HAIR ANALYSIS FOR DRUGS OF ABUSE

By

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A thesis submitted to the University of Central Lancashire in fulfillment of the requirement for the degree of PhD (by Published Work)

School of Forensic and Investigative Sciences

University of Central Lancashire

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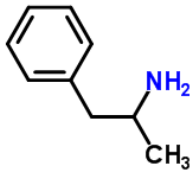
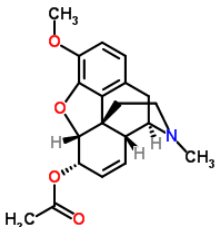
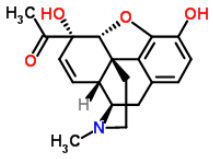
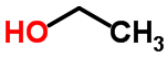
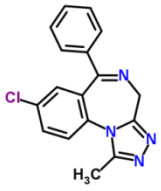
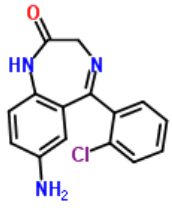
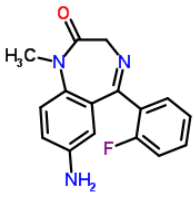
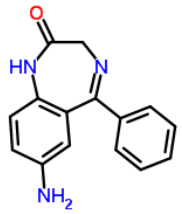
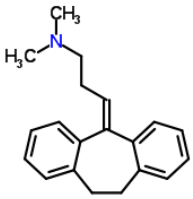
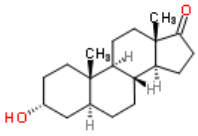
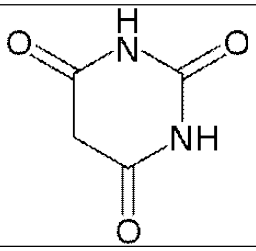
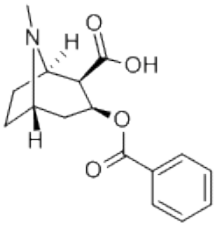
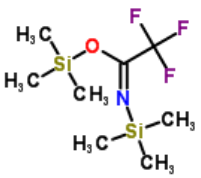
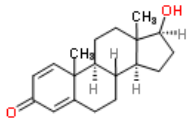
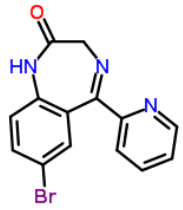
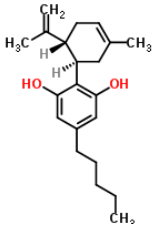
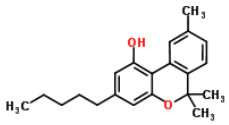
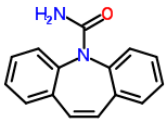
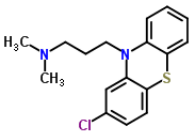
Compounds	Chemical Structures
Amphetamine	
Acetylcodeine	
6-Acetylmorphine	
Alcohol	
Alprazolam	
7-aminoclonazepam	

7-aminoflunitrazepam	
7-Aminonitrazepam	
Amitriptyline	
Androsterone	
Barbiturate	
Benzoylecgonine	
N,O-Bis(trimethylsilyl) trifluoroacetamide	

Boldenone	
Bromazepam	
Cannabidiol	
Cannabinol	
Carbamazepine	
Chlorpromazine	
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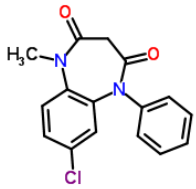
Clobazam	
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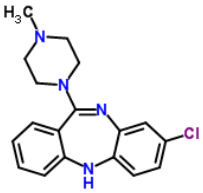
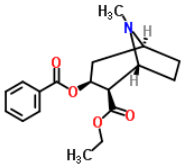
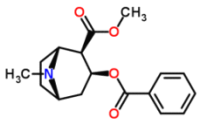
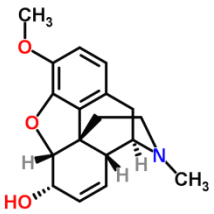
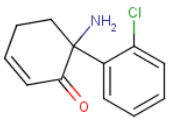
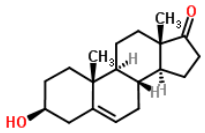
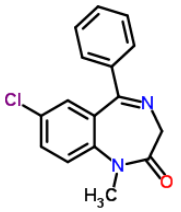
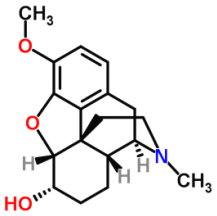
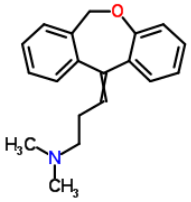
Clozapine	
Cocaethylene	
Cocaine	
Codeine	
5, 6- Dehydronorketamine	

Dehydroepiandrosterone	
Diazepam	
Dihydrocodeine	
Doxepin	
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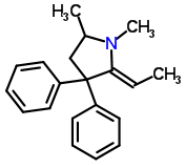
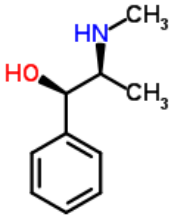
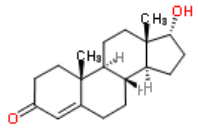
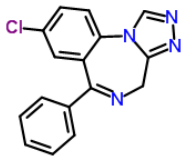
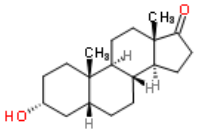
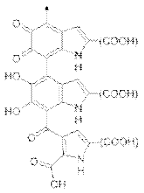
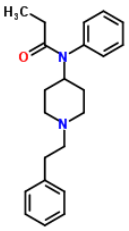
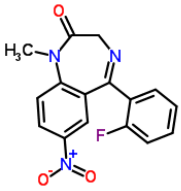
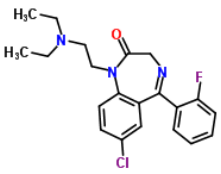
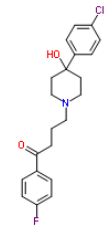
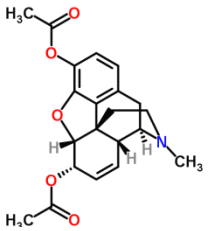
EDDP	
Ephedrine	

Epitestosterone	
Estazolam	
Etiocholanolone	
Eumelanin	
Fentanyl	
Flunitrazepam	
Flurazepam	

Haloperidol	
Heroin	
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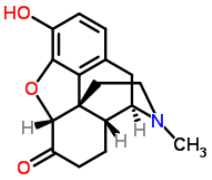
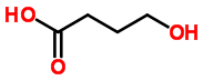
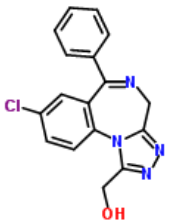
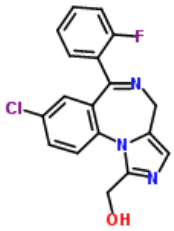
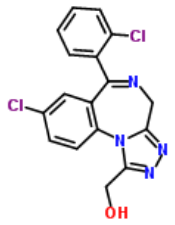
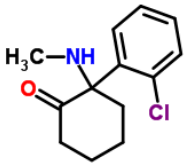
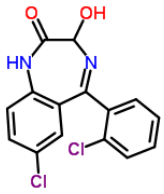
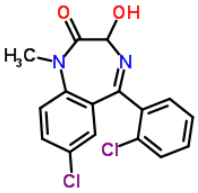
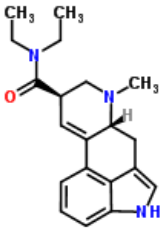
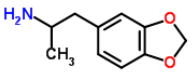
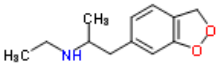
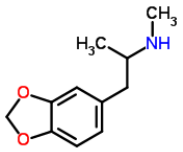
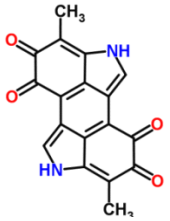
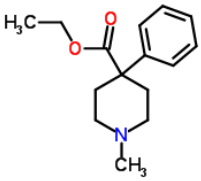
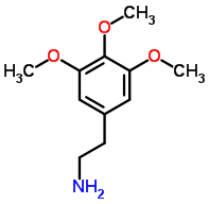
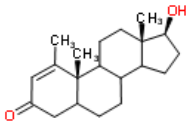
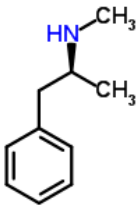
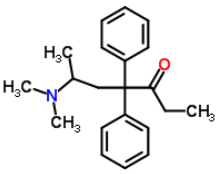
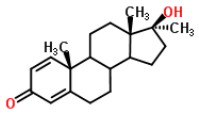
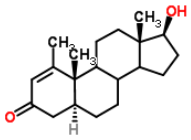
Hydromorphone	
γ-Hydroxybutyric acid	
α-Hydroxyalprazolam	
α-Hydroxymidazolam	

α-Hydroxytriazolam	
ketamine	
Lorazepam	
Lormetazepam	
LSD	
MDA	
MDEA	

MDMA	
Melanin	
Meperidine	
Mescaline	
Metenolone	
Methamphetamine	
Methadone	

Methandienone	
Methenolone	
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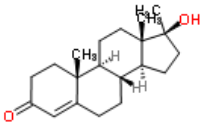
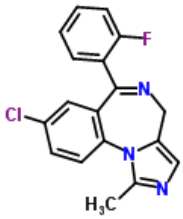
17α-Methyltestosterone	
Midazolam	
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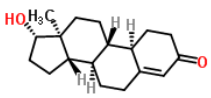
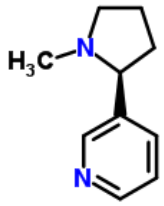
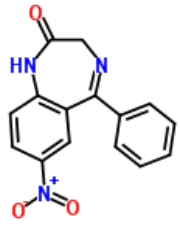
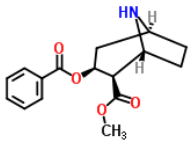
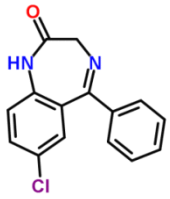
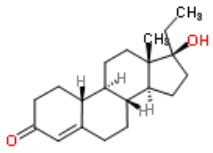
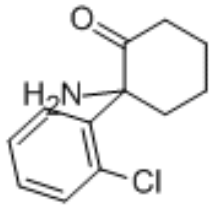
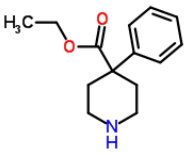
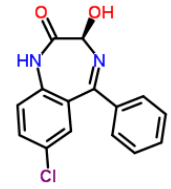
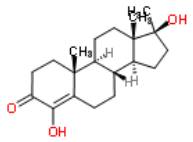
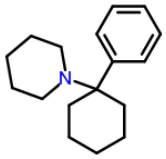
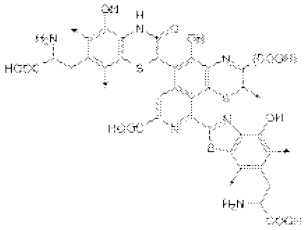
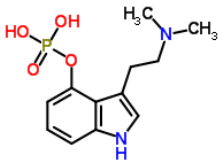
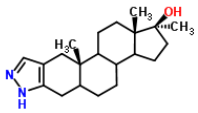
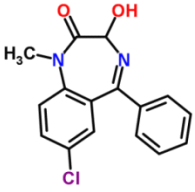
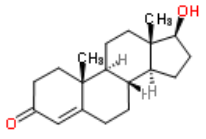
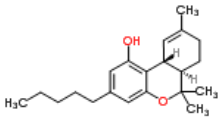
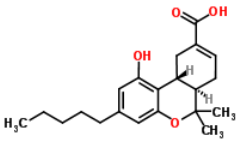
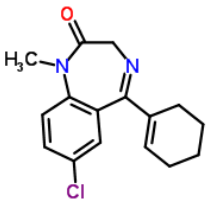
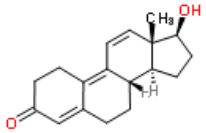
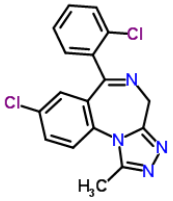
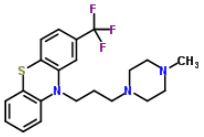
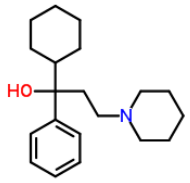
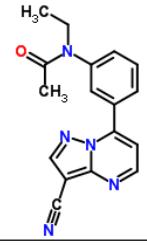
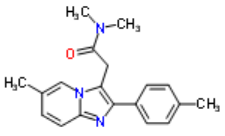
Nandrolone	

Nicotine	
Nitrazepam	
Norcocaine	
Nordiazepam	
Norethandrolone	
norketamine	
Normeperidine	

Oxazepam	
Oxymesterone	
Phencyclidine	
Pheomelanin	
Psilocybin	
Stanozolol	
Temazepam	

Testosterone	
Δ^9-Tetrahydrocannabinol	
Δ^9-Tetrahydrocannabinol-9-carboxylic acid	
Tetrazepam	
Trenbolone	
Triazolam	
Trifluoperazine	

Trihexyphenidyl	
Zaleplon	
Zolpidem	

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Many thanks to my colleagues Zhuo Xianyi, Liu Wei, Shen Baohua, Bu Jun, Ma Dong, and Yan Hui and to several former postgraduate (MSc, MPhil and PhD) students Jiang Yan, Wang Mengye, Sun Qiran, and Shi Yan. My thanks and appreciations are also extended to the technical and secretarial staff members of the University of Central Lancashire.

