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Review

Hair analysis for abused and therapeutic drugs

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Abstract

This review focuses on basic aspects and recent studies of hair analysis for abused and therapeutic drugs and is discussed with 164 references. Firstly, biology of hair and sampling of hair specimens have been commented for the sake of correct interpretation of the results from hair analysis. Then the usual washing methods of hair samples and the extraction methods for drugs in hair have been shown and commented on. Analytical methods for each drug have been discussed by the grouping of three analytical methods, namely immunoassay, HPLC–CE and GC–MS. The outcomes of hair analysis studies have been reviewed by dividing into six groups; morphine and related, cocaine and related, amphetamines, cannabinoids, the other abused drugs and therapeutic drugs. In addition, reports on stability of drugs in the living hair and studies on drug incorporation into hair and dose–hair concentration relationships have been reviewed. Applications of hair analysis to the estimation of drug history, discrimination between OTC drug use and illegal drug use, drug testing for acute poisoning, gestational drug exposure and drug compliance have also been reviewed. Finally, the promising prospects of hair analysis have been described. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Hair analysis; Drugs of abuse; Therapeutic drugs

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1. Introduction

Nowadays, hair is being recognized as a third fundamental biological specimen for drug testing besides urine and blood. Although more than 450 papers on hair analysis for drugs have been published since 1954, most of them have appeared only in this decade. Due to the progress of separation techniques and detection sensitivity and selectivity, drugs in hair can be detected and determined at the levels of pico-mole/mg. The largest number of papers on hair analysis have dealt with cocaine, followed by opiates and, third, by amphetamines. The top three groups of drugs comprehended almost 50% of all papers on hair analysis. However, recent hair analysis studies have been changing to other kinds of drugs, for example doping agents like clenbuterol, therapeutic drugs like benzodiazepines, methadone and carbamazepine, and tobacco components (Table 1).

The fields in which hair analysis has so far been applied are mainly forensic toxicology and drug abuse studies, followed by clinical toxicology and clinical chemistry. Although most of the reports until 1990 dealt only with the detection of drugs in hair, recent studies have tried to find more information from hair analysis and have dealt in more detail with drug incorporation mechanism and drug behavior in hair.

In the field of drug testing, great interest has been taken in hair analysis in recent years because of its wide window of detection. However, there are very few reviews written about both basic aspects and applications of hair analysis. Therefore, this review describes these basic matters and applications concerning abused and therapeutic drugs in hair.

2. Biology and sampling of hair

Although hair looks like a primitive structure, it is actually a very complex part of human body and its

Table 1
Drugs found in hair[a]

Abused drugs:	Cocaine (88)	Phencyclidine (PCP) (8)
	Morphine, 6-acetylmorphine, codeine (74)	Fentanyl (5)
	Amphetamine/methamphetamine (37)	LSD
	Cannabinoids (14)	Propoxyphene
	MDMA/MDA (11)	Methaqualone
Therapeutic drugs:	Methadone (11)	Amitriptyline
	Benzodiazepines (11)	Pholcodine
	Haloperidol (8)	Ethylmorphine
	Ofloxacin and related (5)	Ephedrine
	Buprenorphine (4)	Extromoramide
	Barbiturates (4)	5-Fluorouracil
	Carbamazepine (3)	Furosine
	Zipeprol (2)	Thiopental
	l-alpha-Acetylmethadol (2)	Chloroquine
	Meprobamate	
Tobacco ingredient:	Nicotine (22)	Cotinine
Doping agents:	Clenbuterol (11)	Stanozol

[a] Parentheses mean the number of papers until October 1998.

biology is still unclear at many points. Each hair shaft grows up via the synthesis of hair matrix cells accompanied by keratinization. The hair shaft consists of an outer cuticle, an inner medulla and a central cortex. Generally, the cuticle is less intact toward the distal end of the hair shaft than the proximal side.

Hair grows in predictable patterns, lengths and textures in different body areas. The lengths and textures are determined by the type of hair which in turn is dependent on gender and age.

The patterns, colors, textures, hair diameter and hair growth rates considerably vary between the genders and among races. There are three stages in the hair growth cycle. Following a long period of active hair growth (anagen phase), the hair follicle enters a short transition stage (catagen phase: 4~6 weeks). After the transition stage, the hair follicle enters a resting period (telogen phase) in which the hair shaft stops growing completely and can be removed easily by pulling. For scalp hair, the resting phase is relatively short, about 10 weeks. On the scalp of an adult, it is reported [1] that approximately 15% of the hairs are in a resting stage and the remaining 85% in the anagen phase.

It is usually stated [1] that the average rate of hair growth is 0.44 mm per day (range, 0.38–0.48) for men and 0.45 mm per day (range, 0.4–0.55) for women in the vertex region of the scalp. However, the rate of growth is also dependent to a certain extent on anatomical location, race, gender and age.

Although the mechanisms of drug incorporation into hair have still not been sufficiently clarified, drugs are thought to be distributed into hair by two processes: incorporation into the growing hair shaft from blood and/or adsorption from other media such as sweat and smoke or powders from the environment.

The most important considerations in hair sampling are: collecting hair from a preferable anatomical location (posterior vertex) where hairs are relatively uniform [1], collecting the hair a uniform distance from scalp (especially if sectional analysis is to be performed), collecting a sufficient sample for the number of tests to be performed, preventing contamination and accurately identifying the sample.

3. Extraction method of drugs from hair

3.1. Washing of the hair sample to remove external contamination

The washing of hair samples has been well investigated mostly for the analysis of cocaine.

Washing solvents for hair samples containing cocaine are generally divided into three categories; MeOH or EtOH [2,3,6,9,15], 0.1% sodium dodecylsulfate (SDS) or other detergents [4,7,8,10,11,13], and dichloromethane [12,14,16] (Table 2). The hair samples are incubated, briefly washed or stirred in these solvents at room temperature or 37°C for up to 15 min. In most cases, water is additionally used as a rinse. The goal of washing is to remove only the external contamination or unnecessary dirt and grease from the surface of hair. Therefore, over-washing should be avoided so that drugs inside the hair shaft remain unaffected. Wilkins et al. [17] have reported that even incorporated drugs in hair are removed during the washing process. Nakahara et al. [18] investigated the effect of removing external methamphetamine (MA) contamination using 0.1% SDS under ultra-sonification. When control hair was soaked in an aqueous solution of 10 μg/ml of MA hydrochloride for 24 h, MA contaminated on the hair surface could be removed easily by washing with 0.1% SDS. However, hair soaked in more than 20 μg/ml of MA solution for 24 hours left a few ng/mg of MA in the hair even after washing.

3.2. Extraction procedures

Extraction procedures for drugs from hair are mainly divided into three modes, namely, digestion with alkali [25,27], acid extraction [5,18,19,22,27] and enzymatic treatment [8,20,21,28]. In Table 3, the extraction methods are summarized as well as the derivatization for GC–MS. Intact hair, fine cutting, powdering and homogenization have been reported as the form of the sample for extraction.

3.2.1. Digestion (alkaline agent)

The general alkaline digestion method involves incubation of the hair sample in 0.1~2.5 *M* NaOH at 37°C overnight. After adjustment to pH 9 with acid, the aqueous solution is extracted with solid phase extraction. Such alkaline methods [25,26] are applicable to alkaline stable compounds such as morphine, amphetamines and cannabinoids, but generally cannot be used for the analysis of cocaine, heroin/6MAM and other ester compounds in hair.

