ten times, were detected in three sample. Since this finding seems unexplainable by the pharmacological properties of heroin, external contamination must be considered in theses cases.

The average morphine concentrations in the hair samples were much lower than those of 6-MAM and heroin. This may be due to a lower extraction rate of morphine with methanol according to the results of Rothe and Pragst [7] who found decreasing extractability in the order

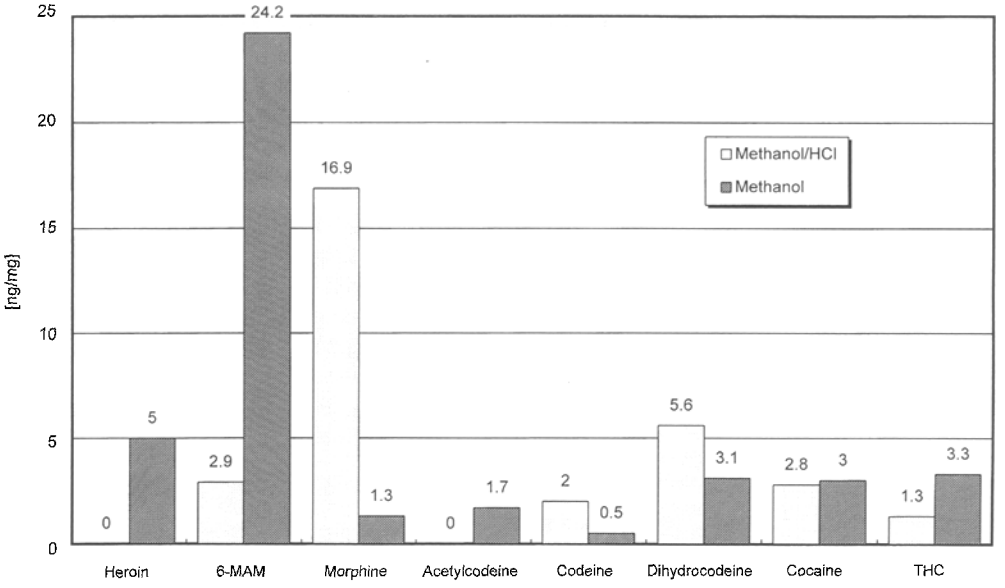

Fig. 3 Comparison of the hair extraction procedures with pure methanol and methanol/hydrochloric acid

Table 2 Drug concentrations detected in 850 hair samples

	THC [ng/mg]	Cocaine [ng/mg]	6-MAM [ng/mg]	Heroin [ng/mg]	Morphine [ng/mg]
Mean	1.501	6.738	5.462	5.237	0.857
Median	0.477	1.070	1.260	1.300	0.421
Standard deviation	2.955	15.090	12.037	10.764	1.222
Minimum value	0.009	0.037	0.028	0.045	0.011
Maximum value	16.700	129.680	79.820	53.140	7.800
n	104	230	141	38	87

heroin > 6-MAM > morphine. An alternative explanation could be a higher incorporation rate of heroin and 6-MAM as compared to morphine. It has been demonstrated in several investigations [10–13] that concentrations of cocaine in hair clearly exceed those of benzoylecgonine or ecgoninemethylester. It has been presumed that the permeability of cocaine from plasma into hair is much higher than that of benzoylecgonine or ecgonine methylester, similar to the permeation through the blood-brain barrier [13]. Likewise morphine, which is also more polar may be incorporated less effectively into hair than heroin and 6-MAM. Therefore the application of concentration ratios between a parent drug and its metabolites seems to be difficult.

Figure 5 presents the distribution of cocaine concentrations found in the hair samples. The range of cocaine concentrations as well as the distribution of the values are very similar to those of 6-MAM with slight differences in the range 0.1–1 ng/mg. This might be due to the dosage relationships between cocaine and heroin, which are also rather similar in view of their pharmacological effectiveness. Compared to cocaine or 6-MAM levels the range of THC concentrations in the hair samples were about 5 to 10 times lower (see Fig. 6). Although various biophysical properties of a compound such as membrane permeability, melanine affinity or basicity may influence its incorporation in hair, two main reasons seem to be important to ex-