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Finally, I am indeed grateful to my husband, Xu Che, my son Xu Jingtian, and my parents for their tolerance, understanding and support during the course of my research work over the several years and preparation of this thesis.

DECLARATION

I declare that this thesis has been composed by myself and while registered as a candidate for the degree of PhD (by Published Work) to which this submission is made, I have not been a registered candidate for any other higher degree at this or any other University or other institution of learning. I also declare that I am the first author for eleven publications (Pub 1, 6, 9, 10, 11, 12, 13, 16, 23, 26, and 27) and a joint author for the rest of the submission. I declare that I have participated fully in all joint publications submitted in the thesis. My participation included original ideas and working hypotheses, awards of several projects grants and post-graduate studentships, supervision of 6 postgraduate research students, experiential designs, literature search, experimental work, analysis, and interpretation of data, preparation and presentation of the data, writing, correcting, and submission of manuscripts for publication purposes.

DEDICATIONS

This thesis is dedicated to my family, my husband, Xu Che, my son Xu Jingtian, my parents, and all my research collaborators, especially Professor Shen Min.

Index of Publications on Hair Analysis for Drugs of Abuse

1. **Xiang Ping**, Shen Min, Wu Hejian, Huang Zhongjie. Determination of heroin matabolites in biological fluids, tissues and hair of addicts using GC/MS-SIM. *Journal of Chinese Mass Spectrometry*. 1999, 20(3): 73-74
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4. Jiang Yan, Shen Min, Ziqin Zhao, Yonghong Ye, **Xiang Ping**, Shen Baohua. Study of distribution and metabolism of methamphetamine in hair of guinea pig. *Journal of Forensic Medicine*. 2001, 17(4):214-217
5. Shen Min, Wu Hejian, **Xiang Ping**, Shen Baohua, Huang Zhongjie. Detection of psychotropic drugs and their metabolites in human hair. *Journal of Chinese Mass Spectrometry*. 2001, 22(1): 32-39
6. **Xiang Ping**, Shen Min, Shen Bao-hua, Liu Wei, Bu Jun, Wu Hejian. Simultaneous quantification of cannabinoids and the major metabolite, THC-COOH in human hair. *Journal of Forensic Medicine*. 2002, 18(4): 216-219
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9. **Xiang Ping**, Shen Min, Shen Bao-hua, Bu Jun, Liu Xiaoqian, Jiang Yan, Zhuo Xianyi. Analysis of Ketamine in Hair. *Journal of Forensic Medicine*. 2005, 21(4): 290-293
10. **Xiang Ping**, Shen Min, Shen Bao-hua, Ma Dong, Bu Jun, Jiang Yan, Zhuo Xianyi. Determination of opiates in biological human samples by liquid chromatography-tandem mass spectrometry. *Journal of Forensic Medicine*. 2006, 22(1): 52-54
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12. **Xiang Ping**, Shen Min, Shen Bao-hua, Ma Dong, Zhuo Xianyi. Simultaneous Screening for 59 Drugs of Abuse in Biological Samples by LC-MS/MS-MRM. *Journal of Chinese Mass Spectrometry*. 2006, 27(4): 237-241
13. **Xiang Ping**, Shen Min, Zhuo Xianyi. Hair analysis for ketamine and its metabolites. *Forensic Sci Int*. 2006,162: 131-134
14. Yan Hui, Shen Min, **Xiang Ping**, Shen Baohua. Hair analysis of anabolic steroids in doping control. *Chinese Journal of Forensic Sciences*. 2006, 6:21-25
15. Yan Hui, **Xiang Ping**, Wang Mengye, Shen Min. Analysis of 10 Kinds of Anabolic Androgenic Steroids in Human Hair by High Performance Liquid

Chromatography-Electrospray Tandem Mass Spectrometry. *Chinese Journal of Analytical Chemistry*. 2007, 35(7): 949-953

16. **Xiang Ping**, Shen Min, Shen Baohua, Yan Hui. Determination of stannozolol in hair using liquid chromatography tandem mass spectrometry. *Chinese Journal of Chromatography*. 2008, 26(4): 469-472
17. Shen Min, **Xiang Ping**, Shen Baohua, Wang Mengye. Determination of endogenous anabolic steroids in human hair by gas chromatography–tandem mass spectrometry. *Chinese Journal of Chromatography*. 2008, 26(4): 454-459
18. Sun Qiran, **Xiang Ping**, Yan Hui, Shen Min. Determination of cocaine and benzoylecgonine in guinea pig's hair after a single dose administration by LC-MS/MS. *Acta pharmaceutica Sinica*. 2008, 43 (12) : 1217-1222
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23. **Xiang Ping**, Shen Min, Zhuo Xianyi. Application of liquid chromatography-mass spectrometry in drugs and poisons analyses. *Shanghai scientific & Technical Publishers*. Shanghai, China. 2009
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26. **Xiang Ping**, Sun Qiran, Shen Baohua, Shen Min. Evaluation of segmental hair analysis after a single dose of benzodiazepines. *Forensic Sci Int*. 2011. 204:19–26
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Abstract

The work presented in this thesis involved the development of a series of analytical methods to detect trace amounts of drugs in hair and also investigated the mechanisms by which drugs may be incorporated into hair. The major areas covered in this study can be summarized as follows:

1. The methods for the identification and quantification of opiates, amphetamines, ketamine, cannabis, cocaine, benzodiazepines, antidepressants, antipsychotics, and anabolic steroids in hair were developed using gas chromatography–mass spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry (LC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS). With GC-MS methods, the limits of detection were 0.1-0.5 ng mg^{-1} of hair for antidepressants and antipsychotics. For illegal drugs, hair specimens were analyzed by GC-MS with limits of detection of 0.02-2ng mg^{-1}. GC/MS/MS is more sensitive than GC-MS to detect these drugs in hair. The lower limits of detection ranged from 0.001 to 0.020 ng mg^{-1} for 21 anabolic androgenic steroids and their esters in hair using liquid chromatographic-tandem mass spectrometric method. And the limits of detection ranged from 0.2 to 5 pg mg^{-1} for benzodiazepines in hair. Tandem mass spectrometry is characterized by its sensitivity, selectivity and specificity, which makes it particularly suitable for the analysis of trace amount of target analytes in hair.

2. Usually, screening for drugs of abuse is the first step in clinical and forensic

toxicology. There are a large number of controlled substances and doping agents and novel compounds, which have yet to be characterised. A series of screening methods for drugs of abuse in hair were developed using LC-MS/MS and GC-MS/MS. Using our own library of MRM transitions, the optimum collision energies selected for each transition and retention times were set up. These methods have been applied successfully in forensic casework.

3. Of growing importance to the field of hair analysis is the detection of metabolites related to the parent drugs. Demonstrating the presence of a metabolite of a drug (such as, heroin, amphetamines, cocaine, meperidine, ketamine, triazolam or psychotropic drugs) provides compelling evidence for exposure to the parent drug, and permits distinction between external contamination from ingestion and facilitation of the interpretation of results. The presence of antidepressant and antipsychotic drugs and their metabolites in the hair of psychiatric patients was investigated using GC-MS-EI and GC-MS-PCI. The parent drug and its major metabolite, such as opiates (morphine, 6-acetylmorphine), methamphetamine (methamphetamine, amphetamine), ketamine (ketamine, norketamine), cocaine (cocaine, benzoecognine), meperidine (meperidine, normeperidine), triazolam (triazolam, α-hydroxytriazolam), and clonazepam (clonazepam, 7-aminoclonazepam) were quantified in authentic hair samples simultaneously. The differences were finding in the ratio of parent drug to metabolite. For illegal drugs, the concentrations of parent drugs were higher than that of their metabolites. The results of triazolam and clonazepam were contrary. These data are suitable reference values and are the basis for the interpretation of results.

4. The mechanisms by which drugs are incorporated into hair are not fully understood. Based on experiments with guinea pigs with black, white, or brown hair, the mechanisms of incorporation of cocaine, methamphetamine, ketamine, triazolam and anabolic steroids into hair were investigated. The concentrations of drugs in hair were found to be related to physicochemical properties of drugs. The parent drugs were the predominant analytes in hair. There was an obvious relationship between the concentration of drugs in hair and hair pigmentation. The concentrations of drugs deposited in black hair was found to be higher than that in brown and white hair samples, even when comparing results using hairs on the same multicoloured animal body. This work confirmed that melanin affinity is a governing factor in drug incorporation into hair shafts. These studies on the distribution of drugs in the hair shaft and how their concentration changes along the shaft provide information relevant to the time of ingestion and substance use/abuse.

5. In recent years an increase in drug-facilitated sexual assault (DFSA) has been reported. Segmental hair analysis has proved useful in widening the window of detection, as blood and urine analyses are of limited use, due to the long delays between the actual assaults and obtaining samples from suspects that are frequently encountered in investigations of such crimes. In China, benzodiazepines are the most frequently observed compounds in cases of drug-facilitated crime. In a paper reported here, 14 volunteers ingested a single 1-6 mg estazolam tablet to permit the evaluation of segmental hair analysis after a single drug dosage. Hair was collected one month after administration of the drug. All the proximal segments tested positive for estazolam. With increased dosage, estazolam could be detected in the 2-4 cm

segments nearest the hair root in some subject's hair shafts. In some cases, the 4-6 cm segments also tested positive. Hair analysis was applied to samples from two authentic criminal cases. A significant variation was observed between those obtained from previous studies and the results presented here. The intersubject variability in segmental analysis can be explained mainly due to melanin content and diffusion from sweat or other secretions during formation of the hair shaft. However, more substantial procedural and interpretation guidelines are required to use segmental hair analysis in drug-facilitated crimes. On the other hand, the minimal dosage for detection, which is a critical but previously unknown threshold value of fundamental importance in hair analysis, was determined for triazolam and ketamine in guinea pig hair.

6. Doping with endogenous anabolic steroids is one of the most serious drug issues in sports today. The measurement of anabolic steroid levels in human hair permits the distinction between pharmaceutically produced steroids and naturally occurring steroids. Full-length hair samples were taken at the skin surface from the vertex of 39 males, 30 females and 11 children from China. None of the subjects were professional athletes. Testosterone and dehydroepiandrosterone were detected in all the hair segments. The physiological concentrations of testosterone were in the range 0.8-24.2 pg mg^{-1}, 0.1-16.8 pg mg^{-1} and 0.2-11.5 pg mg^{-1} in males, females and children, respectively. However, the mean values of dehydroepiandrosterone were much higher than those for testosterone. This is the first investigation into the physiological concentrations of anabolic steroids in human hair in Chinese subjects. These data provide suitable reference values and form the basis for the interpretation

of results from investigations into the abuse of endogenous anabolic steroids.

In conclusion, the work presented in this study demonstrates that there was a good correlation between the concentration of drugs in hair and drug dosage. There was an obvious relationship between hair drug concentration and hair colour. Melanin affinity is shown to be a governing factor in determining drug incorporation into hair, and the concentration of drugs deposited in black hair was found to be higher than that in brown and white hair samples. This thesis provides data that will be useful in the application of hair analysis regarding drugs of abuse and in the interpretation of toxicological results.

Introduction

This study concentrated mainly on hair analysis for drugs of abuse. Testing for drugs of abuse is important in many clinical and forensic toxicological situations, both for assessing the level of intoxication and for evaluation of the level of drug impairment. Compared to more traditional biological materials, such as blood and urine, hair has particular advantages for toxicological analysis, such as ease of collection, non-invasiveness, stability and storage of samples, and a long detection period, which can be up to several weeks, months, or even years, depending on the length and type of the hair sample. The major advantages of hair analysis are summarized below.

1) If a urine specimen is positive for the presence of drugs, hair analysis can discriminate between a single exposure and long-term use.

2) Segmental hair analysis can provide a more accurate record of drug use and be used to indicate the history and frequency of drug intake.

3) The stability of parent drugs in hair can help to confirm exogenous sources.

4) It is possible to collect subsequent hair samples and segments for reanalysis.

Hair analysis has been gaining increasing attention and recognition in forensic science. Baumgartner (1979) first reported the detection of morphine in the hair of heroin abusers using radioimmunoassay (RIA) techniques. Since then, more than 400 papers have been

published on drugs of abuse in hairs. Gas chromatography–mass spectrometry (GC-MS) was then the method of choice for detection. Now, with the progress of separation techniques and the development of tandem mass spectrometry (GC-MS/MS or LC-MS/MS) [Villain, 2005; Moore, 2007; Chèze, 2007], drugs in hair can be detected and determined at levels of pg mg^{1} of sample.

However, hair drug analysis is a complex scientific undertaking, involving hair specimen collection, decontamination, drug solubilization, analysis, quality control and interpretation of results. A great deal of research has focused on the legitimacy of drug testing and the studies of this technology comprise the accumulation of knowledge and application, criteria for analytical methods and interpretation of results, and the relationship between drug dose and hair concentration.

Structure and Growth of Human Hair

Hair is a fibrous outgrowth from the skin of mammals which grows from papillae embedded in the bases of follicles situated in the dermis or true skin. A single hair shaft is not a homogeneous fibre, but consists of keratinized cells held together by the cell membrane complex, and this arrangement forms three concentric radial structures in hair shafts: cuticle, cortex and medulla (Fig. 1a) (Pragst, 2006). The pigmented cortex is responsible for the elasticity and colour of the hair, whereas the 5–10 layers of shingle-like cells of the non-pigmented cuticle are responsible for the high chemical and physical resistance properties and the glossiness or shine of the hair.