3.2.2. Enzymatic treatment

Moeller et al. [28] reported a hydrolysis method with β-glucuronidase/arylsulfatase (glusulase) for

Table 2
Washing procedures of hair sample for hair analysis (cocaine cases)

Hair[a]	Sample volume	Sample preparation	Wash procedure [times of washing]	Ref.
Cocaine				
Human	10–100 mg	cut	MeOH (1 ml), inc.[b], 37°C, 15 min [3]	[2]
Human	5 mg	cut	MeOH, vort., 0.5 min [1]	[3]
Human	10 mg	cut	1% SDS (50 ml) [1]→H_2O (50 ml) [10] →MeOH (30 ml) [3], stir.[b], 5 min each	[4]
Rat	50 mg	intact	H_2O [3]	[5]
Human	100 mg	not mentioned	EtOH (2 ml) [1], →buffer (pH 7) [2], 37°C, 15 min each	[6]
Mice	50~100 mg	intact	5% detergent (10 ml) [1]→H_2O (500 ml)	[7]
Human, Rat	10~30 mg	cut	0.1% SDS [3]→H_2O [3], sonic. 1 min each	[8]
Human	2 mg	intact	EtOH [3]	[9]
Human	25~100 mg	intact	0.3% Tween 20 [1]→H_2O [1]	[10]
Human	8~12 mg	powder	0.05% SDS [3]→H_2O [3]→EtOH [3]	[11]
Human	50 mg	powder	CH_2Cl_2 (5 ml) [1]→water (5 ml) [1]→CH_2Cl_2 (5 ml) [1]	[12]
Human	50 mg	cut	0.1% Tween 80 (5 ml) [1]→H_2O (5 ml) [1]→acetone (1 ml) [1]	[13]
Human	50 mg	powder	buffer (5.6) [2]→Ch_2Cl_2 [2], inc., 3 min each	[14]
Human	30~40 mg	homogenization	MeOH [?]→buffer (pH 7)	[15]
Human	100 mg	powder	CH_2Cl_2 (5 ml) [1]→water (5 ml) [1]→CH_2Cl_2 (5 ml) [1]	[16]

[a] All hair samples are pigmented hairs except Rat* hair (white hair).
[b] inc.: Incubation, vort.: vortexing, stir.: stirring, sonic.: ultrasonification.

Table 3
Summary of extraction and derivatization for GC–MS analysis

Hair sample	Extraction	Derivatization	Ref.
Cocaine, BE, EME			
	0.05*M* H_2SO_4 (37°C, stir. ON[a])→pH 4, SPE[b] 3	BSTFA-TMCS (99:1) (60°C, 30 min)	[19]
	Proteinase K (40°C, inc. ON)→SPE	MTBSTFA (40°C, 10 min)	[20]
	Proteinase K (25°C, inc. ON)→SPE	PFPAA+HFIP (60°C, 20 min)	[8]
	0.1*M* HCl (45°C, inc. ON)→pH 7, SPE	MSTFA (70°C, 15 min)	[5]
	MeOH (60°C, 2h)→Ev.[c]→dissolved in 0.1*M* HCl ≠		
	≠→washed with hexane→pH 9.2 Ext. with hexane-isoamyl alcohol (99:1)		[7]
6-MAM, morphine			
	Glusulase[c] (45°C, inc. 2 h)→SPE	PFPAA (70°C, 30 min)	[21]
	MeOH-TFA (9:1) (rt*, sonic. 1 h+rt, ON)→Ev.→SPE	BSA (80°C, 20 min)	[22]
	Protease E or VIII (37°C, in. ON)→SPE	Silylation	[23,24]
	1*M* NaOH (37°C, inc. ON)→pH 9→SPE	TFAA (70°C, 30 min)	[25]
Methamphetamine, MDMA			
	5*M* HCl-MeOH (1:20) (rt*, sonic. 1 h+rt, ON)→Ev.→SPE	TFAA (55°C, 20 min)	[18]
	2.5*M* NaOH (80°C, 20 min)→Ext. CH_2Cl_2	TFAA (70°C, 15 min)	[26]
	0.6*M* HCl (, ON)→Ext.	TFAA (55°C, 20 min)	[27]
	Glusulase[c] (40°C, 2 h)→SPE	PFPA (60°C, 30 min)	[28]

[a] ON=overnight.
[b] SPE=solid phase extraction.
[c] Ev.=evaporation, glusulase=glucronidase/sulfatase.

the destruction of the hair structure. Hair (10~30 mg) was hydrolyzed with 75 μl glusulase solution for 2 h at 40°C. After centrifugation, the supernatant was extracted by SPE. Recently, other enzymes like proteinase K [8,20], protease E [23] and VIII [24] have been used for the mild degradation of the hair fiber structure. The merit of these enzymatic methods is to solubilize the hair sample without degradation of the unstable compounds like cocaine and heroin/6MAM. The demerit of this method is that it is relatively expensive.

3.2.3. Acid extraction and acidic methanol

The acidic extraction of drugs from hair has been reported by several groups [5,19,27]. The extraction in 0.1~0.6*M* HCl or 0.05*M* sulfuric acid is generally carried out at room temperature or 37°C overnight. After acid treatment, the solution is neutralized and extracted with SPE.

The author's group [18] have reported an effective method for the direct extraction of hair with methanol–5N hydrochloric acid (20:1) under ultrasonification for 1 h with storage overnight. In addition, it was also found that methanol–trifluoroacetic acid (9:1) is good for extraction of 6MAM from heroin users' hair [22]. The method using methanol–trifluoroacetic acid (9:1) minimizes hydrolysis of acetylmorphine and maximizes its extraction efficiency.

4. Analytical methods for drugs in hair – choice of analytical methods

4.1. Immunoassay

In the early time of hair analysis studies, Baumgartner et al. [29,30] reported the radioimmunoassay (RIA) for opiates [29], cocaine/benzoylecgonine [30] and phencyclidine [31] in hair. Using RIA, the assay for cocaine/benzoylecgonine [32–34] and methadone [35,36] in hair have also been reported elsewhere. Franceschin [37] reported the detection of morphine in hair with the Abbott TDx. Nakahara et al. [38] reported an ELISA method using monoclonal antibodies for detection of methamphetamine in hair. Other drugs which have been detected by immunoassay methods include clenbuterol [39] and fentanyl

[40]. Segura [41] reported a simple immunological technique for hair analysis, which included a suitable extraction with methanol–trifluoroacetic acid.

4.2. HPLC and CE

There are not many reports on the analysis of drugs in hair by HPLC or CE because of its relatively low sensitivity and lack of confirmatory ability. Uematsu et al. [42] reported the detection of haloperidol in human hair with HPLC-UV. Tagliaro et al. [43,44] detected cocaine and morphine in hair with HPLC with fluorometric detection. Takayama et al. [45] reported a high-sensitive HPLC method with chemiluminescence detection for methamphetamine and amphetamine in hair. In addition, phenytoin [46], carbamazepine [46], thiopental [47] and ketamine [47] have been identified in human hair by HPLC methods. The utilization of capillary electrophoresis for hair analysis has been recently attempted. Tagliaro et al. [10,48] succeeded to detect cocaine and morphine in hair with capillary electrophoresis. Plaut et al. [49] applied CE to the detection of methaqualone in hair.