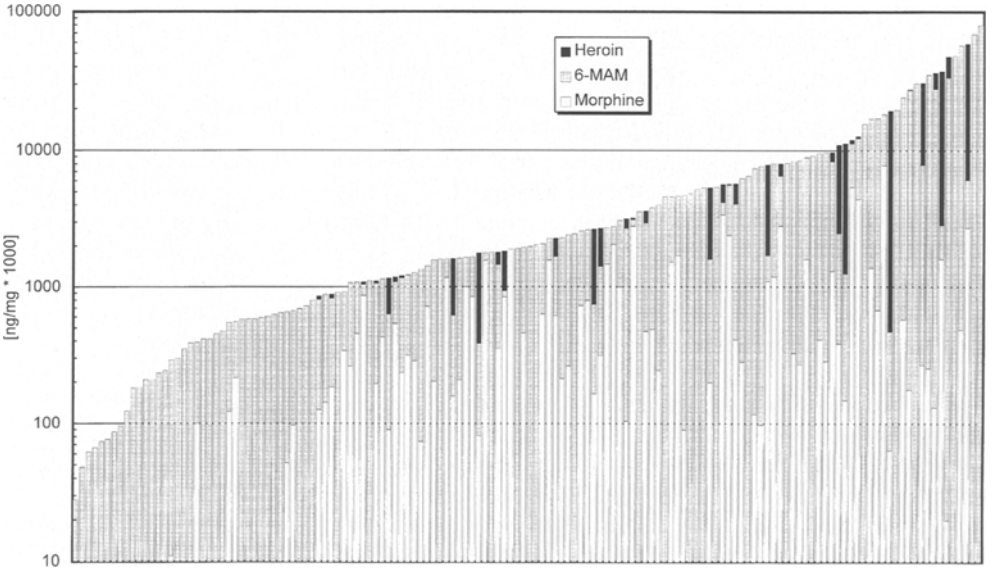

Fig. 4 Heroin, 6-MAM and morphine concentrations detected in hair samples of heroin users in ascending order of total opiate concentrations (n = 142)

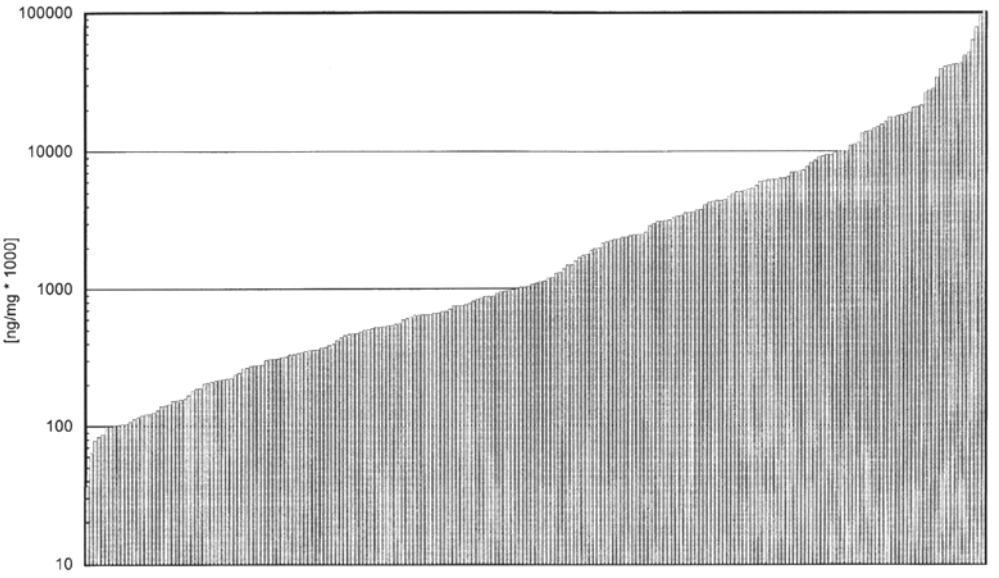

Fig. 5 Cocaine concentrations detected in hair samples of cocaine users in ascending order (n = 230)

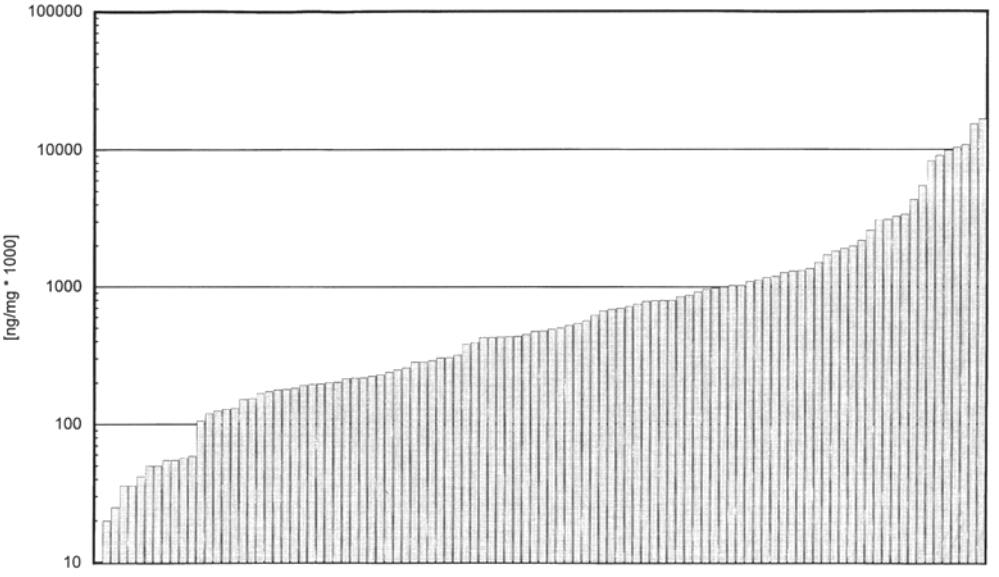

Fig. 6 THC concentrations detected in hair samples of cannabis users in ascending order (n = 104)