Hair originates from the hair follicle (Fig. 1b), which is located 3–5 mm below the skin surface. The hair follicle is surrounded by a rich capillary system that provides the growing hair with the necessary metabolic materials for its formation and growth. The germination center around the hair bulb papilla is formed by matrix cells (keratinocytes and melanocytes) present on the basement membrane. This association gives rise to the different hair shaft layers including the cuticle, cortex and medulla. The matrix cell cycle is one of the most rapid of all human tissues. Rapid cellular mitosis forces a migration of the upper zones in the direction of the hair root mouth. In the next higher zones the genes for formation of keratine are expressed at a high level. Cortex cells change from a spherical shape at the germinative level to a spindle-like form. Protein filaments are synthesized, which fill the cells and fuse together. In the zone of hardening, where disulphide bonding, resorption and dehydration occur, all cytoplasmic organelles disappear leaving cellular residues coupled by membrane structures.

Cuticle cells originate from matrix cells of the outer sphere of the papilla. These cells change to a shingle-like structure and contain amorphous proteins. The cell membrane complex consists of proteins and a protein–lipid complex originating from previous cell membranes. This part of hair is most vulnerable to chemical and mechanical attack and is the primary diffusion point for incorporation and elimination of drugs. As can be expected, lipophilic drugs are preferentially deposited in the cell membrane complex (Pragst, 2006).

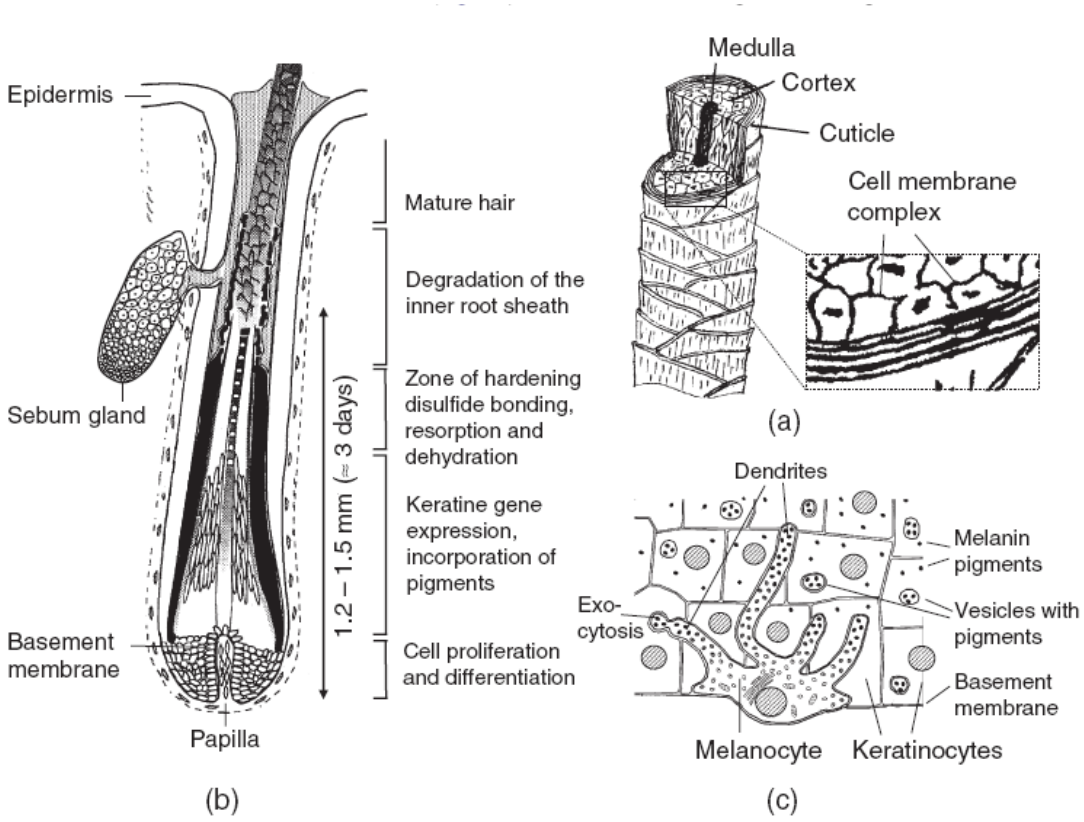


Fig. 1. a) Structure and constituents of the human hair shaft. b) Formation of hair in a follicle from matrix cells on the basement membrane to the mature hair shaft. Drug incorporation from blood should occur in a 1.2–1.5 mm zone before completion of keratinisation. c) Melanocytes on the basement membrane of the cortex synthesize melanine in melanosomes that are discharged in vesicles into the keratinocytes by an exocytotic mechanism. There, the membranes of the vesicles and melanosoms are digested and remain the melanin pigments. (Pragst, 2006)

Hair grows in a cycle composed of the anagen (active growth), catagen (transition) and telogen (resting) stages. The individual length of hairs depends on stage duration and growth rate. The average values for the above stages are 4–8 years, a few weeks, and 4–6 months, respectively. Scalp hair growth ranges 0.6–1.4 cm per month in general (Pragst, 2006). At any one time, approximately 85% of adult scalp hair is in the growing phase (anagen) with the remaining 15% in the resting phase (telogen). This high rate of hair production is the consequence of high metabolic activity and rapid cellar proliferation. Matrix cell kinetics shows that the hair matrix cell cycle is probably the most rapid of all normal tissues. In human cyclical hair growth is asynchronous and has been found to vary in different follicles from 0.1-0.45 mm per day. The rate of hair growth shows vestiges of seasonal change and is undoubtedly affected by endocrine dysfunction, metabolic and genetic disorders, nutrition, hormones and drugs which target follicular segments and interfere with the cell kinetics of human hair growth. The dynamics of the hair growth cycle vary between different species, different individuals, between different body sites in the same species and between different follicle types in the same body site (Pötsch, 1996).

Hair Pigmentation

Melanins (a diverse but related group of pigments found in skin, hair and eyes) in mammals are formed in specialized cells called melanocytes (Fig. 1c), which enclose distinct cytoplasmic organelles known as melanosomes. Pigment formation (follicular melanogenesis) takes place in the melanosomes in four stages. In the first stage, the basic structural unit consists of tyrosinase and protein, which is then followed by formation of

an inner membranous structure in which melanin is biosynthesized and accumulates. Finally, the melanosome then transforms into a uniformly dense melanin particle. The melanized melanosome is then transferred into cortical and medulla keratinocytes, which then form the pigmented hair shaft. This activity is regulated by a series of enzymes, structural and regulatory proteins, transporters, and receptors and their ligands during the anagenic stage of the hair growth cycle (Slominski, 2005). The hair bulb is the only site of pigment formation for the hair shaft. The active melanocytes, which exist in the upper hair matrix of the anagen hair follicle, transfer melanin mainly to the hair shaft cortex, to a lesser extent to the medulla, and only rarely to the hair cuticle (Kintz, 2007). Melanocytes and pigmentation play an important role in the incorporation of basic drugs into hair.

A partial scheme of the melanin synthesis is presented in Figure 2. Eumelanins are heterogeneous polymers consisting of 5, 6-dihydroxyindole (DHI) and 5, 6-dihydroxyindole-2-carboxylic acid (DHICA) units. The structural components of pheomelanins are benzothiazine, benzothiazole, and isoquinoline units. In the initial stages, cysteine is required for the synthesis of pheomelanin but not for eumelanin (Prota, 1972; Thomson, 1974).

Hair colur is genetically controlled and is among the most diverse of the pigmentation phenotypes. Four types of melanin are thought to be responsible for this diversity, namely eumelanin, oxyeumelanin, pheomelanin, and oxypheomelanin (Prota, 2000). Oxyeumelanin and oxypheomelanin are formed as oxidative products of the pigment monomer units. In this respect, black to dark brown hair contains virtually intact

eumelanin. As the intensity of brown coloration lightens, the hair is found to contain more of an oxidative breakdown product of eumelanin, namely oxyeumelanin. The oxidative process is induced by the presence of hydrogen peroxide. Hair containing large Amounts of oxyeumelanin are blond. The broad spectrum of hair color variations in Caucasians can be attributed to two pigments, eumelanin and pheomelanin, but at different stages of structural integrity (Prota, 2000).

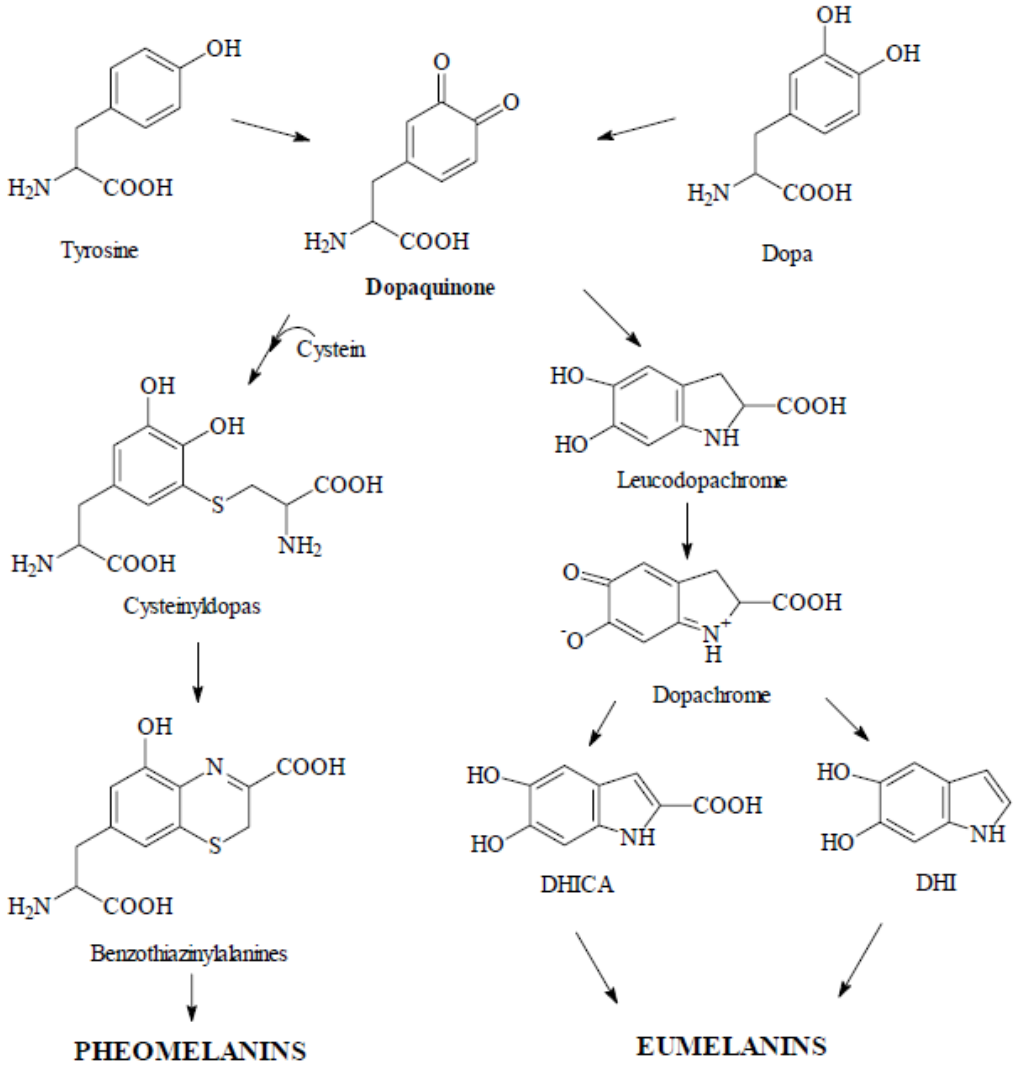


Fig. 2. Simplified scheme illustrating in vivo biosynthesis of eumelanins and pheomelanins. (Kintz, 2007)

Mechanisms of Drug Incorporation into Hair

The precise mechanisms by which drugs incorporate into hair remain unclear and need further investigation. Three models for incorporation have been proposed: drugs can enter the hair through (1) active or passive diffusion from the capillaries feeding the dermal papilla, (2) diffusion from sweat and other secretions bathing the growing or mature hair fiber, or (3) external drug deposition from vapours or powders that diffuse into the mature hair fibre (Fig. 3) (Kintz, 2007). Indeed, a combination of these three routes is probably most realistic, and a full picture of the incorporation of drugs into hair shafts is likely to involve all three to greater or lesser extents.

Three additional key chemical and physical factors, namely the melanin content of hair and the lipophilicity and the basicity of the drug itself, also influence drug incorporation (Pragst, 2006). The degree of disposition of a drug in hair closely relates to these three key factors, and Nakahara *et al*. (1995) have reported that a number of drugs have an affinity for melanin *in vitro*. Nakahara concluded that numerous factors, including pH, the presence or absence of various functional groups within the specific molecular configuration of the scrutinized drugs, and the presence of eumelanin (as opposed to pheomelanin) are important in this respect.