4.3. GC–MS (GC–EIMS, GC–CIMS), MS–MS, LC–MS

The analytical method most frequently used for hair analysis is GC–MS. In hair analysis, GC–MS is superior to other analytical methods in terms of sensitivity, specificity and selectivity. Usually electron impact mass detection is used, but positive and negative chemical ionization mass detectors have also been used. In recent years, tandem mass (MS–MS) and LC–MS [50,51] have been used for hair analysis in order to increase sensitivity and detect GC-unstable compounds. Generally, drugs in hair are quantitated by selected ion monitoring (SIM) due to the low amounts of drug present. Therefore, the deuterated target drugs are often used as the internal standard (IS). As the deuterated ISs behave in the same way as the target drugs during the extraction, purification, derivatization and chromatography, they bring about not only good quantitative results but also more definite qualitative results on account of the constant relative retention times.

5. The outcome of hair analysis

5.1. The outcome of hair analysis for morphine and related drugs

It is said that hair analysis studies on abused drugs started from the time when Baumgartner et al. [30] succeeded to detect opiates in the hair of heroin abusers by RIA and estimate their opiate abuse histories by sectional hair analysis. In 1986, Marigo et al. [44] detected morphine in the alkaline digestion of heroin addicts' hair using HPLC with fluorometric detection. The choice of GC–MS for the identification of morphine and 6-acetylmorphine(6MAM) in hair started from 1991. Goldberger et al. [52] identified 6MAM (av. 0.90 ng/mg, n=20) and heroin (av. 0.17 ng/mg, n=7) in hair samples from 20 heroin users by GC–MS. Nakahara et al. [53] showed that hydrolytic extraction of morphine analogs in hair with 10% HCl for 1 h at 100°C gave a quantitative recovery of morphine. In their experiments, the total morphine level in hair from monkeys administered with heroin was six times higher than from those administered with morphine. From hair of monkeys and humans intoxicated with heroin, they detected 6MAM using a methanolic extraction at the levels of 0.7–7.2 ng/mg as the major component in hair together with morphine but without heroin. Mangin and Kintz [54] showed variability of opiates concentrations in human hair according to their anatomical origin: head, auxiliary and pubic regions. Moeller et al. [28] developed a new extraction method using SPE after incubation of powdered hair samples with β-glucuronidase/aryl-sulfatase in phosphate buffer. Tagliaro et al. [48] reported an analytical method with capillary electrophoresis for morphine in hair. Welch et al. [11] have demonstrated that extractions with 0.1N HCl are efficient at removing morphine from hair. Nakahara et al. [22] compared the efficiency of extraction of 6MAM and morphine from hair between five extraction methods; methanol, 0.1 M HCl, methanol–5 M HCl (20:1), helicase and methanol–trifluoroacetic acid (TFA) (9:1). Their findings show that methanol–TFA was the best solvent for extracting 6MAM and morphine with minimum hydrolysis and maximum efficiency of 6MAM (Fig. 1). Wang et al. [55] reported the simultaneous assay of heroin and metabolites in hair,

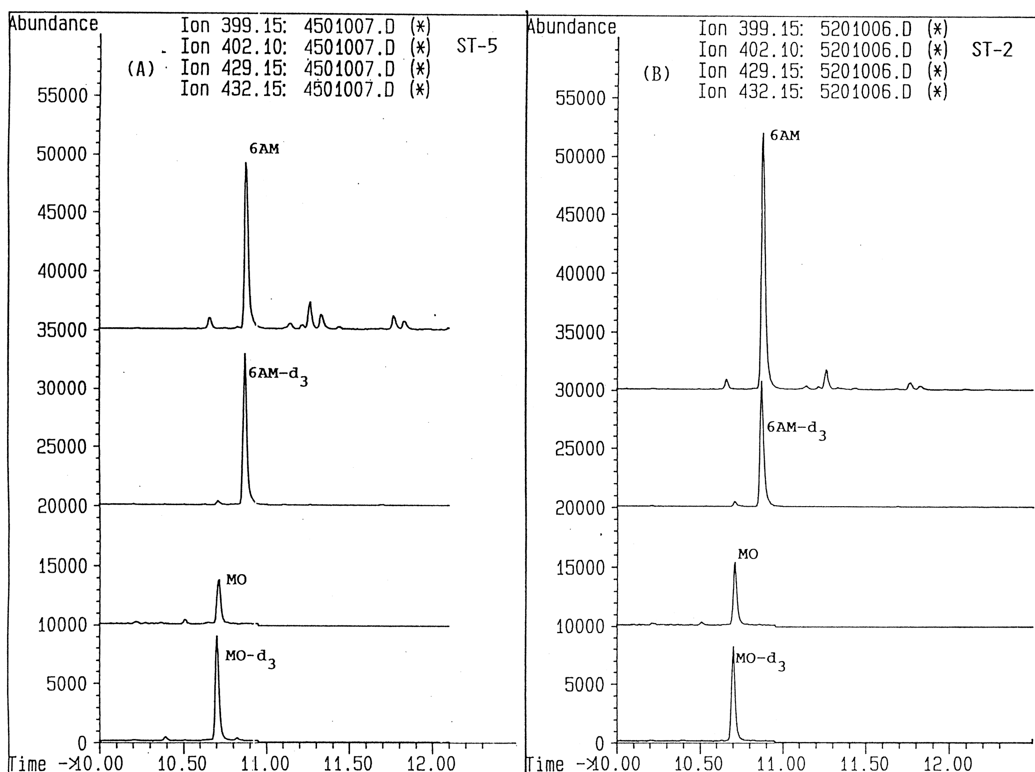

Fig. 1. GC–MS chromatograms obtained from the hair of heroin abusers using methanol–trifluoroacetic acid (9:1) as an extraction solvent. (Reproduced with permission of the authors of Ref. [63].)

plasma, saliva and urine by GC–MS. Wilkins et al. [56] reported the disposition of codeine in female human hair after multiple-dose administration and found a difference of drug concentration in distal hair between female and male subjects. Cirimele et al. [57] reported supercritical fluid extraction of codeine, morphine and 6MAM in drug addict hair. Gygi et al. [58] found that after controlled administration, the incorporation of codeine and its metabolites, morphine, into rat hair occurs in a distinct dose-proportional manner. Jurado et al. [59] and Kintz and Mangin [60] reported simultaneous quantification of opiates, cocaine and cannabinoids in hair by GC–MS. Nakahara et al. [22] also reported a method for confirmation of heroin use and determination of heroin abuse history. Wilkins et al. [25] developed a new method using PCI–MS for the determination of codeine and its metabolite, morphine. Gaillard and Pepin [14] developed a new SPE method on C18 cartridges which allows a very simple protocol of manipulation and a single elution of opiates and cocaine homologs from human hair samples. Polettini et al. [61] evaluated the recovery of extraction of opiates from the hair samples of heroin over-dose corpses and the extent of hydrolysis of acetylated opiates (6-acetylmorphine, acetylcodeine), using alkaline hydrolysis, acid hydrolysis and methanol.

Hair analyses of polydrug poisonings including opiates have been discussed as case reports. Hold et al. [24] developed a sensitive method for the combined extraction of cocaine, opiates and their metabolites from human head hair using an enzyme-based digestion technique. Potsch and Skopp [62] found that cosmetic treatments such as bleaching and perming make the drug concentration in hair decrease. Tagliaro et al. [63] reported the findings from hair analysis regarding heroin overdose death.