plain the lower THC concentrations compared to those of cocaine or 6-MAM. One explanation for this finding might be the clearly lower dosage of THC. A second reason may arise from the more lipophilic properties of THC. This could lead to a decreased incorporation of THC in the hair follicle which, because of its distribution kinetics, is mainly incorporated into the fat compartment. THC has a higher volume of distribution than cocaine (4–14 L/kg vs. 1–2 L/kg).

Consumption behaviour

How are the drug concentrations in hair related to the consumption behaviour of the user? To answer this question the results of the hair analyses were correlated with self-reports of the drug abusers. However, several factors have to be considered which may influnece drug concentrations in hair such as individual variability in drug metabolism, hair growth cycles, residual drug concentration in katagenic or telogenic hairs, exogenous contamination, route of administration or loss of drug from hair etc. But the major problem is the very limited reliability of data from self-reported drug consumption. However, the amount of evaluated data seems to be high enough to provide statistical evidence. Moreover, all analytical results were obtained by the same procedure so that the drug concentrations are comparable. Thus, a careful approach towards a correlation of consumption behaviour and drug concentrations in hair has been attempted.

From a correlation of self-reported cannabis use and THC levels found in the hair samples, a rough classification into two user groups seems to be possible. THC concentrations in the range 0.1 ng/mg–1 ng/mg are suggestive of weekly up to daily cannabis consumption. THC concentrations above 1 ng/mg seem to be linked with multiple daily cannabis use. For cocaine as well as heroin abuse three groups of consumption behaviour were evaluated. Below

Table 3 THC concentrations in hair samples of 6 marihuana smokers correlated with self-reported cannabis use

Individual No.	Smoker behaviour	THC [ng/mg]
1	Daily 1 marihuana cigarette, occasionally hashish	0.152
2	Daily 1 joint hashish	0.463
3	1–2 marihuana cigarettes per week	0.087
4	Almost daily one marihuana cigarette	0.111
5	1 joint hashish only	Not detectable in the corresp. 1 cm hair segment
6	During a three week vacation in the Caribbean Sea daily smoking of marihuana	0.569

1 ng/mg only a weekly use may be presumed. Cocaine or 6-MAM concentrations between 1 ng/mg and 10 ng/mg suggest a weekly to daily drug abuse and above 10 ng/mg a multiple daily use and/or extremely high dosage. This first classification proposed can provide only an approximate and preliminary approach, which should be supplemented by additional surveys. Possible factors which may influence the result of a hair analysis have been disregarded in the proposed classification, however, they must be included in the interpretation of a single result, for instance, in forensic work.

Six representative cases are shown in Table 3. In contrast to the other cases, these individuals had not been accused of drug abuse by the authorities. They freely reported their drug consumption behaviour which suggests that the reports are more reliable. All six individuals smoked preferably marihuana and occasionally hashish. Their drug use behaviour is in agreement with the proposed classification.

We suggest that the maximum concentrations of the drugs observed in the hair samples must be linked with a maximum durg intake behaviour, which may be expressed in a 5 to 10 times drug intake per day assuming a high drug quality. Good evidence for this assumption is the detection of heroin in remarkable concentrations up to 53.1 ng/mg hair in spite of the extremely short half-life of this compound (~ 3 min). This means that the contact time of heroin with the hair follicle supplied with blood must be very frequent in one day and/or the dosage must be extremely high. It has been proposed that a morphine concentration of 100 ng/mg in hair corresponds to a maximum daily dose of 200 mg (heroin?) [14]. In our opinion, this dose seems to be too low to produce such a high morphine level. The maximal THC concentrations found in the hair samples must also be caused by multiple daily cannabis consumption using hashish with an high content of THC. On the other hand, our data indicate that one drug intake a week seems to be detectable in a hair sample, which was also suggested by Möller et al. [14].

An interesting phenomenon is that the frequency of positive drug findings in hair was inversely proportional to common urine findings. In the hair samples cocaine was found most frequently, followed by 6-MAM and finally by THC, whereas usually in urine samples primarily cannabinoids, then opiates and finally cocaine and benzoylecgonine were detected. If the biophysical properties of these compounds and/or pharmacokinetic reasons are not responsible for this discrepancy, this finding may suggest that the majority of cannabis users are light to moderate smokers of marihuana products and hair samples do not necessarily contain measurable THC concentrations.

In future work standardization of methodology of hair analysis must be established in order to obtain comparable results which allow uniform interpretations. In particular, analytical priorities should be considered, for instance, for the question: Is it better to optimize the recovery of morphine from hair or that of 6-monoacetylmorphine and heroin?

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