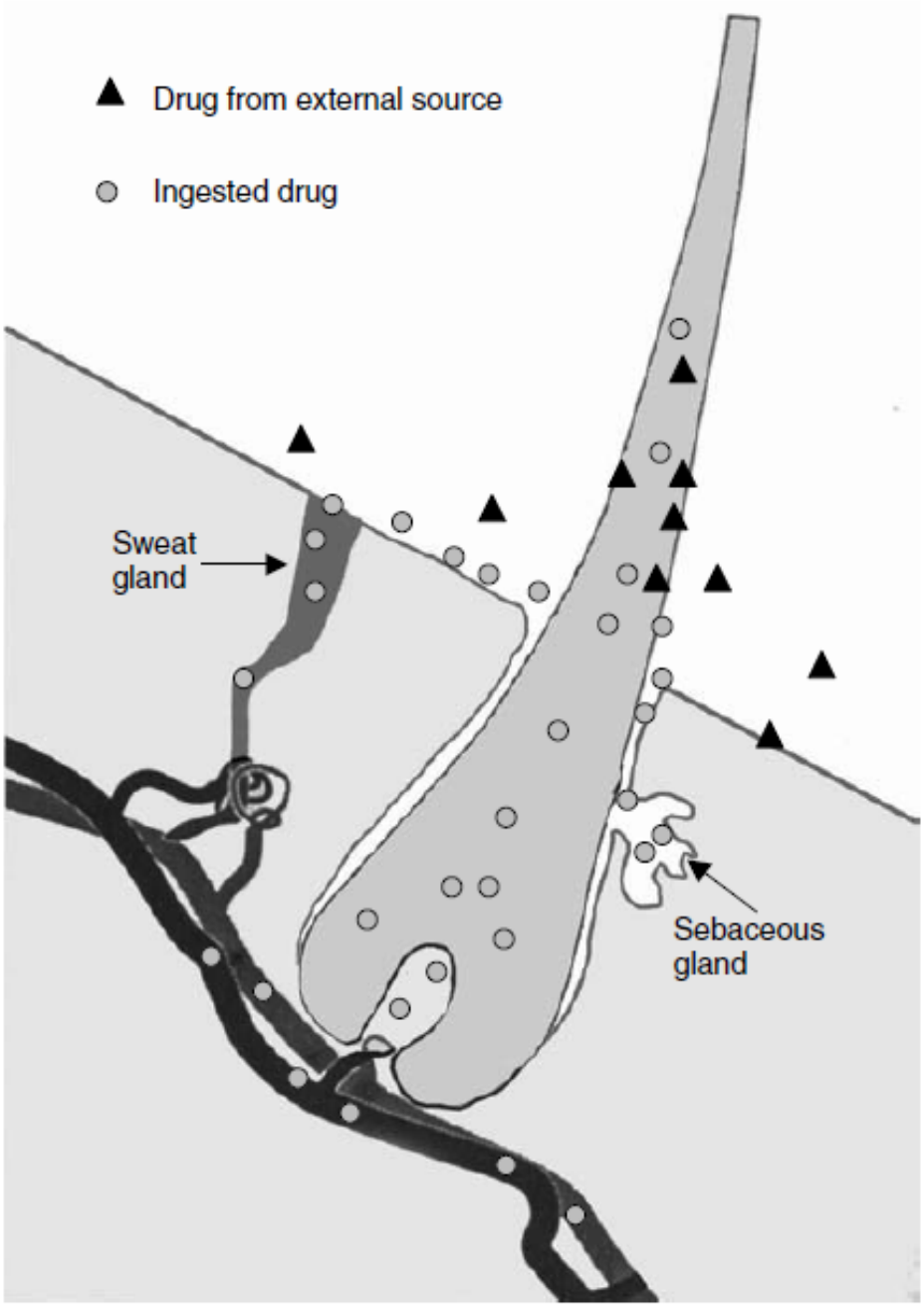


Fig. 3.Three models of drug incorporation. Ingested drugs can enter the hair from the bloodstream feeding the dermal papilla as well as by sweat and sebum bathing the mature hair fibre. External drugs from vapours or powders may also incorporate into the mature hair fibre. (Kintz, 2007)

However, the mechanism of drug incorporation into hair is very complex and still not fully clear. This cannot be the only relevant mechanism, since drugs are trapped into the hairs of albino animals, which lack melanin pigment. Another mechanism proposed is the binding of drugs with sulphydryl containing amino acids present in hair. There is an abundance of amino acids such as cysteine in hair, which forms crosslinking S-S bonds between polypeptides to stabilize the protein fibre network. Drugs diffusing into hair cells could also be bound in this way (Kintz, 2004).

Because of the ethical constraints, relevant pharmacological and toxicological assessments have to be extensively studied in laboratory animals, rather than in humans. Although there are some species similarities and differences in absorption, distribution, metabolism, and excretion with humans (Lin, 1995), scientists continue to search for an animal species in which the pattern of drugs is consistently the same as in humans. In studies to investigate the mechanisms of drug incorporation into hair, the guinea pig is usually chosen as the experimental animal because its hair growth cycle is quite similar to that of the human (Chase, 1954).

Specimen Collection

Collection procedures for hair drug analysis have not been standardised in forensic labs. In most published studies, the samples are obtained from random locations on the scalp. The recommendation of the Society of Hair Testing (2004) is to cut the sample from the posterior vertex region of the head, as close as possible to the scalp, since this is the region with the least variation in growth rate (approximately 1.0 cm $month^{1}$). In addition,

the number of hairs here in the growing phase is more constant, and the hair is less subject to age- and sex-related influences. Head hair is the preferred sample of choice but occasionally, alternative hairs such as axillary or pubic hairs have been tested. The sample size varied considerably among laboratories and ranged from one single hair to 250 mg dry weight and depends on the drug to be analysed and the test methodology. Once collected, hair samples may be stored at ambient temperature in aluminium foil, a paper envelope or a plastic tube.

Stability of Drugs in Hair

One of the advantages of hair analysis is the excellent stability of hairs over time. Cartmell *et al.* (1991) tested the scalp hair of 8 Chilean mummies with ages ranging from 2,000 BC to 1,500 AD. Benzoylecognine was found to be very stable in these samples and could be consistently found in mummy hair. However, popular hair cosmetic treatments, such as bleaching or permanent waving, were found to affect the stability of incorporated drugs. For example, commercially available bleaching as well as perming formulas (Poly Blonde Ultra@, Poly Lock@; Henkel, Dusseldorf, Germany) were applied *in vitro* to the hair strands of both groups under investigation (Potsch, 1996). After these treatments, the detectable drug concentration had decreased for hairs treated by both bleaching and permanent waving. In the spiked hair, only 2-18 % of the starting solution could be found after bleaching. About 20-30 % of the drug substances could still be detected after perming. Yegles *et al.* (2000) treated hair with a bleaching product for 20 minutes. The results obtained showed that the concentrations of all the drugs detected decreased in bleached hair in comparison with untreated hair. Repeated shampooing was

found to have no significant action on the drug content of hair. After cosmetic treatments, drug concentrations decline dramatically by 50–80% from their original concentration.

On the other hand, long-term effects of weather (sunshine, rain, wind) may also cause damage to the hair shaft, with subsequent impacts on detectable drug concentrations (Skopp, 2000).

Hair Analysis

Hair analysis has become routine in forensic toxicology. In 2004, the Society of Hair Testing (SOHT) published new recommendations for hair testing in forensic cases. Because of potentially serious consequences that may arise from inappropriate procedures, the analyst assumes a high responsibility for obtaining a correct result. Therefore, the whole process, from sampling to result interpretation, must be well organized and precisely performed to avoid any potential error. Pragst (2006) suggested practical steps of hair analysis as below (Fig. 4).

Segmental Analysis of Hair

Segmental hair analysis can provide important information with respect to time course and frequency of drug use. For example, in date-rape cases and those cases involving criminal poisonings, the detection of a particular drug in a hair segment corresponding to the time when the intake was alleged to have occurred may give information important for the police investigation and criminal procedings. Also, segmental hair analysis can

provide detailed temporal mapping of the drug abuse pattern, which can be compared with the levels of the drug(s) in body fluids such as blood or urine (Kintz, 2007).

Decontamination

Since external contamination by drugs is a big problem in hair analysis, as this may lead to false positive results, decontamination must be used before analysis. There is no general consensus with respect to hair washing procedures. The generally accepted procedure comprises one washing sequence of 0.1% sodium dodecyl sulphate in aqueous solution, followed by rinses indistilled water and acetone. Kintz *et al.* (1995) decontaminated hairs by washing the specimens twice in 5 ml dichloromethane for 2 minutes at room temperature. This procedure proved sufficiently efficient to remove external contamination, since the GC-MS analysis of a third wash remained negative, although the former wash tested positive for the targets.

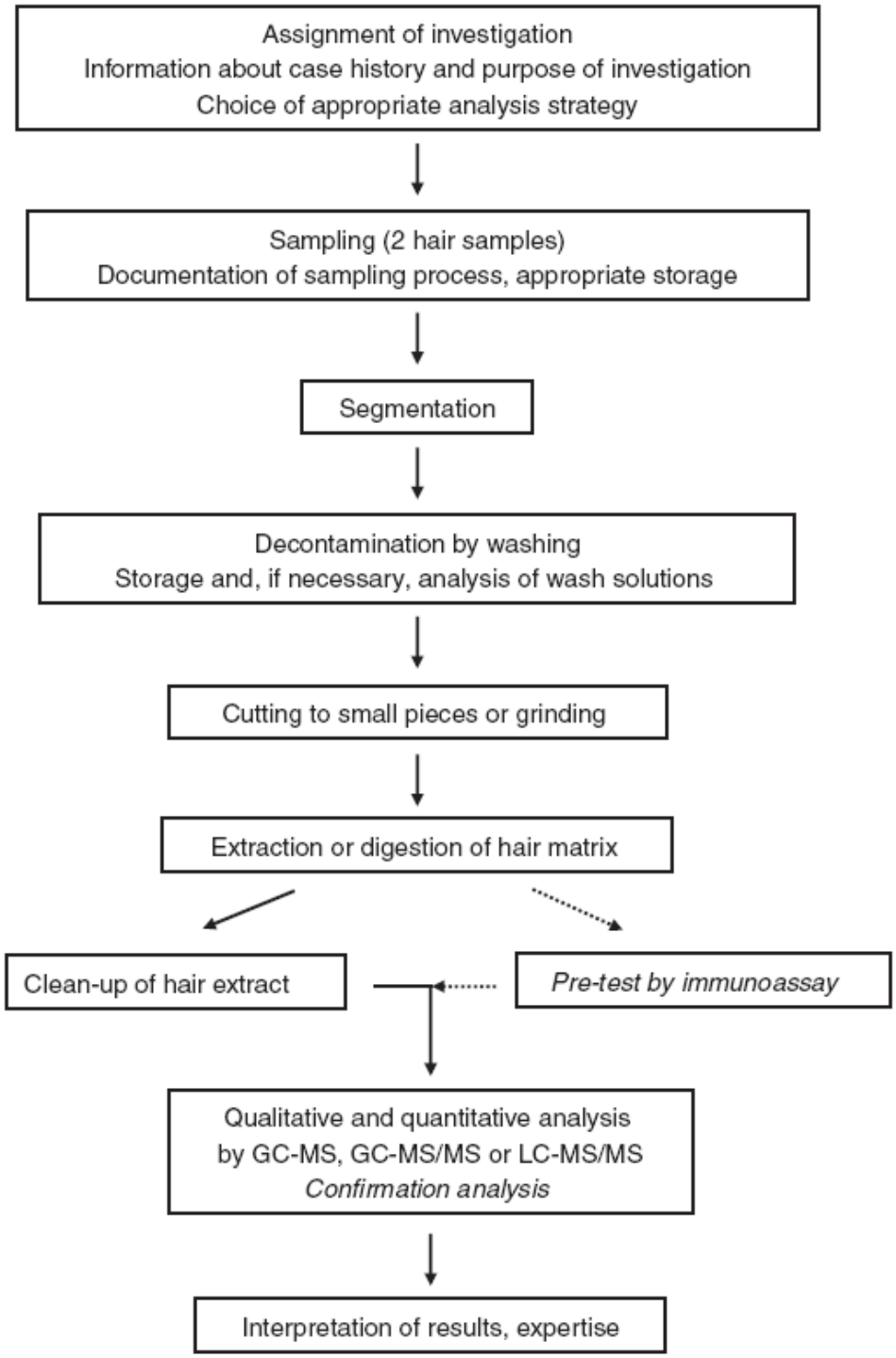


Fig. 4. Steps of hair analysis. (Pragst, 2006)

Incubation

Drugs in hair must be extracted into solution and then detected using analytical instruments. Such extraction procedures require sufficient contact between the drugs within different compartments of the hair and the solvent. Mechanical treatment of the hair to increase the contact area must therefore be applied. The major incubation methods comprise methanolic sonication, digestion with alkali or acid or enzymatic treatments. Methanolic sonication is simple and can be used for drugs present at high concentrations. The methanol extract can be directly injected for GC-MS. Despite extended methanol extraction in an ultrasonic bath, drug recovery is incomplete and frequently lower in comparison with other procedures (Pragst, 2006). Another disadvantage of this approach is the relatively high levels of impurities in the extract. Therefore, a secondary clean-up procedure involving liquid/liquid extraction or solid-phase extraction is generally recommended.