5.2. Hair analysis for cocaine and related drugs

In the early 80s, both European [64] and American groups [29] independently reported the detection of cocaine metabolites in hair using RIA. Smith and Lue [65] found subnanogram levels of cocaine metabolites in human hair with RIA. Although some papers concerning hair analysis of cocaine were reported before 1990, in most cases the target compound was benzoylecgonine, especially in cases of the use of RIA. Since the use of GC–MS for hair analysis in the 1990s, some papers [2,8,66] have showed that the major component in hair of cocaine users is in fact cocaine and not its metabolite.

5.2.1. Cocaine in hair as a major component

Nakahara et al. [8] demonstrated, using animal model experiments, that cocaine is overwhelmingly incorporated into hair in preference to its metabolites when compared with their plasma AUCs.

Nakahara and Kikura [66] demonstrated by using a combination of normal and deuterated cocaine, BE and EME for rat experiments, that after administration, cocaine is predominantly incorporated into hair over the metabolites, BE and EME. In addition, BE appears to be produced after incorporation into the hair shaft, from the fact that almost no BE was incorporated into hair from blood and only BE-d3 was detected in hair after the administration of both cocaine-d3 and BE (Fig. 2). They concluded that cocaine should be a primarily target compound in hair.

5.2.2. Hair of coca leaf chewers

Henderson et al. [20] and Moeller et al. [67] detected cocaine, BE and EME from the hair of five coca leaf chewers. The former authors [20] reported that the mean cocaine concentration in the hair of these subjects was 15.2 ng/mg, the mean BE concentration was 2.8 ng/mg and mean EME concentration was 1.6 ng/mg. The latter authors [67] noted that in 95% of the cases, cocaine exceeded BE and EME in concentration. In contrast Springfield et al. [68] reported the detection of cocaine and metabolites in the hair of ancient Peruvian coca leaf chewers and found the two metabolites in higher concentration than the parent drug.

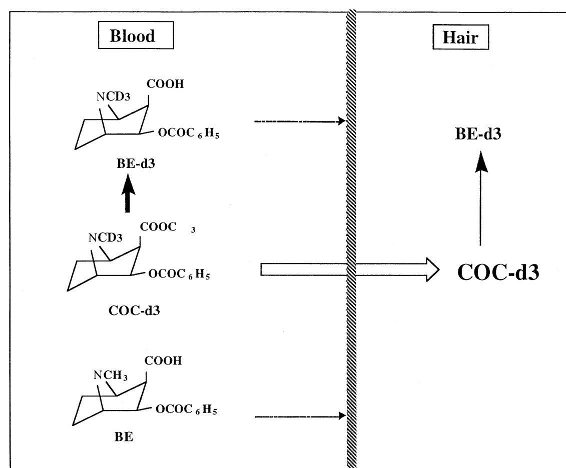

Fig. 2. The speculation on preference of cocaine incorporation into hair to the major component in the blood, benzoylecgonine after cocaine administration to rats. (Reproduced with permission of the authors of Ref. [66].)

5.2.3. Cocaethylene

DiGregorio et al. [69] investigated the prevalence of cocaethylene in the hair of 18 pregnant women. Out of the 15 cocaine positive hair samples, nine samples were positive for cocaethylene and of these nine, eight reported using both cocaine and alcohol. It has been reported [7,14,24,56] that cocaethylene which is produced from the combination of cocaine and alcohol can be detected in the hair of cocaine abusers. Reid et al. [70] reported hair concentration differences of cocaine and cocaethylene between pigmented and nonpigmented hair of 29 gray haired cocaine abusers. They found statistically significant differences between pigmented and senile white sections of paired samples. Particularly they found out that the hair concentration difference of cocaethylene (pigmented/white=6) was much larger than that of cocaine (pigmented/white=2).

5.2.4. Melanin and cocaine

The relationship between hair melanin and cocaine has been noted in many papers regarding hair analysis. Reid et al. [71] found the incorporation tendency of cocaine/BE into hair to be black>brown>blond. Joseph et al. [72] speculated that melanin was considered the most likely binding site for cocaine in hair. Their Scatchard analysis indicated that dark hair had a 5- to 43-fold greater

binding affinity than light hair. Nakahara et al. [73] showed in vitro that cocaine was the most affinitive drug to melanin of 20 major drugs of abuse.

5.2.5. Analytical methods

Hold et al. [24] developed a positive ion chemical ionization gas chromatography–mass spectrometry method for the simultaneous quantitation of cocaine, opiates, and their metabolites in human hair. They applied their method to analysis of the hair samples obtained from cocaine and heroin users and detected COC, BE, EME, norcocaine, cocaethylene, codeine, 6MAM, and morphine in the samples. Clauwaert et al. [51] reported a LC–MS method for cocaine and metabolites in human hair.

5.3. Hair analysis for amphetamines

5.3.1. Methamphetamine/amphetamine

Due to the high incidence of methamphetamine abuse in Japan, hair analysis for methamphetamine/amphetamine has been reported mostly by Japanese scientists. Ishiyama et al. [74] analyzed MA and AP in hair with trifluoroacetyl (TFA) derivatization using *N*-ethylbenzylamine as an internal standard. The concentrations of amphetamines in MA abusers' hair ranged from 4 to 120 ng/mg. Niwaguchi et al. [75] detected MA in Wistar rat hair (non-pigmented hair) after single, 5-days and 14-days repeated administration of 20 mg/kg/day of MA. The concentrations of MA in the hair were relatively low (0.5~1.9 ng/mg). Suzuki et al. [76] demonstrated that MA and AP in a single hair could be detected using CI–MS. Nakahara et al. [18] showed the usefulness of stable isotope dilution GC–MS for precise determination of MA and AP in hair using MA-d4 and AP-d4. In addition, they [77] demonstrated that the distribution of MA in 1- or 2-cm sectional hair nearly corresponded to reported drug histories. Furthermore, the Nakahara group [78,79] reported the movement of methoxyphenamine along human hair shaft with hair growth and studied the excretion of methoxyphenamine into human beard hair by stable isotope dilution-GC–MS. Takayama et al. [44] reported the determination of MA and AP in a single hair sample by HPLC with chemiluminescence detection.

5.3.2. MDMA/MDA

Several hair analysis studies on MDMA and its analogs have been reported. Kintz et al. [80] reported the simultaneous determination of amphetamine, MA, MDA and MDMA in human hair by GC–MS. Kikura et al. [81] investigated the disposition of MDA, MDMA, MDEA, MMDA and metabolites of MDMA into hair using animal models. The order of drug-hair incorporation tendency was MMDA>MDEA>MDMA>HMMA>MDA>HMAP, with levels about 6 times larger than those of amphetamine analogs, suggesting that the methylenedioxy group on the benzene ring raises the hair incorporation tendency very positively. They also analyzed hair samples of seven MDMA abusers. MDMA and MDA were simultaneously detected in all the samples by GC–MS along with MDEA in the two samples. Nakahara and Kikura [82] evaluated hair root samples as a specimen for proving acute MDMA poisonings using an animal model. They found that MDMA can be detected from 5 min after administration at high concentrations (~150 ng/mg) accompanied with MDA. They also demonstrated that at lethal doses, the increase in the MDMA concentrations and the ratios of metabolite (MDA) to parent drug (MDMA) stop due to the cessation of the hair growth, the incorporation of drug into hair shaft and the activity of metabolism after death. Rohrich and Kauert [83] reported the determination of AP and MDA-derivatives in hair. Rothe et al. [84] analyzed hair samples of 20 volunteers of the techno-music scene, who regularly consumed ecstasy tablets and amphetamine. In 20 hair samples, they found AP (17 cases), MDA (16 cases), MDMA (16 cases), MDEA (13 cases) and MBDB (2 cases).

