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Review article

Detection of drugs in human hair for clinical and forensic applications

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Summary. While hair has for sometime been analyzed for assessment of trace elements, it is only in recent years that attention has been focused on this matrix as a possible means of evaluating drug impregnation. This technique was applied to treated subjects and drug abusers for determination of drug consummation. The method involved decontamination in ethanol, solubilization in sodium hydroxide at 100°C for 10 min, extraction in chloroform/isopropanol/n-heptane (50:17:33, v/v), separation on a BP-5 capillary column (GC) and detection by mass spectrometry. Hair samples were analysed for barbiturates, antidepressants, benzodiazepines, β-blockers, nicotine, opiates, benzoylecgonine, cannabis and amphetamines.

Key words: Hair analysis – Chronic drug use – GC/MS

Zusammenfassung. Die schon lange zum Nachweis von Schwermetallvergiftungen angewendete Haaranalyse wird seit einigen Jahren auch zum Nachweis des Drogenmißbrauchs eingesetzt. – Die hier beschriebene Methode wurde für die Untersuchung von Haarproben von mit den entsprechenden Arzneistoffen behandelten Patienten und von Drogenabhängigen eingesetzt. Dazu werden die zuvor mit Ethanol gewaschenen Haare in 1 N Natronlauge (100°C) gelöst und mit Chloroform/Isopropanol/n-Heptan (50/17/33; v/v/v) extrahiert. Nach gaschromatographischer Trennung an einer BP-5-Kapillare wurden die Substanzen massenspektrometrisch detektiert. Es wurden Barbiturate, Antidepressiva, Benzodiazepine, Betablocker, Nicotin, Opiate, Benzoylecgonin, Cannabis und Amphetamine nachgewiesen.

Schlüsselwörter: Haaranalyse – Drogenmißbrauch – GC/MS

Introduction

Although it has been a long time since the evidence first appeared in the literature, only recently has particular attention been devoted to the use of hair as a sample for detection of illicit drugs.

Morphine for example, can be detected in biological fluids only within a few days of heroin intake, and the morphine levels determined are strongly influenced by the dose and the time of the last injection. In contrast, hair appears to be a particularly interesting substrate for the investigation of chronic drug abuse. Drugs pass from the body fluids into the hair and remain firmly bound. It is therefore possible, depending on hair length, to trace the drug intake of an addict over periods longer than 6 months.

Radioimmunoassay, fluorescence polarization, liquid chromatography and gas chromatography coupled to mass spectrometry have been used to identify and quantify opiates, phencyclidine, phenobarbital, amphetamines, methadone, cocaine, marijuana, digoxin, haloperidol, nicotine and antidepressants [1–16].

This paper presents the results obtained from drug analysis in hair during a 2 year period at the Forensic Institute of Strasbourg, France.

Materials and methods

Hair samples consisting of 30–40 strands and weighing at least 50 mg, were cut as close as possible to the scalp on the back of the head. The hair was decontaminated by washing in 5 ml ethanol for 15 min at 37°C. The protein matrix of the hair was destroyed by incubation in 1 ml 1 *M* NaOH for 10 min at 100°C and neutralized with HCl after cooling and centrifugation.

β-blockers were extracted with 3 ml ether/dichloromethane (80:20, v/v) in the presence of 25 µl oxprenolol (25 mg/l) as internal standard. After agitation and centrifugation, the organic phase was purified with 200 µl 0.1 N H_2SO_4. After shaking and centrifugation, the acidic layer was removed and 140 µl injected into a 10 µm RSil CN (Alltech, 300 mm × 4.1 mm i.d.) column. The mobile phase consisted of water, acetonitrile, and 0.1 *M* buffered NaH_2PO_4 solution (60:30:10, v/v) adjusted to pH 3.0. The flow rate was 1.0 ml/min and the detection was carried out at 215 nm. Betaxolol was not confirmed by GC/MS.

Benzodiazepines, barbiturates, antidepressants, fenfluramine and nicotine were extracted with 5 ml chloroform/isopropanol/n-heptane (50:17:33, v/v) after suitable pH adjustment and addition of 20 µl SKF 525 A (10 mg/l) as an internal standard. After agitation and centrifugation, the organic phase was evaporated to dryness. Opiates, benzoylecgonine, cannabis and amphetamine were

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extracted in the same manner in the presence of 20 µl levallorphan (10 mg/l) as internal standard and derivatizated with BSTFA + 1% TMCS, except for amphetamines where trifluoroacetic anhydride was used. The residue was dissolved in 20 µl dichloromethane and 2 µl was injected into a BP 5 capillary column (12 m × 0.22 mm i.d.). The flow of carrier gas (helium, purity grade N 55) through the column was 1.8 ml/min. The column oven temperature was programmed to rise from an initial temperature of 60°C to 280°C at 30°C/min and kept at 280°C for the final 10 min. Splitless injection was employed with a split valve off-time of 1 min. The GC system consisted of a Perkin Elmer (8500) chromatograph with an Ion Trap Detector, operated at 70 ev. The electron multiplier voltage was set at 1450 V. Selected ion monitoring was used to quantify drugs.