Bal´ıkova *et al.* (2003) evaluated the efficiencies of neutral (Söerensen buffer, pH 7.4), acid (0.1 M HCl) and basic (1 M NaOH) digestion of the hair matrix and compared the relative recoveries for morphine, codeine, dihydrocodeine, and hydrocodone. Comparing the three methods of incubation of authentic hair samples, those methods using 1 M NaOH or 0.1 M HCl yielded higher recoveries of total equivalents of morphine or codeine, whereas the incubation in Söerensen's buffer allowed the reflection of real ratios of labile metabolites and/or parent compounds in an original sample. For enzymatic treatment, for example, the hair samples were then cut into approximately 1 mm segments with surgical scissors and 0.5 ml of 6 mg ml^{-1} dithiothreitol (DTT) (in Tris

buffer at pH 7.2) were added to 50 mg of hair, after 2 hr, 0.5 ml of 1 mg ml^{-1} Pronase E (in Tris buffer at pH 7.2), then incubated at 37°C for 12 hr for hydrolysis.

Extraction

A variety of extraction procedures have been described, including solid phase extraction (SPE), liquid-liquid extraction (LLE), solid-phase microextraction (SPME) and headspace solid-phase microextraction (HS-SPME), and molecularly imprinted solid-phase extraction (MISPE) (Kintz, 2007). Anderson *et al.* (2008) compared the benzodiazepine results for 10 post-mortem scalp hair samples using a classical solid-phase extraction and a molecularly imprinted solid-phase extraction system. Overall, the MISPE and SPE hair results were in good qualitative agreement. For all the samples, where both extraction methods were used to detect nordiazepam, temazepam, and oxazepam, the concentrations obtained were always higher for SPE. This is probably due to the MIP procedure producing extracts with fewer matrix interferences than the extracts produced using the classical SPE method.

Detection and Quantification

There was no difference for instrumental methods between hair analysis and other biosamples (blood, urine, tissues, etc). Immunochemical technique， GC-MS and LC-MS/MS are commonly used.

Capillary gas chromatography–mass spectrometry is the method most frequently used in

hair analysis. The advantages of GC-MS include a higher resolution of the capillary gas chromatography and high specificity of electron impact ionization (EI) mass spectra. These features are enhanced by measurement in the selected ion monitoring mode (SIM) and use of deuterated internal standards. Together, these features enable the development of specific and sensitive procedures for a large variety of drugs or metabolites with sufficient accuracy at very low concentrations (Pragst, 2006). However, many drugs of abuse and their metabolites are polar with free amino ($-NH_2$), hydroxyl (-OH) or carboxyl (-COOH) groups. Therefore, the hair extract must be derivatized to improve chromatographic behavior prior to GC-MS analysis.

Recently, liquid chromatography-tandem mass spectrometry (LC-MS) has been developed as a very practical technique due to its high sensitivity and selectivity without derivatisation. LC-MS is a valuable complementary method for gas chromatography/mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC).

The outstanding advantage for hair analysis is the high sensitivity of the new LC-MS/MS instruments, which can detect concentrations in the range of picograms per milligram of hair. For example, Villain *et al.* (2005) presented results for the screening of 16 benzodiazepines and hypnotics in human hair by LC-MS/MS (alprazolam, 7-aminoclonazepam, 7-aminoflunitrazepam, bromazepam, clobazam, diazepam, lorazepam, lormetazepam, midazolam, nordiazepam, oxazepam, temazepam, tetrazepam, triazolam, zaleplon, and zolpidem). The limits of quantification for all benzodiazepines and hypnotics range from 0.5 to 5 pg mg^{-1} using a 20 mg hair sample. Che`ze *et al.* (2005)

developed an approach for hair analysis by liquid chromatography–tandem mass spectrometry using a triple stage quadrupole with an electrospray ionization (LC-ESI-MS/MS). Separation was performed on an Uptisphere ODB C18 column using a gradient of 2 mM formate buffer and acetonitrile. For the 23 compounds studied, detection limits were lower than 2 pg mg^{-1}.

Quality Control

One of the requirements for the correct application of an analytical method in toxicological analysis is to set quality standards which provide confidence in the results obtained. The general guidelines and recommendations for quality management in analytical laboratories can be applied to hair analysis. General requirements include training of laboratory staff, certification of instruments and equipment, and procedures for sample handling, chain of custody and documentation.

International guidelines for mass spectrometric identification and quantification of drugs have to be taken into account (Stolker, 2000; Rivier, 2002). Detailed and current values concerning chromatographic retention times and identification criteria, such as the number of detected ions and peak area ratios (of at least three ions in gas chromatography–mass spectrometry (GC-MS)) along with other characteristics, are essentially comparable between laboratories. Suppression or enhancement of analyte ionisation by co-eluting compounds is a well known phenomenon in LC-MS (/MS) analysis, mainly depending on the sample matrix, the sample preparation procedure, the quality of chromatographic separation, mobile phase additives, and ionisation type.

Studies of ion suppression/enhancement should be an integral part of the validation of any LC-MS (/MS) method (Peters, 2007).

Cut-off Values

A cut-off level is a value (typically a concentration) at or above which a sample may be termed positive. Cut-off levels for drugs in hair are a crucial part of the hair testing process. If results are slightly below this level, a sample must be reported as negative, even though a drug may be present, albeit at a lower concentration. According to Pragst (2006), cut-off values are generally used for two reasons: firstly, to avoid false positive analytical results for methods such as immunoassays where matrix effects and cross reactivity to other compounds may set a lower limit for an accurate use, and secondly, cut-off values are used to decide whether a correct analytical result is caused by a certain reason (i.e., by relevant drug use) or not. Substance Abuse and Mental Health Services Administration (SAMHSA), after consulting mainly with commercial hair-testing companies, has proposed the cut-off levels for hair testing that are listed in Table 1. The Society of Hair Testing (2004) has proposed somewhat different cut-off levels, and their criteria are also given in Table 1. Higher cutoff levels reduce the risk of environmental exposure and false positives from the presence of drugs rather than active use. With lower cutoff levels, more drug users would be identified but at the risk of identifying more passively exposed individuals.

Table 1 Proposed Cut-off Levels for Various Drugs in the Confirmation Assay

Drug	**Analyte**	**SAMHSA Cut-off Level (ng drug/ mg hair)**	**SOHT Cut-off Level (ng drug/ mg hair)**	**Comments**
Marijuana	delta-9-tetrahydro cannabinol- 9-carboxylic acid	0.05	0.0002(THC-COOH) 0.05(THC)	Although this is a specific metabolite, it is recommended that delta-9 THC be measured to determine contamination levels and external removal; achieving these trace levels requires extraction of a considerable amount of hair
Cocaine	Cocaine	0.5	0.5	BE/cocaine ratio must be greater than 5%, or CE or NC must be present; the SOHT does not make a recommendation as to the BE/cocaine ratio
	Benzoylecogonine	>5% of cocaine level	0.05	
	Cocaethylene	0.05	0.05	
	Norcocaine	0.05	0.05	
Opiates	Morphine	0.2	0.2	Morphine must be present if 6-MAM is detected
	Codeine	0.2	0.2	
	6-Acetylmorphine	0.2	0.2	
Amphetamines	Amphetamine	0.3	0.2	Amphetamine must be also present in hair from a methamphetamine user
	Methamphetamine	0.3	0.2	
	MDMA	0.3	0.2	
	MDA	0.3	0.2	
	MDEA	0.3	0.2	

Scope of this Study

This study was designed mainly to investigate the following:

(a) The application of GC-MS/MS and LC-MS/MS for drugs of abuse in hair.

(b) The relationship between hair concentration and dosage.

(c) The relationship between hair concentration and colours.

(d) The relationship between hair concentration and physicochemical properties of drugs.

(e)The metabolites related to the parent drugs in hair.

(f) Time courses of drugs in hair.

(g) Segmental hair analysis to determine the time of drug exposure.

(h) Physiological concentrations of anabolic steroids in human hair.

Original and Distinct Contribution to the Advancement of Scientific Knowledge and Understanding in the Area of Hair Analysis for Drugs of Abuse

This thesis covers a range of important issues in hair analysis and includes 27 scientific works in which the name of the candidate was either listed as the first author or as the major contributor. The studies described here focus on the profiles of drug incorporation into hair; a number of factors that need to be evaluated in the interpretation of hair analysis; the minimal amount of drug detectable in hair after administration; and the physiological levels of anabolic steroids in human hair in Chinese subjects.

(a) The application of GC-MS/MS and LC-MS/MS for drugs of abused in hair

MS/MS is recognised for its high specificity and sensitivity in the determination of drugs and metabolites in biological fluids. A sensitive and specific screening GC-MS/MS method was developed for the simultaneous determination of 13 drugs of abused in human hair (Publication 8). The compounds analysed were amphetamine, methamphetamine, 3, 4 - methylenedioxymethamphetamine (MDMA), MDA, morphine, 6-monoacetylmorphine (6-MAM), codeine, meperidine, normeperidine, cocaine, benzoylecgonine, methadone and EDDP. Hair samples were washed with 0.1% sodium dodecyl sulphate (SDS), and then rinsed with deionized water and acetone. After being air-dried, the segments were cut into about 1 mm lengths. One ml of HCl (0.1 M) was added to 50 mg of segments and incubated overnight at 45 °C. After cooling to room temperature, the resulting digests were adjusted to pH 9.2 using 0.4% NaOH and mixed

with 2 ml ethyl acetate, vortexed, centrifuged (3000 rpm), and evaporated to dryness after adding 1 drop of 2% acid methanol. The residue was derivatized with 25 µl N-methyl-N-trimethylsilytrifluoroacetamide (MSTFA) for 30 min at 70°C. After cooling down, the residue was injected to GC-MS/MS, as shown in Fig 5. Ethyl acetate was the optional extraction solvent for all the 13 drugs for best recovery and fewer matrix interferences. MSTFA is more active than bis (trimethylsilyl) trifluoroacetamide (BSTFA) for derivatization.

Fig. 5. GC-MS/MS chromatogram of 13 abused drugs spiked in hair (1. amphetamine-TMS; 2. methamphetamine-TMS; 3. MDA-TMS; 4. MDMA-TMS; 5. meperidine; 6. normeperidine-TMS; 7. EDDP; 8. methadone; 9. cocaine; 10. benzoylecgonine-TMS; 11. codeine-TMS; 12. morphine; 13. 6-monoacetylmorphine-TMS). (X axes: Retention time; Y axes: Peak intensity)

A liquid chromatographic-tandem mass spectrometric method for the simultaneous determination of anabolic androgenic steroids and their esters in hair has been developed (Publication 21). The hair sample was treated with methanol to extract the esters, followed by alkaline digestion for optimum recovery of the anabolic androgenic steroids. After liquid-liquid extractions, the extract was dried, re-dissolved and analyzed by multiple reactions monitoring with a quadrupole mass spectrometer, as shown in Fig 6. The lower limits of detection ranged from 0.001 to 0.020 ng mg^{-1} for the 21 analytes.

LOD and LOQ ranged between 0.001- 0.02 and 0.002- 0.04 ng mg^{-1}, respectively, as determined by LC-MS/MS under positive mode.

To determine trace endogenous anabolic steroids in hair, a more sensitive GC-MS/MS method was developed for the simultaneous identification and quantification of testosterone, epitestosterone, androsterone, etiocholanolone, dehydroepiandrosterone in hair (Publication 17). After basic hydrolysis, hair sample was extracted with diethyl ether, derivatized with MSTFA/iodotrimethyisilane/ DL-dithiothreitol (1000:5:5, v: v: w) and detected using GC-MS/MS in the multiple-reaction monitoring mode. The limits of detection for five endogenous anabolic steroids were in the 0.1~ 0.2 pg mg^{-1} range. All analytes showed good linearity and the extraction recoveries were 74.6~ 104.5 %. The intra-day precision was determined by assaying six spiked hair samples at each concentration level on the same day and inter-day precision was assayed for 6 replicates on each of 4 days. Inter-day and intra-day precisions were less than 20%.