5.4. Hair analysis for cannabinoids

Hair analysis for cannabinoids is one of the most difficult analyses due to detection and interpretation problems because of the low concentration in hair and the external contamination. In 1991, Cirimele et al. [85] reported the detection of PFP derivatized THC [0.26–2.17 ng/mg] and THCA [0.07–0.33 ng/mg] with GC–MS from the alkaline digests of drug abusers' head and pubic hair (100 mg). The same group have also reported the detection of PFP derivatized THCA with GC–NCIMS in a range of

0.02–0.39 ng/mg [86]. Wilkins et al. [87] developed a quantitative analysis of TMS derivatives of THC, 11-OH-THC, and THCA in human hair by GC–NCIMS and detected only THC in the head hair of marijuana smokers. They pointed out the decrease (up to 50%) of THC levels in human hair by dichloromethane washing [87]. Cirimele et al. [88] also reported the simultaneous identification of THC, CBN and CBD in human hair. Jurado et al.'s report [89] showed that there were large differences (up to 73.7%) in the quantitative results of cannabinoids in human hair between Spanish and French laboratories and suggested that the determination of cannabinoids in hair varies widely among laboratories.

5.5. Hair analysis for other abused drugs

5.5.1. PCP, LSD, fentanyl

Baumgartner et al. [31] reported the detection of PCP in human hair with RIA and Kidwell [90] also detected this compound in human hair by tandem MS. Sakamoto et al. [91] developed an analytical method for the simultaneous detection of PCP and its metabolites, 4-phenyl-4-piperidino-cyclohexanol (PPC) and 1-(1-phenylcyclohexyl)-4-hydroxy-piperidine (PCHP), in rat hair. Their results suggest that hair could be a very useful specimen for confirmation of active past PCP use because PCP and its metabolites can be detected simultaneously. Nakahara et al. [92] found PCP [0.33–14 ng/mg], PCHP [0.02–0.12 ng/mg], and trans-PCPdiol [0.09–0.45 ng/mg] in eight human hair specimens and showed that *trans*-PCPdiol was the major metabolite in the hair of PCP users despite *trans*-PCPdiol being only a minor metabolite in the hair specimens of rats administered with PCP. The GC–MS chromatogram obtained from the hair sample of a PCP abuser is shown in Fig. 3. Slawson et al. [93] investigated the incorporation of PCP into pigmented and nonpigmented rat hair and found that PCP is incorporated into pigmented hair more than nonpigmented hair.

Nakahara et al. [94] reported the detection of lysergic acid diethylamide (LSD) and its metabolites in rat and human hair. With HPLC with fluorometric detection and GC–MS, LSD was detected in the hair of rats receiving doses of more than 0.05 mg/kg, whereas norLSD was detectable only in the hair of rats receiving a higher dose (2 mg/kg). The same GC–MS and HPLC assays were applied to analysis of hair from 17 self-reported LSD users and LSD was detected in only two of the samples.

The detection of fentanyl in hair has been reported by four different groups. Wang et al. [39] analyzed thirteen hair samples collected from patients following intravenous administration of 1–6 mg of fentanyl. Eight of the fentanyl patients' hair samples contained fentanyl concentrations ranging from 0.013–0.048 ng/mg of hair in the 'root' end. Selavka et al. [95] reported a case in which hair analysis was used to identify a chronic abuser of fentanyl in a State Crime Laboratory. Sachs et al. [96] reported the analysis of fentanyl and sufentanyl in human hair using GC–MS–MS and Stout et al. [97] investigated the accumulation of fentanyl in mouse hair.

5.5.2. Clenbuterol

Since the detection of clenbuterol in hair of calves was reported by Adam et al. [98] in 1994, a few studies concerning detection of clenbuterol in calf hair have been reported [37,99,100]. In most cases, hair analysis has been used for detection of illegal use of clenbuterol for meat production.

5.5.3. Nicotine

In 1985, Haley and Hoffman [101] reported an analysis for nicotine and cotinine in hair to determine cigarette smoker status. They determined the amounts of nicotine and cotinine correlated with individual smoking habits and exposures. Kintz et al. [102] evaluated the amounts of nicotine in hair of smokers and nonsmokers. The same authors [103] detected nicotine in the hair of neonates born from smoking mothers. Mizuno et al. [104] analyzed the nicotine content of hair for assessing individual cigarette-smoking behavior and found a significant positive correlation between the hair concentration of nicotine and the number of cigarettes smoked. Balabanova et al. [105] found nicotine in the scalp hair of naturally mummified bodies from the Christian Sayala (Egyptian–Nubian). Eliopoulos et al. [106] investigated hair concentrations of nicotine and cotinine in women and their newborn infants. They detected hair mean concentrations of 19.2 ng/mg for nicotine and 6.3 ng/mg for cotinine in smoking mothers, which were significantly higher than the

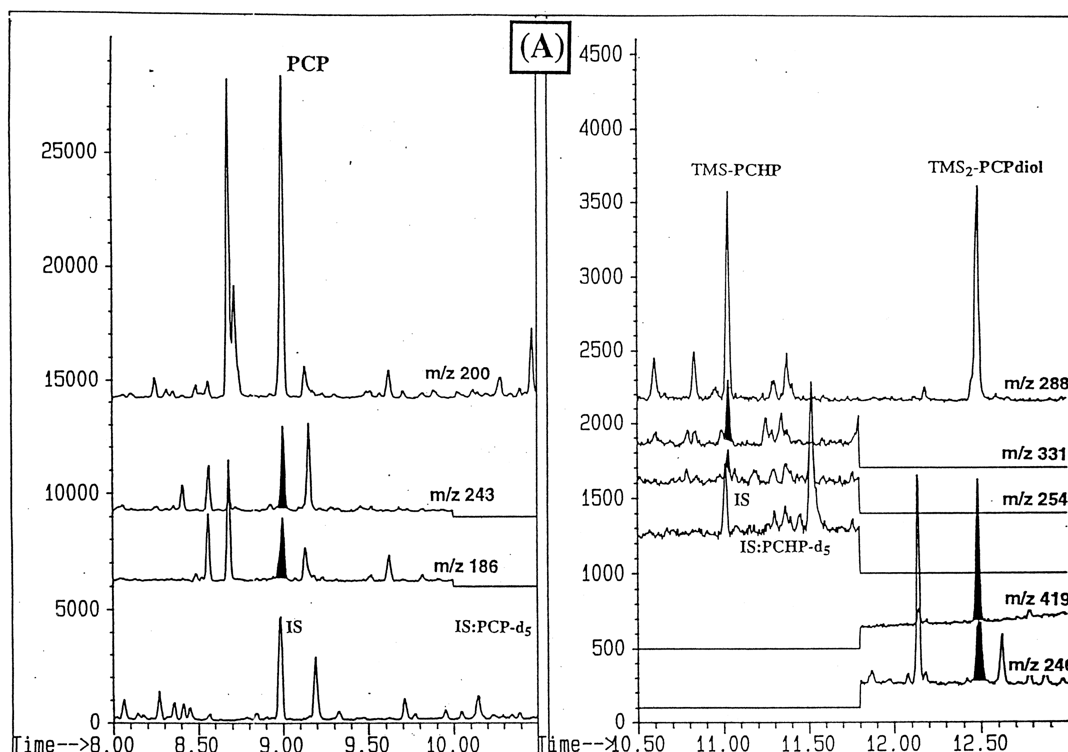

Fig. 3. GC–MS chromatograms obtained from the hair of two PCP abusers, Detection of (A) 6MAM (6.2 ng/mg) and morphine (1.8 ng/mg) in Section 6 (14–16 cm from the root) of ST-5 hair and (B) 6MAM (14.1 ng/mg) and morphine (3.2 ng/mg) in Section 7 (16–18 cm from the root) of ST-2 hair. The peaks of the selected sub-ions are expressed as black peaks. (Reproduced with permission of the authors of Ref. [93].)