For each analyte, recovery and limit of sensitivity for the assay was 65–92%, and approximately 0.03–0.1 ng/mg hair, respectively.

As a strong alkaline procedure was employed, no cocaine was detected. When spiked in a drug-free hair preparation, benzoylecgonine, remained stable during boiling in NaOH.

Results and discussion

The absence of drug metabolism in hair and the fairly uniform growth rate of 1 ± 0.3 cm per month [17] may provide an historical account of drug use by analysis of hair samples. Hair is easily collected without trauma on the part of the donor, it can be stored without deterioration, and its contents can be analyzed with standard methods. Drug concentrations found in hair samples examined in this institute, including post mortem cases, are summarized in Table 1. Concentrations found in hair were within the range of previously published results [1–16]. Since many drugs have been identified in hair, it can reasonably be supposed that other chronically used drugs could also be screened in human hair. Thus, the application to toxicological investigations in hair will surely increased.

Table 1. Concentrations of drug in human hair (ng/mg)

Drug	Level
Diazepam (1)	1.37
Nordiazepam (3)	1.04–2.41
Flunitrazepam (1)	0.41
Nitrazepam (1)	0.37
Secobarbital (3)	21.6 –58.9
Amobarbital (2)	31.4 –41.6
Phenobarbital (4)	21.7 –137.3
Amitriptyline (14)	0.04–1.89
Clomipramine (2)	0.37–0.79
Morphine (29)	0.09–27.10
Codeine (3)	0.31–4.21
Benzoylecgonine (3)	1.21–3.41
Amphetamine (3)	0.96–12.71
Fenfluramine (1)	14.1
11-nor-delta 8-THC-9COOH (32)	0.27–2.91
Betaxolol (5)	0.6 –2.8
Nicotine (22 non smokers)	0.06–1.82
Nicotine (42 smokers)	0.91–33.89

( ), Number of cases

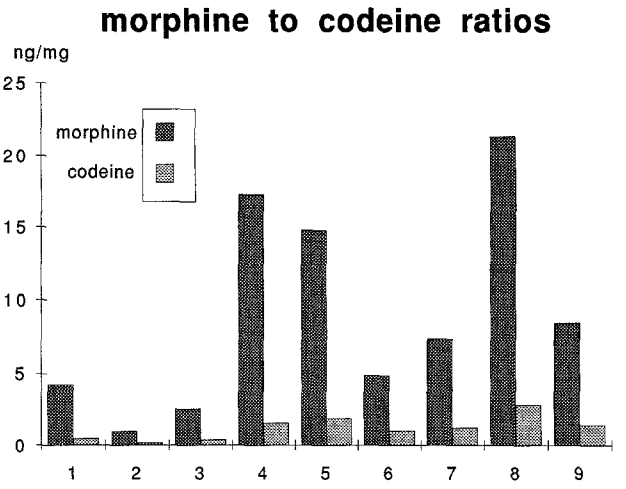

Fig. 1. Morphine and codein contents in 9 fatalities involving heroin

Table 2. Morphine concentrations (ng/mg) in hair of the head, pubic and axillary regions in 5 fatalities involving heroin

Case	Head	Pubis	Axillea
1	0.88	2.46	0.40
2	1.30	1.32	0.75
3	27.10	41.34	24.20
4	0.62	0.80	0.51
5	14.21	19.07	11.48

Table 3. Results of analysis of hair from babies with known in utero exposure to heroin and nicotine

Case	Morphine (ng/mg)	Case	Nicotine (ng/mg)
A	0.81	E	0.37
B	0.96	F	0.40
C	2.40	G	1.12
D	1.21	H	1.37
–	–	I	0.88

Table 4. Evaluation of nicotine in smokers and non-smokers. Influence of environmental smoke exposure

Population	Nicotine (ng/mg)
Smokers	0.91–33.89
Non smokers A	0.54– 1.82
Non smokers B	0.06– 0.33

A, With environmental smoke exposure (12 subjects); B, with no environmental smoke exposure (10 subjects)

In cases of heroin abuse, codeine was also identified. Figure 1 shows the repartition between morphine and codeine in 9 fatalities involving heroin overdose. The codeine could be due to its presence in the opium used for preparing heroin but since the morphine levels in the 9 cases was clearly higher than the codeine level, heroin