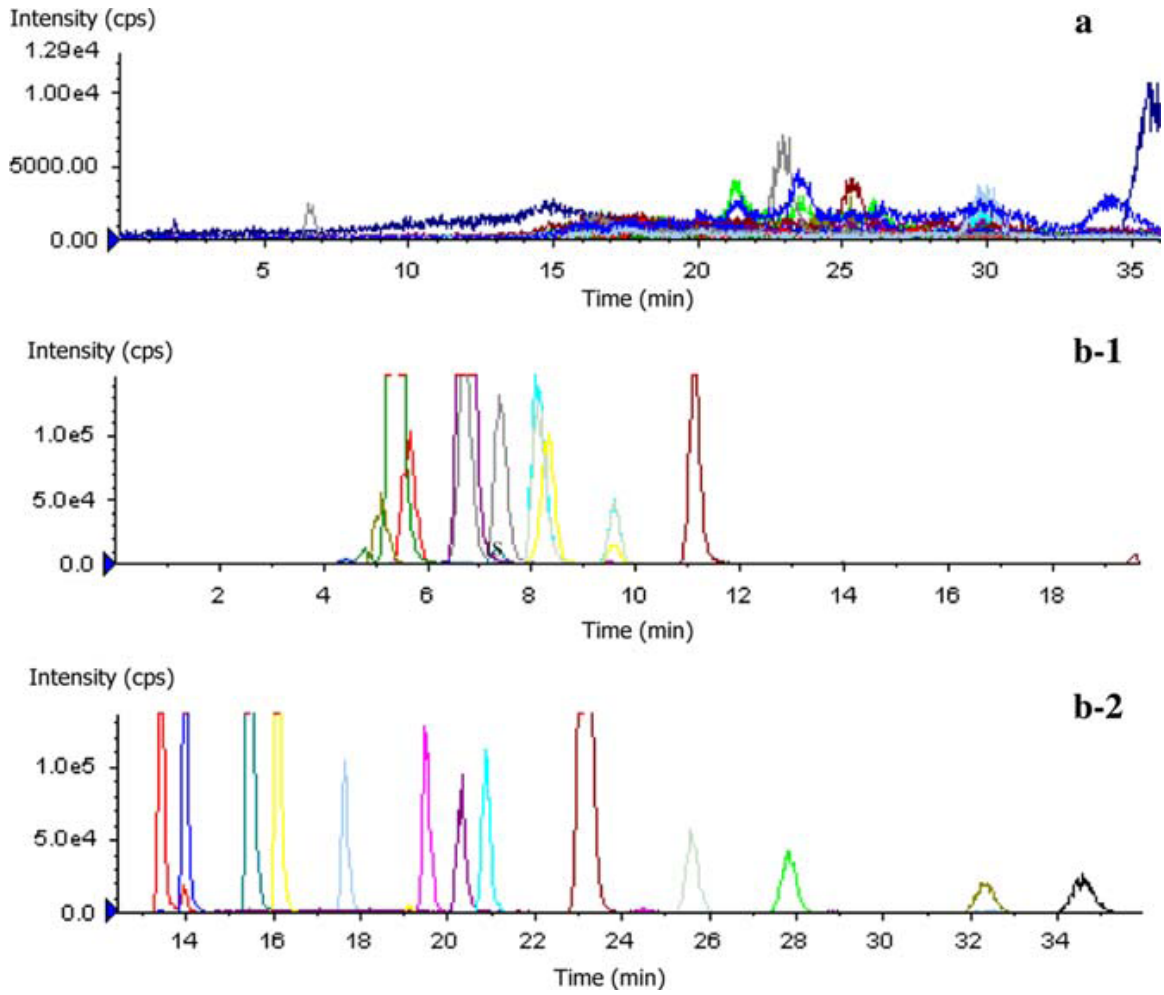


Fig. 6. LC-MS/MS chromatograms acquired from (a) blank hair (b) hair spiked with 21 analytes and IS (b-1: 0.2 ng mg^{-1} for methandienone, oxymesterone, epitestosterone, metenolone, norethandrolone and stanozolol, boldenone, nandrolone, testosterone, and 17a-methyltestosterone; b-2: 0.4 ng mg^{-1} for metenolone-acetate, -enanthane, testosteronepropionate, -phenylpropionate, -isocaproate, -enanthate, -cypionate, androlone-phenylpropionate, -decanoate, -laurate and boldenone-undecylenate). The retention time of IS was 7.5 min.

It is a considerable technical challenge to achieve the separation of complex mixtures of 59 drugs of abuse in a single run using LC-MS/MS. An Allure PFP Propyl column was found to be the most suitable of 4 different columns (SHISEIDO C18 MGII 5 μm

2.0×250 mm，COSMO SIL 5C18-MS-II 2.0×150 mm，RESTEK Ultra IBD 5 μm 2.1×50 mm and Allure PFP Propyl 5 μm 100×2.1 mm). Those drugs which have the same MRM transitions, such as, codeine and hydrocodone, Δ^9-tetrahydrocannabinol and cannabinol (CBD), separated well. The applied LC gradient ensured the elution of all the drugs examined within 20 min. Our own MS/MS library was developed containing two MRM transitions, declustering potential (DP), collision energy (CE), and the retention time (RT). Compounds included opiates, cocaine, amphetamines, cannabinoids, antidepressants, benzodiazepines, hypnotics, neuroleptics, and β-blockers. The method was sufficiently selective and sensitive to detect illicit drugs and applied in forensic casework (Publication 12).

Above all, the developed methods extend the scope of detecting drugs of abuse in hair and promote the applications of MS/MS in China.

(b) The relationship between hair concentration and dosage

Several studies (see Publications 4, 7, 9, 13, 18, 26) undertaken by this author and her collaborators have demonstrated that there is a good relationship between hair concentration and dosage.

After shaving an area of back hair (8 cm × 4 cm) from guinea pigs, from these three groups were administered intraperitoneally once a day for 7 successive days, at 1, 5, and 20 mg kg^{-1} ketamine (low, medium, and high dosage), respectively. After dosing for 7 consecutive days, there was a 2-day induction period before hair segments with different

colours were collected. Significant differences were seen between the groups when compared with each other ($\rho<0.05$), as shown in Fig 7; higher dosage resulted in greater hair concentration of ketamine (Publications 9, 13).

There was a good correlation between the concentration of cocaine (COC) and benzoylecgonine (BZE) and dose. Comparing the black hair samples of two dose groups, it was shown that the higher the dose of cocaine that guinea pigs received, the higher concentration of cocaine and BZE could be detected in their hair (Publication 18).

The same results were established from the hair segments of guinea pigs after administration of methamphetamine (Publication 4). The concentration of methamphetamine and amphetamine were significantly related to the administered dose (5, 10, 20 mg kg^{-1}).

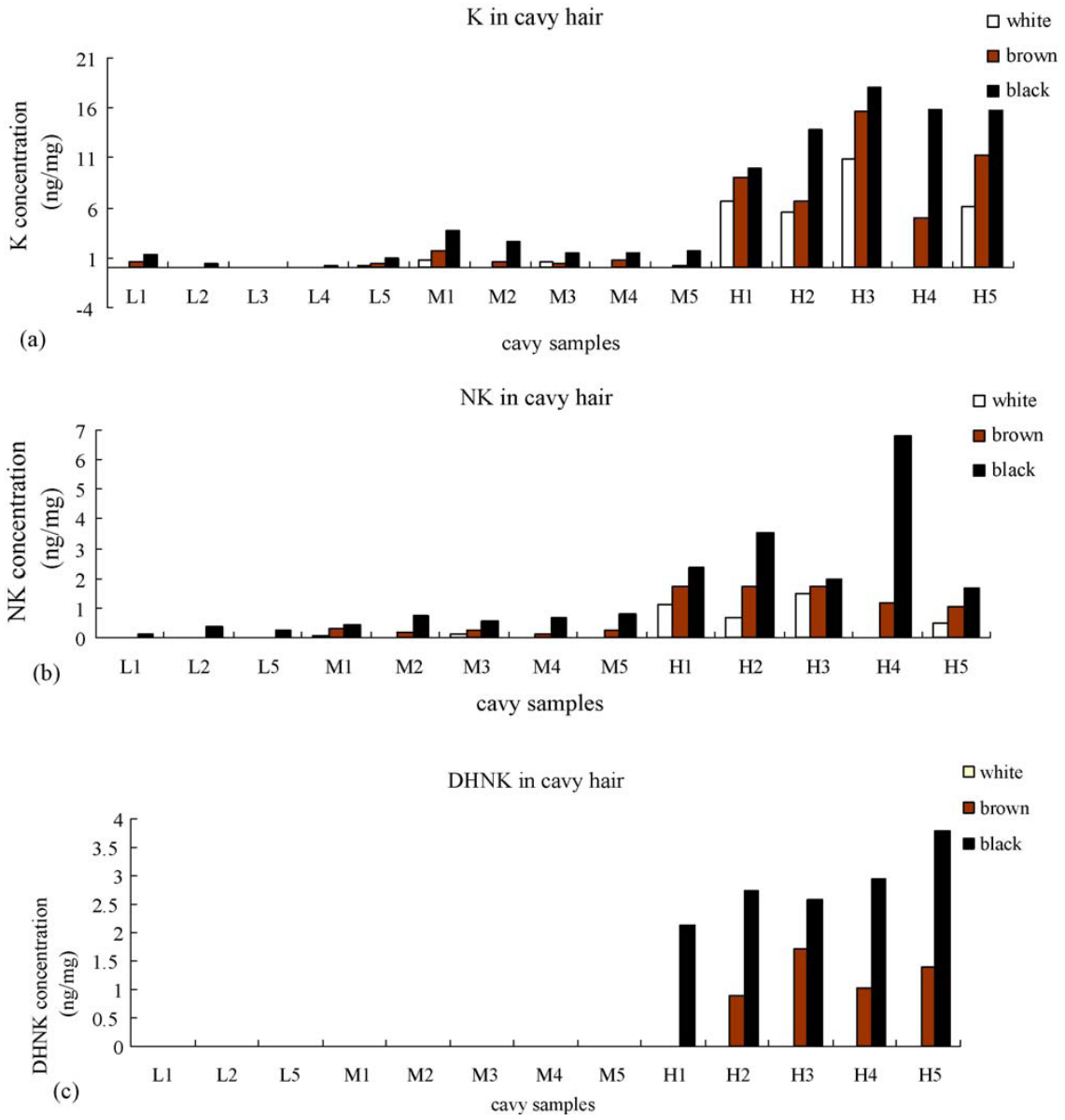


Fig. 7. Concentration profiles found in hair samples from the 15 cavies: (a) ketamine (K), (b) norketamine (NK), and (c) dehydronorketamine (DHNK).

Fig. 8. Relationship between the daily dose of chlorpromazine and clozapine and their concentration in human scalp hair. A, chlorpromazine; B, clozapine.

The hair root samples (taken 2 cm from the root) of 35 psychiatric patients (29 males and 6 females) were measured for antipsychotic and antidepressant drugs by GC-NPD after GC-MS identification. Six cases tested positive for carbamazepine, three cases for amitriptyline, five cases for doxepin, seven cases for trihexyphenidyl, and sixteen cases for chlorpromazine and clozapine, respectively, and samples from one case were positive for chlorprothixene, trifluoperazine and haloperidol, respectively. Data from 16 hair samples which tested positive for chlorpromazine was statistically analysed. It was found that the accumulation of chlorpromazine in the hair was dependent on the dose, and that the concentrations of chlorpromazine were significantly correlated ($\rho<0.001$, $n=16$) with the daily doses and that the correlation coefficient was 0.8047 (as shown in Fig 8). Sato *et al.* (1993) previously demonstrated that the concentration of chlorpromazine in black hair collected from 23 Japanese patients who had been taking chlorpromazine in fixed daily doses (30- 300 mg per day) ranged from 1.6 to 27.5 ng mg^{-1}, and significantly correlated

with the daily dose (r=0.788, ρ<0.001, n=23). This was in agreement with our results (Publication 7).

Statistical analysis of hair samples containing clozapine also showed a correlation between clozapine concentrations in the hair and the daily doses, with a correlation coefficient of 0.7097 (ρ<0.001, n=16). So far, no data has been published on clozapine other that produced by Cirimele *et al.* (2000), who also observed a close dose-concentration relationship between daily dose and hair clozapine concentration (r=0.542, n=23) (Publication 7).

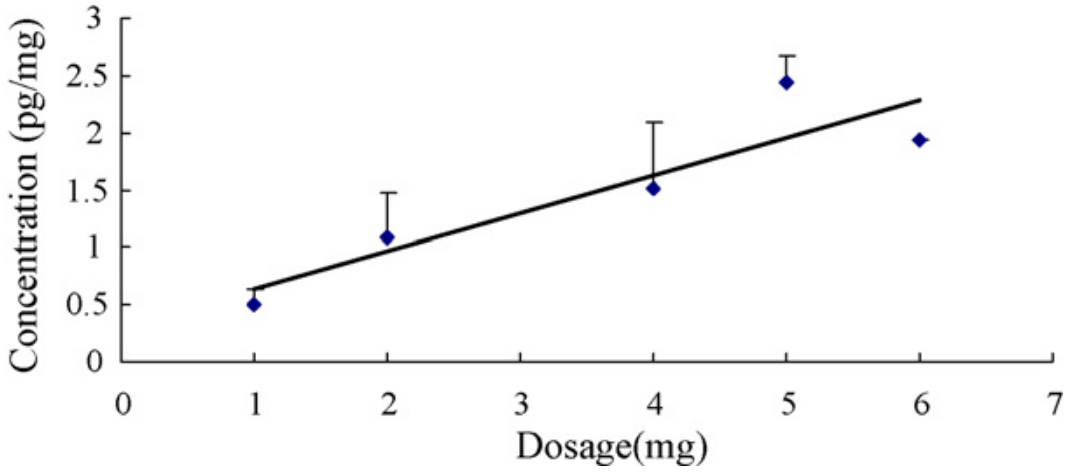


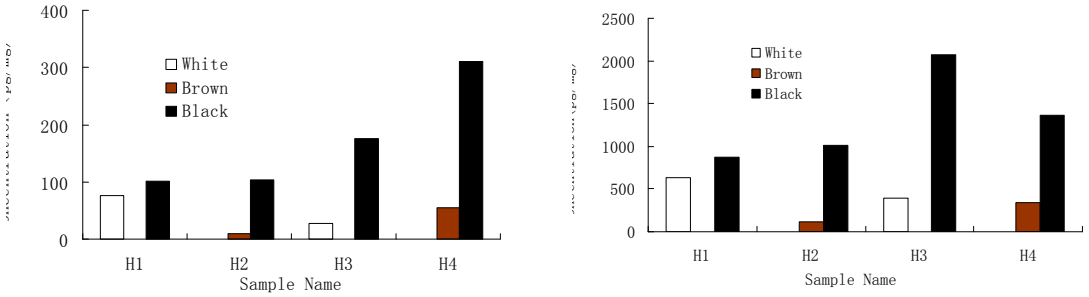
Fig. 9. Estazolam concentrations in hair at different doses.