concentrations found in nonsmokers (1.2 ng/mg for nicotine and 0.3 ng/mg for cotinine). They also detected mean concentrations of 2.4 ng/mg of nicotine and 2.8 ng/mg for cotinine in infants of smoking mothers, which were significantly higher than concentrations in infants of nonsmokers (0.4 ng/mg for nicotine and 0.26 ng/mg for cotinine). Nilsen et al. [107] investigated passive smoking using hair from smokers and nonsmokers who had been exposed in a dynamic exposure chamber to an atmosphere containing nicotine. They concluded that environmental nicotine is the dominating contributor to the overall nicotine found in hair both from smokers and nonsmokers from the hair segment analysis of nicotine. However, Zahlsen and Nilsen [108] demonstrated significant differences in the concentrations of nicotine in hair from smokers and nonsmokers. Gerstenberg et al. [109] showed nicotine and cotinine accumulation in pigmented and nonpigmented rat hair and found the nicotine concentration to be approximately 20 times higher in pigmented than in nonpigmented hair. Nafstad et al. [110] reported a practical and valid method for the detection of nicotine in hair to estimate environmental tobacco smoke exposure in children. Gwent et al. [111] reported time course of appearance of cotinine in human beard hair after a single 30-min buccal administration of nicotine as a chewing gum. Cotinine levels in beard peaked on day 5, and cotinine was not detected after day 7. Uematsu et al. [112] reported nicotine content along the hair shaft as an indicator of changes in smoking behavior. Pichini et al. [113] evaluated extraction procedures and examined the effect of hair treatments on nicotine and cotinine levels in hair.

There are several reports on hair analysis of nicotine in infants' hair for measuring exposure to environmental tobacco smoke (Pichini [114], Naf-

stad [110]). Knight et al. [115] revealed the racial differences in systemic exposure to cotinine by hair and urine analysis. Zahlsen et al. [116] investigated interindividual differences in hair uptake of air nicotine and showed that the number of cigarettes smoked was a poor indicator for the estimation of individual exposure to environmental tobacco smoke. Dimich–Ward et al. [117] and Jaakkola and Jaakkola [118] reported hair analysis in the work place for assessment of exposure to environmental tobacco smoke (ETS). They concluded that nicotine measured in hair was useful as a biological marker for exposure to ETS from multiple sources.

5.5.4. Hair analysis for therapeutic drugs

5.5.4.1. Benzodiazepines. In 1992, Sramek et al. [119] reported the detection of diazepam with RIA and in 1993 Kintz and Mangin [120] detected diazepam and oxazepam from the hair of 11 neonates. The detection of nordiazepam [121], oxazepam [122], flunitrazepam [122,123] and 7-aminoflunitrazepam [122,123] in human hair by GC–NCIMS has also been reported. Hold et al. [124] developed a new method for detection of alprazolam in hair with GC–NCIMS and applied it to the determination of the hair of rats administered with alprazolam. Yegles et al. [125] detected nordiazepam, diazepam, oxazepam and flunitrazepam in the hair obtained from 21 corpses that had died from an overdose of drugs. The concentrations found ranged from 1.8 to 9.5 ng/mg.

5.5.4.2. The other therapeutic drugs. The Uematsu group reported several papers [41,127–129] regarding the hair analysis of haloperidol as evidence of individual dosage history. They also applied their method to antimicrobial quinolones [130–134]. In 1954, the detection of barbiturates in the hair of guinea pigs was reported [135]. Smith et al. [136] reported the detection of phenobarbital in hair for the purpose of forensic interests. Goulle et al. [137] pointed out that phenobarbital in hair yields good information over a long period, especially when blood collection has not been made. Gygi et al. [138] showed from an experiment using pigmented and nonpigmented rat hair that hair concentrations of phenobarbital are not affected by pigmentation. Fujii et al. [139] found the usefulness of a kind of protease, Biopurase, for the digestion of hair and recovery of phenobarbital and phenytoin. Mei and Williams [46] reported the simultaneous determination of phenytoin and carbamazepine in human hair by HPLC for testing the compliance of patients during anti-epileptic therapy. Saris et al. [140] reported the HPLC determination of carbamazepine and metabolites in human hair for compliance and a long term change regarding pharmacokinetics. Pragst et al. [141] successfully analyzed amitriptyline, clomipramine, doxepine, imipramine and maprotiline as well as their nor-metabolites in the hair samples of 56 patients undergoing permanent treatment with tricyclic antidepressants. Wilkins et al. [17] developed a quantitative determination of l-α-acetylmethadol (LAAM) and its demethylated metabolite in hair using positive chemical ionization GC–MS. Couper et al. [126] showed in their paper that the alkaline digestion procedure was significantly more effective in recovering a range of antidepressants and antipsychotic drugs from hair than either the acidic, methanolic or enzymatic treatments. Gaillard and Pepin [142] detected thiopental along with other drugs from polydrug abuser's hair and also identified lidocaine as a case report of an unusual use of lidocaine during episodes of self mutilation. Williams et al. [143] and Tsatsakis et al. [144] reported the usefulness of hair analysis of carbamazepine as a potential index of therapeutic compliance in the treatment of epilepsy. Frost et al. [145] succeeded to enantioselectively determine methadone and its primary metabolite in hair by capillary electrophoresis.

6. Stability of drugs in hair

The Potsch and Skopp's group have studied the stability of drugs in hair under various conditions [23,146,147]. They found [23] that after storage of natural hair in soil or in water for 4 weeks, the opiate levels had dramatically decreased. They also showed [146,147] that the opiate concentrations in hair decreased by both bleaching and permanent waving. Jurado et al. [148] also reported the influence of cosmetic treatment on hair for drug testing of cocaine, opiates, cannabinoids and nicotine. Their data showed that bleaching produced greater decreases than dyeing.

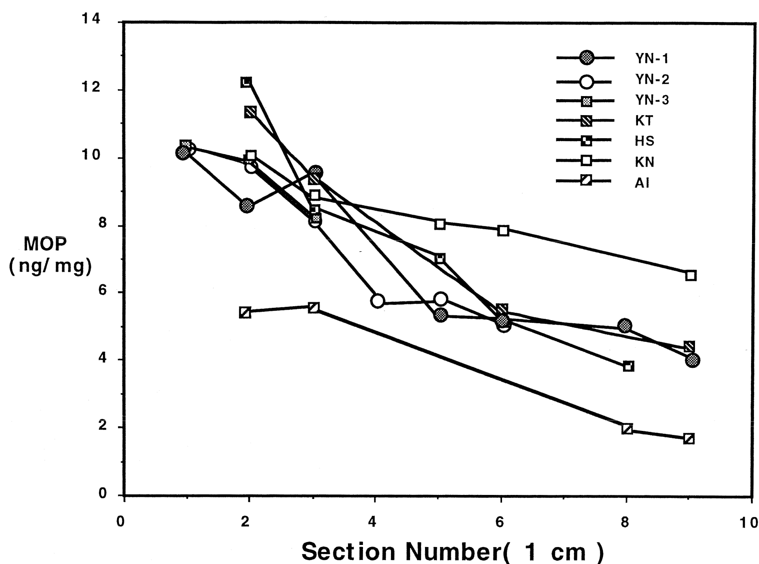

Fig. 4. Decay of the concentration of methoxyphenamine in the living hair over 5 months. All subjects took the same oral dosage of MOP (50 mg×7). Only bands entirely in one (1-cm) section were selected; drug bands divide into two sections were omitted. (Reproduced with permission of the authors of Ref. [77].)