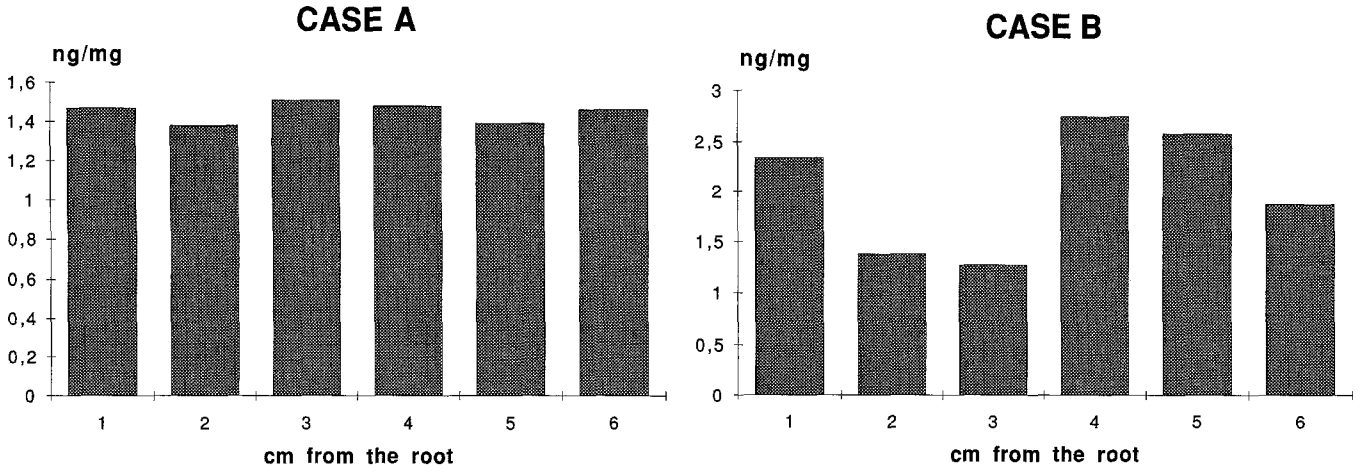

Fig. 2. Pattern of morphine use. *A*: constant use *B*: with variation

abuse is highly probable. This conclusion is in accordance with those of Sachs and Arnold [2], and Moeller and Fey [18].

It is of particular importance to specify the nature of the hair, since drug concentrations are different in head hair, axillary hair and pubic hair. The highest morphine concentration was found in pubic hair, followed by head hair and axillary hair (Table 2). These differences may be explained by the different growth rates and length of the hair. In a study of methadone, Balabanova and Wolf [10] demonstrated that the highest drug concentration was found in axillary hair, followed by pubic hair, and head hair. The variation in incorporation of morphine and methadone in hair is still unclear, but the secretion of drugs in perspiration, resulting in a loss of drugs, certainly plays an important role in this mechanism.

Determination of in utero drug exposure by hair analysis is another application of this technique which was recently proposed for cocaine exposure [19]. Neonatal hair from 7 infants whose mothers were known cocaine users ranged from 0.2 to 27.5 ng benzoylecgonine per mg hair. Table 3 shows morphine and nicotine concentrations in neonatal hair from infants whose mothers were known to be heroin and heavy tobacco users. Hair analysis may identify intrauterine exposure to drug in babies when a maternal drug history is not available or in doubt.

To validate data on tobacco use, hair samples were investigated to quantify nicotine from 22 non-smokers and 42 smokers [20]. Hair from both non-smokers and smokers contained nicotine (Table 4), but although it was difficult to determine an absolute cut-off level, an amount greater than 2 ng nicotine per mg hair can be used to differentiate smokers from non-smokers. In the non-smoker population, it was possible to distinguish passive smokers from other non-smokers. In passive smokers the nicotine content was greater than 0.5 ng/mg and lower in non-exposed non-smokers. The presence of varying amounts of nicotine in hair from passive smokers can be explained by deposition of atmospheric smoke, which is rich in nicotine. The high nicotine concentrations in the hair of passive smokers were confirmed by a questionnaire indicating their habits. Cotinine concentration was also determinated, but cannot be used to classify the population, since the differences are not statistically significant.

Because head hair grows at approximately 1.0 ± 0.3 cm per month, the window of detection for hair analysis can range from many months to years. Furthermore, if the strands of hair are cut into sections (for example one month intervals, about 1 cm), it is possible to obtain information on the pattern of use, i.e. whether drug use has decreased, is constant, or has increased (Fig. 2).

During control tests of hair fragments, a drug addict is not able to hide the fact of drug abuse, even by deliberately abstaining for several days before sample collection, although this might not be detectable by urine analysis.

Hair analyisis is, therefore, particularly useful for the screening of personnel in highly sensitive positions, such as the army, administration or aviation.

In summary, it appears that the value of hair analysis is steadily gaining recognition in clinical toxicology for evaluation of treatment compliance and in forensic toxicology for legal applications.

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