The hairs of 14 volunteers who had been administrated with a single dose of estazolam (1-6 mg) were decontaminated, segmented, analyzed and quantified. A single 1 mg dose of estazolam could be detected in the hair up to a month after administration of the drug. As Fig 9 shows, there was a good correlation between the dosage and the 0-2 cm segment

concentration (r=0.766), with narrow variations for subjects at the same dosage (less than 40%) (Publication 26).

(c) The relationship between hair concentration and pigmentation

Publications 4, 13, 18, and 25 indicated that there was a good relationship between the drug levels and that of melanin in hair. Publication 18 showed that higher concentrations of cocaine and BZE were found in black hair than in white and brown hair samples (Fig. 10). One suggested explanation is that analytes are incorporated with eumelanin, a major subtype of melanins, in hair by electrostatic interaction. Black hair has more eumelanin than other coloured ones. On the other hand, it was discovered that white hair, which contains no melanin, also incorporated cocaine and BZE. This observation supports the notion (Kintz, 2004) that melanin is not the only dominant factor involved in the mechanism(s) for incorporation of cocaine into hair.



(a) (b)

Fig.10. Cocaine (a) and BZE (b) determined in hair samples from guinea pigs which received high dose (10 mg kg^{-1}) of cocaine.

In another experiment (Publication 25), 18 guinea pigs were divided into three groups of 6 animals. Before the experiment, an 8 cm ×4 cm area of hair on the animals' backs was shaved to the skin with an electric hair shaver and drug-free hair from these zones were preserved. Then, triazolam was administrated intragastrically at doses of 10 μg kg^{-1}, 100 μg kg^{-1} and 500 μg kg^{-1} (n=6). Hair newly grown from the shaved areas was collected 7, 14, 21, 28 and 35 days after administration. Since guinea pigs are naturally covered with two or three coloured hairs (white, brown or black), shaved hair of each colour were collected separately. In the 500 μg kg^{-1} dosage group, where triazolam in 56% of hair samples could be quantified, triazolam concentration in black hair was found at higher levels than in brown or white hair, as shown in Fig. 11. This observation agrees with the hypothesis that the degree of drug disposition in hair closely relates to its affinity with melanin due to electrostatic interaction (Nakahara, 1995). For methamphetamine, the incorporation rate into white and brown hair is much poorer than that of black hair (Publication 4).

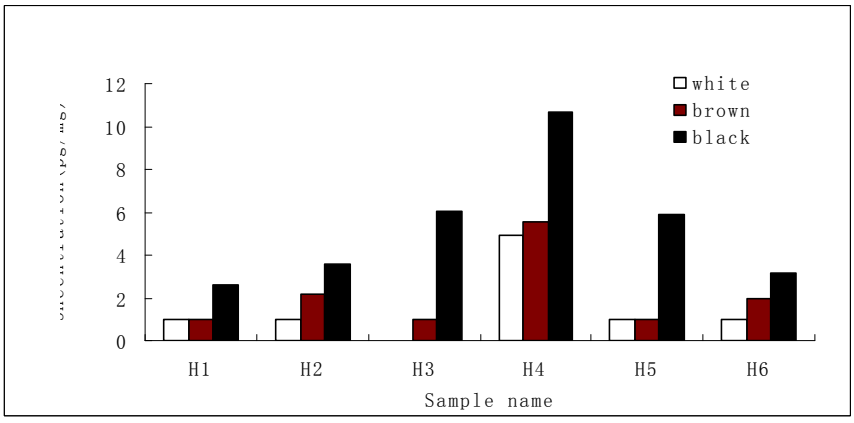


Figure.11. Triazolam determined in guinea pig hair in 500 μg kg^{-1} dosage group.

Ketamine is weakly basic (pKa = 7.5) and exists as a cation at physiological pH. As melanin is a polyanionic polymer, melanin and ketamine interact strongly with it, which facilitates its incorporation into hair. Just as concentrations of melanin increased in the order of white > brown > black hair, so concentrations of ketamine and norketamine also increased accordingly (Fig. 7). As shown in Publication 13, the concentration of ketamine deposited in black hair was also significantly high. The average ratio of concentrations of ketamine in black to brown hair was 1.87:1, and the ratio of incorporation in black to white hair was 2.24:1 in the high dosage group. As differences in hair colour may contribute to deviation in the analytical results, Rollins (2003) suggested normalizing the results from different hair colours according to their concentrations of melanin. The mean total concentrations of melanin in black and brown hair of Caucasians were 7.11 and 3.57 μ g mg^{-1}, respectively. Assuming these values hold in the correction of ketamine concentration in guinea pig hair, it was found that the corrected ketamine concentrations in black and brown guinea pig hair were similar, supporting melanin affinity as a governing factor in determining ketamine incorporation into hair. Although hair colour may complicate the interpretation of hair testing in humans, this possibility is less significant in Chinese subjects as their hair colour is almost all black (Publication 13).

In our experiment, the presence of hair with different colours in the same guinea pig hair samples effectively minimized variability due to individual differences and other inter- and intra-factors such as living condition, age and health condition.

(d) The relationship between hair concentration and physicochemical properties of drugs

The degree of disposition of drug in hair closely relates to its physicochemical properties (Musshoff, 2007) and is reflected in our experimental results using guinea pig hair. Relative to the metabolites norketamine (NK) and dehydronorketamine (DHNK), the lipophilicity of ketamine is higher. This can be explained by the difference in chemical structures between these three compounds. The change of an N-Me to an N-H group in NK increases its polarity compared with ketamine. The polarity of DHNK is further increased due to the conjugation of double bond(C=C) and the carbonyl group(C=O). It is known that increasing polarity would lead to a decrease in lipophilicity. Thus, the lipophilicity decreases in the order of ketamine > NK > DHNK. After administration of ketamine, blood plasma concentrations of ketamine, NK and DHNK are similar. However, drug disposition in hair depends very much on the lipophilicity of the drug. Therefore, the concentration of ketamine deposited in hair was the highest followed by NK, as shown in Fig. 7. For DHNK, its presence could only be detected in the high dosage group (Publications 9, 13).

In our study, concentrations of cocaine in hair were much higher than those of BZE. This result was consistent with those reported in earlier publications. Nakahara *et al.* (1995) explained that this is because cocaine is more lipophilic and hence better able to penetrate the cell membrane than BZE. Jurado *et al.* (1997) added that, with a higher alkalinity, cocaine is easier to shift from an alkalic blood matrix to an acidic hair matrix (Publication 18).

The presence of therapeutic drugs in the hair of psychiatric patients showed that the order of drug-hair incorporation tendency based on daily dose (concentration in hair-dosage) was trihexyphenidyl > amitriptyline > doxepin > haloperidol > trifluoperazine > chlorprothixene > chlorpromazine > clozapine > carbamazepine. These results suggest that lipophilicity, basicity and low molecular weight facilitate the incorporation of the drug into the hair (Publication 7).

In Publication 24, methyltestosterone, stanozolol, methandienone, nandrolone, trenbolone, boldenone, methenolone and DHEA were administered intraperitoneally to guinea pigs. After the first injection, black hair segments were collected on shaved areas of skin. Except for methenolone, all the anabolic steroids listed above can be incorporated into hair. Although the guinea pigs were dosed with the same amounts and the hair samples collected at the same time, the hair mean peak concentrations (C_{max}) were found to range widely from 105.9 to 3246.6 pg mg^{-1}. According to the C_{max} for guinea pig hair, drug incorporation into hair (greatest to least) was methandienone > boldenone > trenbolone > stanozolol > nandrolone > methyltestosterone >DHEA. Reverse phase high performance liquid chromatography (RP-HPLC) is considered a popular alternative for lipophilicity assessment. Lipophilicity is related to chromatographic retention time (Cimpan, 1998). Except for stanozolol, the order of retention time was trenbolone < boldenone < nandrolone < DHEA < methandienone < methyltestosterone < methenolone, using a mobile phase composed of methanol and 20 mM ammonium acetate buffer containing 0.1% formic acid (73: 27, vv) with Agilent ZORBAX SB-C18 column (150 mm×2.1 mm i.d., 5 μm). This value is not consistent with the order of C_{max} in hair. Like cannabinoids (Nakahara, 1995), methenolone is highly lipophilic, but the levels in hair are quite low.

Methenolone was not detected in guinea pig hair after a single dose, while the concentrations of methenolone in hairs from two bodybuilders were 7.3 and 8.8 pg mg^{-1} (Kintz, 2002). These results indicated that lipophilicity was not the major factor in the incorporation of anabolic steroids into hair. The different concentrations of the drugs in hair depend on their physicochemical properties and on their functional groups (Nakahara, 1995). Nakahara *et al.* (2000) demonstrated that the hydroxyl group showed a negative effect on drug incorporation into hair. All the anabolic steroids in our experiments have one hydroxyl group and are more difficult to incorporate into hair than other drugs of abuse, such as cocaine, morphine, amphetamines. It is believed that the reason why the concentrations of methandienone and boldenone were ten-fold higher than that of other anabolic steroids is due to the particular chemical structure. Conjugated addition occurs with two double bond groups and C-3 ketone in the A-ring of these two drugs. Charge-transfer reactions enhance the ability to bind to melanin when electron-rich drugs interact with the electron-accepting carboxylic groups on the surface of the melanin polymer.

(e) The metabolites related to the parent drugs in hair

Detection of a drug and its metabolite(s) is important in forensic and clinical toxicology. The presence of antidepressant and antipsychotic drugs and their metabolites in the hair of psychiatric patients was investigated using GC-MS-EI and GC-MS-PCI. EI and PCI MS characteristics of 19 drugs and their metabolites are shown in table 2.

The results from the hair root samples (taken 2 cm from the root) of 35 psychiatric patients showed that the deposition of the metabolite into hair is less effective than that of the parents. A possible reason for this phenomenon may be the increase in hydrophilicity of the metabolites (Publication 5).

Experiments (Publication 9) used hair samples obtained from 15 human ketamine abusers for whom, except for one subject whose hair was yellow brown, all hair samples were black. In all samples, ketamine and norketamine were easily detected while only traces of dehydronorketamine were detected in two samples. The average ratio of ketamine to norketamine in human hair was found to be 9.28, which was significantly higher than the average ratio of 6.32 found in black guinea pig hair. The relatively higher concentration of norketamine found in guinea pig compared with human hair may be due to the difference in the frequency of ketamine administration. In guinea pigs, ketamine was continuously administered for 7 days leading to the accumulation of metabolite in the body and hence higher deposited concentrations of norketamine in hair. On the contrary, the common recreational or occasional use of ketamine in abusers resulted in very high ketamine concentration together with low norketamine levels found in human hair. The ratios of norketamine to ketamine in the range of 0.03- 0.88 (mean = 0.32) found in this study is similar to those reported by Leong (2005) (Publication 9).

Table 2 EI and PCI MS Characteristics of 19 Drugs and their Metabolites

Compound	MW	EI (relative abundance)		PCI (relative abundance)	
		M^+	Second peak	$[M+1]^+$	Second peak
carbamazepine	236	236(100)	193(50)	237(100)	473(40)
carbamazepine-M (N-dealkylation metabolite)	193	193(100)		194(100)	395(50)
amitriptyline	277	277(5)	58(100)	278(40)	58(100)
amitriptyline-M1 (N-demethylation metabolite)	263	263(35)	202(100)	264(100)	55(80)
amitriptyline-M2 (ring-hydroxylation metabolite)	293	293(3)	58(100)	294(50)	59(100)
doxepin	279	279(20)	58(100)	280(100)	58(100)
doxepin-M1 (N-demethylation metabolite)	265	265(100)	204(100)	266(100)	
doxepin-M2 (ring-hydroxylation metabolite)	295	295(3)	58(100)	296(35)	58(100)
trihexyphenidyl	301	301(30)	218(100)	302(100)	98(55)
trihexyphenidyl-M(ring-hydroxylation metabolite)	317	317(2)	98(100)	318(60)	98(100)
chlorpromazine	318	318(70)	58(100)	319(60)	58(100)
chlorpromazine-M1 (N-dealkylation metabolite)	334	334(30)	58(100)	335(70)	84(100)

chlorpromazine-M2 (S-oxidation metabolite)	233	233(100)	198(70)	234(100)	199(25)
chlorprothixene	315	315(3)	58(100)	316(20)	58(100)
chlorprothixene-M (ring- oxidation metabolite)	246	246(100)	218(40)	247(100)	55(30)
trifluoperazine	407	407(100)	70(65)	408(85)	113(100)
clozapine	326	326(45)	256(100)	327(100)	99(55)
clozapine-M (N-dealkylation metabolite)	312	312(100)	243(80)	313(100)	
haloperidol	375		237(100)	376(100)	165(85)

Meperidine and its three metabolites normeperidine, N-methoxy normeperidine and acetyl normeperidine, which had been identified in addict urine, were found in hair samples from addicts. N-demethylation is a major metabolic pathway of meperidine and thus normeperidine was detected in almost all hair samples of addicts, EI MS of the metabolite shows a molecular ion at m/z 233 with an abundant ion at m/z 158 (M-C_2H_5OH-CO). N-Methoxy normeperidine [m/z 261 (M), m/z 187 (M-C_2H_5OH-CO)] and acetyl normeperidine [m/z 275 (M), m/z 187 (M-C_2H_5OH-CO-CH_3)] were formed through future metabolism of normeperidine, which were not in all hair samples from addicts. Fig. 12 shows the area ratios of meperidine and its metabolites (normeperidine, N-methoxy normeperidine and acetyl normeperidine) in hair samples from four subjects. There was a clear correlation between meperidine and normeperidine. In contrast, for the other two metabolites, a large inter-subject variation in meperidine metabolism was observed (Publication 2).