Nakahara et al. [77] investigated the decay of MOP levels in hair according to forward movement of drug bands. Only the drug bands entering into a specific 1 cm section were selected. In cases of the same dose, the MOP level at the root side was highest and at the distal side was lowest. MOP levels in the band produced by 7 days-doses decreased to approximately 50% of the original levels after 5 or 6 months (Fig. 4). It is well known that the hair shaft is gradually damaged by various environmental factors. Therefore, it can be assumed that drugs in hair may gradually leak out from the damaged hair by washing, or would gradually be decomposed over long periods, due to the effect of ultraviolet light or heat.

7. Drug incorporation rates into hair (ICR)

7.1. Relationship between AUC or dosage and drug concentration in rat hair

Nakahara et al. [73] have proposed that the ratio of hair concentration to plasma AUC should be used as an index of drug incorporation tendency into hair in order to understand drug–hair incorporation mechanisms. They defined the ratio of hair concentration to plasma AUC under a definite condition as the drug incorporation rate into hair (ICR) and demonstrated that there are big differences of ICRs between 20 drugs; that is, there was a 3600-fold difference between the highest ICR which was for cocaine and the lowest one which was for 11-nor-tetrahydrocannabinol-9-carboxylic acid (Fig. 5).

In order to show the ICR of each drug is constant, regardless of its AUC or dose, it is important that drug concentrations in hair well correlate with their AUC. Some papers [56,149,150] have indicated that there are good correlations between AUCs (or doses) and drug concentrations in hair under the controlled animal experiments.

However, the relationship between dose and hair concentration in drug abusers' hair has not been recognized because the doses used are unclear and the drug metabolism rate of each drug user is highly variable, as well as the pharmacokinetics and pharmacodynamics. Further, labile compounds like heroin and cocaine can be hydrolyzed even in hair for long duration [66].

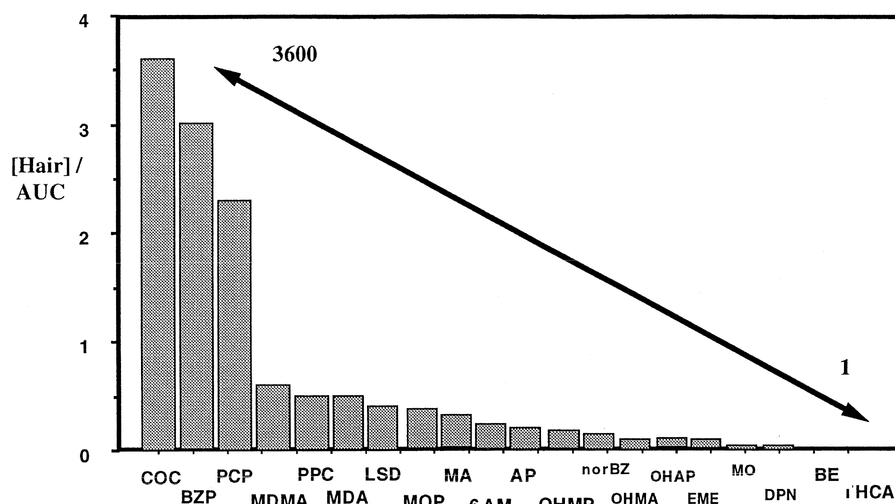

Fig. 5. The drug incorporation rates into hair of 20 drugs of abuse and related. The drug incorporation rates into hair mean the ratios of the rat plasma AUCs to the rat hair concentrations after intraperitoneal administration of drugs usually at 5 mg/kg. The rat hair concentrations were obtained from each hair of equal length which newly grows for 28 days. (Reproduced with permission of the authors of Ref. [73].)

8. Application

8.1. Relationship between drug history and drug distribution in hair

8.1.1. Behavior of drug in hair

Nakahara et al. [78] investigated the movement of methoxyphenamine (MOP) along the hair shaft at the rate of hair growth and the stability of drugs in hair for several months. It was demonstrated that the drug incorporated into the hair moved along hair shaft at a rate of approximately 3 mm/week according to hair growth without any diffusion as shown in Fig. 6. When drug bands were extrapolated according to the sections in which drugs were detected, the drug bands corresponding to 7-days dosage were approximately 6 mm in width which is equivalent to about 14 days-hair growth. The same group [79] also investigated the time course of excretion of MOP into beard hair. The concentration of MOP increased until the day after the last dosage. After the discontinuance of administration, the concentration of MOP in beard gradually decreased. The drug band of 7 days-dosage is estimated to be approximately 6 mm, which means that in this case, it took about 7 days after the discontinuance of the drug use for the clearance of drugs from the hair root to the hair shaft.

8.1.2. Confirmation of past drug use by hair analysis

In 1997, Baumgartner et al. [20] showed the possibility of sectional hair analysis to prove the drug history of a drug addict. The Nakahara group [18,22,77] also compared the drug distribution in hair obtained from the sectional analysis of drug abusers' hair with their self-reported drug histories of methamphetamine- and heroin-abuse. The results of sectional analysis well agreed with the self-reported drug histories.

8.2. Discrimination between OTC drug use and illegal MA use

Using pigmented hair rats administered with amphetamine-like OTC drugs, such as deprenyl (DPN), benzphetamine (BZP) and fenethylline (FNT), the Nakahara group [151–153] showed that hair samples were more useful for distinguishing legitimate drug use from illegal drug use than urine samples. From the hair of rats dosed with DPN, BZP or FNT, the parent or unique compounds were clearly identified

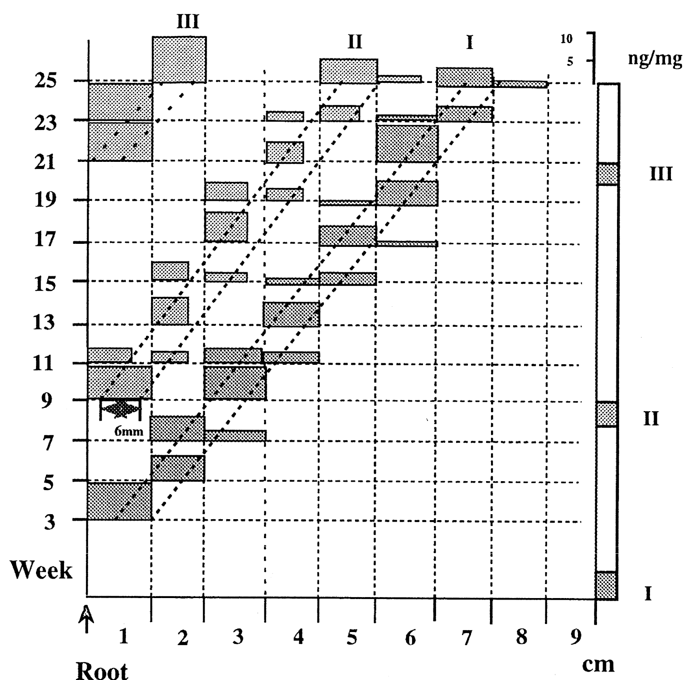

Fig. 6. Movement of each drug-band at every 2 weeks over 6 months. A volunteer subject orally took 50 mg of MOP once a day for 7 days on week 1, 8, 20. About 50 hairs were pulled out on a first day of every two weeks from week 4 to week 25. Each drug band was extrapolated according to the sections in which drugs were detected. (Reproduced with permission of the authors of Ref. [79].)

in the hair samples. For discrimination between the use of OTC drugs and methamphetamine/amphetamine, chiral analysis and comparison of the ratios of metabolite to parent drug in hair were found to be very useful.

8.3. Hair root analysis for acute methamphetamine poisonings

In the field of drug testing, generally hair has been supposed to be a specimen for proving retrospective use from a few days to months. Moreover, it had been thought that hair was not a useful specimen for the detection of acute poisoning. Gygi et al. [150] reported that codeine was found in the hair root an hour after the administration. Nakahara et al. [154] evaluated hair root as a specimen for proving acute drug poisonings using an animal model and fatal cases of MA and MDMA intoxication. From all samples including 5 min after administration, MA and MDMA were detected at high concentrations (~150 ng/mg) with a small amount of desmethyl metabolites. They demonstrated that most of the drugs incorporated into the hair root are still not immobilized at the early stages. In the analysis of the hair roots of four men who died mainly due to the acute poisoning of MA, the results suggested that the hair root is a good specimen for proving acute drug poisoning.

8.4. Gestational drug exposure

8.4.1. 'Cocaine baby'

The Klein and Koren group [9,33,66,155] have reported many cases of hair analysis for 'cocaine babies'. Klein et al. [156] studied hair analysis as a biological marker of intrauterine exposure to cocaine. Their data suggested that both maternal and fetal accumulation of cocaine and its metabolite follow a linear pattern within the regularly used doses. Similarly, they observed a good correlation in animals between maternal dose and fetal hair ac-

cumulation. Chiarotti et al. [13] evaluated cocaine abuse in pregnancy through toxicological analysis of hair from 123 pathological neonates admitted in an intensive care division. DiGregorio et al. [157] investigated the prevalence of cocaethylene in the hair of pregnant women. Sallee et al. [158] compared the neonate hair level to head growth in cocaine-exposed infants.

8.4.2. 'Methamphetamine baby'

'Methamphetamine (MA) babies' in Japan have been confirmed by hair analysis [159,160]. In one of these papers from Nakahara's group [159], MA was detected from all 12 sections (every 1 cm from the root side) of mother's hair at concentrations ranging from 4.0 to 83.8 ng/mg. Also from the baby's hair, MA was detected at 1.0 to 3.0 ng/mg in three sections. Although the MA concentrations in the baby's hair were much lower than that in mother's, the relationship between the sections and the concentrations was perfectly parallel (Fig. 7).

8.4.3. Other gestational drug exposure

Kikura and Nakahara [160,161] also confirmed methylephedrine, dihydrocodeine, caffeine and chlorpheniramine in neonate hair of mother who were abusing BRON syrup containing these drugs during pregnancy. Eliopoulos et al. [162] investigated the influence of pregnant women's smoking habit on their newborn infants using hair analysis. Samperiz et al. [163] detected opiates, cocaine and cannabinoids in the hair samples of 10 infants born from drug-addicted mothers.

8.5. Drug compliance

The compliance of patients to doctor prescription is of great medical importance. Uematsu et al. [164] reported the application of hair analysis to compliance study of 40 patients who had received haloperidol for 4 months. Saris et al. [140] confirmed the compliance of the anti-epileptic drug carbamazepine (CBZ) by hair analysis and found that sectional hair analysis of a patient on a constant dosage of CBZ showed an exponential decrease in hair concentrations of CBZ and its metabolite with increasing distance from the root. An HPLC assay for the simultaneous measurement of phenytoin and carbamazepine in human hair was used to assess therapeutic compliance in a population of patients with epilepsy [46]. Pragst et al. [141] investigated the long-term

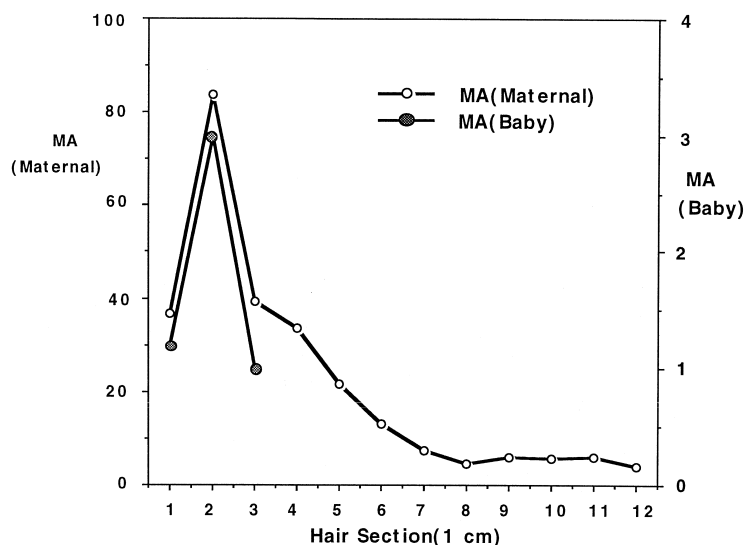

Fig. 7. The MA concentrations in each segment (1-cm) of maternal hair (12 cm) and neonatal hair (3 cm). The hair samples were collected at childbirth. (Reproduced with permission of the authors of Ref. [159].)

compliance of 56 patients, who were under permanent treatment with tricyclic antidepressants, and detected amitriptyline, clomipramine, doxepine, imipramine and maprotiline as well as their nor-metabolites in hair.

9. Promising prospects of hair analysis

In the early stages of hair analysis, it was thought that it was very hard to detect a few ng or sub-ng amounts of drugs contained in hair. We can now detect and determine 0.01 ng/mg or less of drug in hair by GC–MS or LC–MS. Depending on the further development of the analytical instrumentation, we may expect the detection of a few pg/mg or less of drugs in hair in the near future. Therefore, it is clear that we will be able to extract more of the information implicated in hair in the future. So far, the target drugs in hair are mostly drugs of abuse, but in future other chemicals could also be targeted; agricultural chemicals (insecticides, herbicides, etc.), food additives, carcinogens, doping agents (steroids, diuretics, etc.), environmental toxicants, all kinds of therapeutic drugs, nutrients and so on.

A well-known weak point of hair analysis is the problem of making intact hair standards containing precise concentrations of drugs. The question always remains whether drugs were quantitatively extracted from real hair samples or not, although they are easily extracted from control hair samples spiked with them. Thus, this matter makes the standardization of procedures and the comparison of extraction and washing methods difficult. Therefore, it is necessary to develop a way to make hair standards containing precise concentrations of drugs. With the hair standards, a suitable method for each drug may be chosen.

Furthermore, in order to correctly interpret the results obtained from hair analysis more basic studies on the relationship between hair and drugs may be required. When the mechanism of drug incorporation into hair and the drug metabolism and disposition in hair including the pharmacokinetics and pharmacodynamics are well studied, hair testing will occupy an important position in drug testing along with urine and blood testing.

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