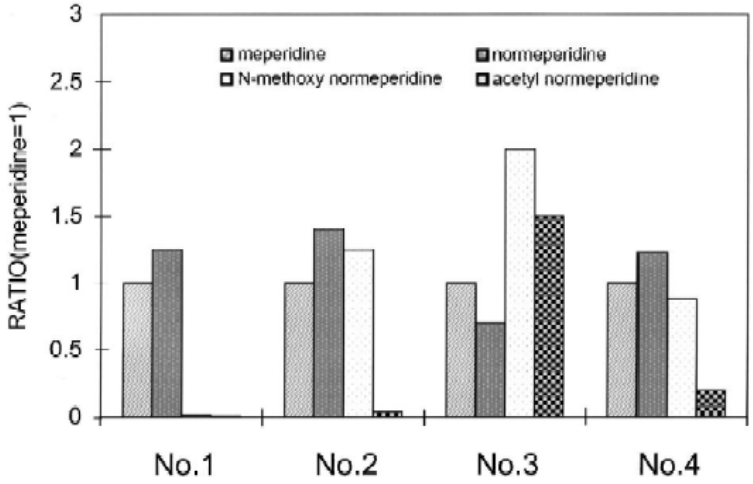


Fig.12. The ratios of meperidine and its metabolites in hair samples from four subject addicts (the relative values are given when the area of meperidine is 1).

Based on animal experiments, the same phenomenon was observed when ketamine and cocaine were the primary analytes in hair (Publications 9, 13, 18, 25). The concentrations of ketamine and its metabolites increased in the order of [ketamine] > [NK] > [DHNK] as observed in the medium and high dosage groups. The difference in concentrations of these three drugs deposited in guinea pig hair is in contrast to similar concentrations found in blood plasma after administration of ketamine. The ratios of the concentrations of ketamine to norketamine in guinea pig hair administered with high dosage of ketamine (i.e. 20 mg day^{-1}) were between 2.33 and 12.94 (mean = 7.37); the ratios of ketamine to dehydronorketamine were between 4.66 and 9.20 (mean = 6.32); and the ratios of norketamine to dehydronorketamine were between 0.44 and 2.3 (mean = 1.19). Except for one black hair sample, where traces of dehydronorketamine were detected, dehydronorketamine disposition was not observed for ketamine dosages at 1 and 5 mg day^{-1} (Publication 13).

In two dosage groups, cocaine and BZE appeared in guinea pig hair in the first week after drug administration, except for sample L1-black (It referred to the black hair sample from No.1 in low dosage). The second week's results, where the concentrations detected were much lower, indicated that the majority of cocaine and BZE were incorporated into hair in the first week after administration. When it came to the second week, BZE could no longer be detected, while cocaine still had a lower concentration in hair samples. Except sample L4-black (It referred to the black hair sample from No.4 in low dosage), all [cocaine]/ [BZE] values were higher than 0.05, ranging from 0.07 to 0.23 (Publication 18).

However, for triazolam and clonazepam (Publications 25 and 26), the results were contrary to expectations. If the fact that α-hydroxytriazolam has a higher limit of detection (LOD) than triazolam is taken into consideration, it is noteworthy that, in the 500 μ g kg^{-1} dosage guinea pig group where both analytes could be detected, the concentration of α-hydroxytriazolam was higher than that of triazolam in most samples. This phenomenon seems to be unusual, since polar metabolite used to be less incorporated into hair than its parent drug (Musshoff, 2007). It has been observed that, the more lipophilic a drug is, the more easily it penetrates the cell membrane into hair. The value of log k'w, which is a lipophilicity index, was 2.73 for triazolam and 2.38 for α-hydroxytriazolam (Toyo'oka, 2001). Therefore, the higher concentration of α-hydroxytriazolam could not be explained from consideration of lipophilicity alone. Another influencing factor is melanin affinity, but triazolam and its metabolites have been shown to have almost the same binding ratio to melanin (Toyo'oka, 2001), and thus melanin affinity could be excluded too. Compared with the present study, Toyo'oka

(2001) found the order of concentration incorporated in DA rat hair shafts after multiple doses was triazolam > α-hydroxytriazolam. However, they also reported that the order of concentration in a Halcion (triazolam tablet) addict's hair was α-hydroxytriazolam > triazolam, which was in accordance with our result. One possible explanation is that of species differences. Guinea pigs, DA rats or human are not only different from each other in their metabolism, but also in the composition of their hair, and the mechanisms of drug incorporated into hair. These factors may induce different predominant analyte, metabolite or parent drug profiles, presenting in their hair.

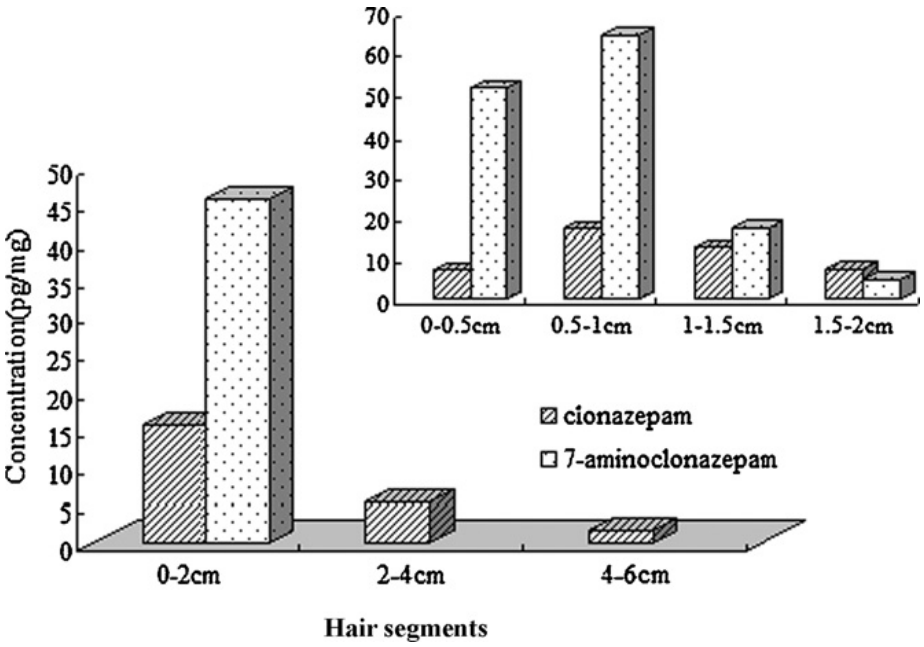


Fig. 13. Hair Segment Analysis of V#1.

In a drug-facilitated sexual assaults case, full-length hair samples of two victims, victim 1 (V#1) and victim 2 (V#2), collected 5 weeks after the offence were cut into segments of 2 cm from the root, and analyzed and quantified, as shown in Fig. 13. Our finding that the

concentration of 7-aminoclonazepam was significant higher than that of clonazepam in hair after a single dose was consistent with that reported by Chèze *et al.* (2004). Nakahara *et al.* (2000) demonstrated that the different concentrations of the drugs in hair depend on their physicochemical properties and on the functional groups. Amino substitution on the benzene ring raised the drug incorporation into hair and hydroxy substitution showed a negative effect (Publication 26).

(f) Time courses of drugs in hair

Based on guinea pig models and successive collection of samples after injection with drugs, time courses of eight anabolic steroids were investigated (Publication 24). As shown in Fig. 14 – Fig. 17, the highest mean concentrations of almost all the targets were reached by days 2 - 4, except for stanozolol, where it was day 10 after administration before peak concentration was reached; the target substance can accumulate in hair as a result of multiple doses. The peak concentrations in the multiple-dose hair were higher than that in the single-dose hair samples, and more time was required for the concentration to decrease. The experimental data indicates a good correlation between the dosage and the peak concentration in hair. Anabolic steroids can be detected in guinea pig hair even 14 days after a single dose. This indicates that anabolic steroids are distributed widely in the hair shaft. The degree and rate of longitudinal diffusion of analytes need to be known to provide a more accurate history of drug use. Studies on the distribution of drugs in the hair shaft and the time course of distribution will have important implications for developing the criteria for the appropriate time and method of

collecting hair samples. This information will be helpful in interpreting any results of hair analysis.

As shown in Fig 14, 15, 16, and 17, the higher concentrations in hair, the higher standard deviations. Except the average concentrations, reasons for these different standard deviations may be the different from inter-individual differences in metabolic capacity, different incorporation rates of drugs, different binding capacities for drugs in hair samples, and the stabilities of durgs in hair (Kintz, 2007).

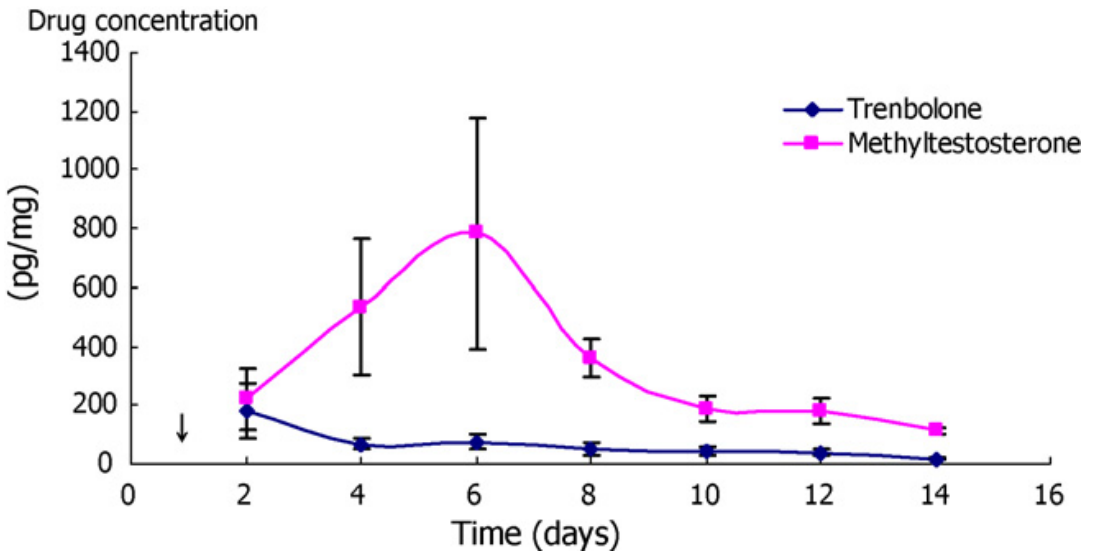


Fig.14. Concentration-time curve of methyltestosterone and trenbolone in guinea pig hair after a single intraperitoneal injection of 60 mg kg^{-1} dosage (n = 6).

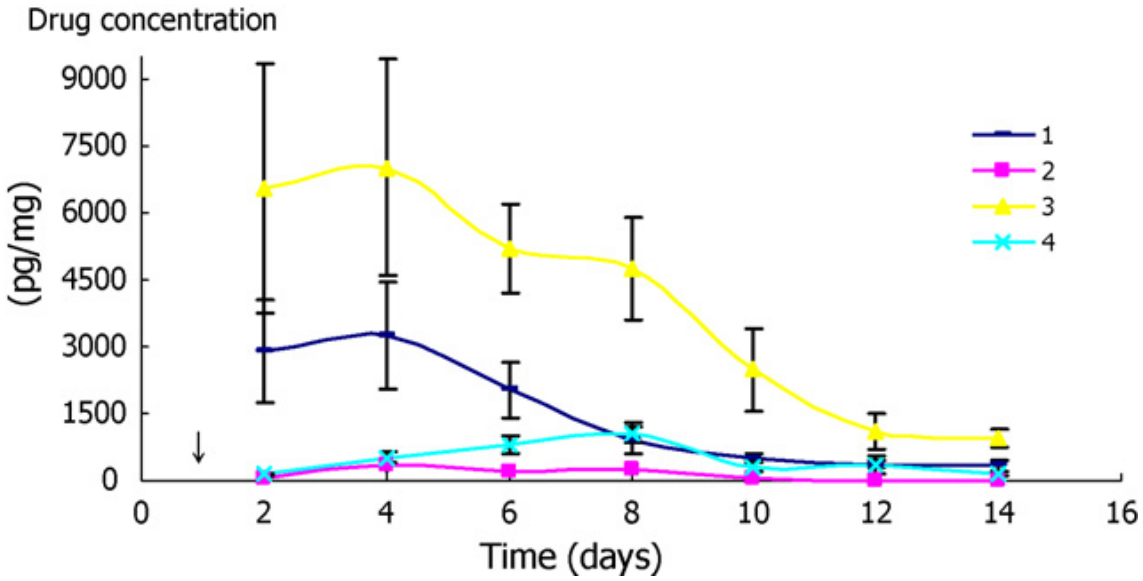


Fig. 15. Concentration - time curve of methandienone and 6-hydroxymethandienone in guinea pig hair after a single- and three-dose intraperitoneal injection of 60 mg kg^{-1} methandienone (n = 6) (1: methandienone in guinea pig hair after a single injection; 2: 6-hydroxymethandienone in guinea pig hair after a single injection; 3: methandienone in guinea pig hair after receiving threedose injection; 4: 6-hydroxymethandienone in guinea pig hair after receiving three-dose injection).

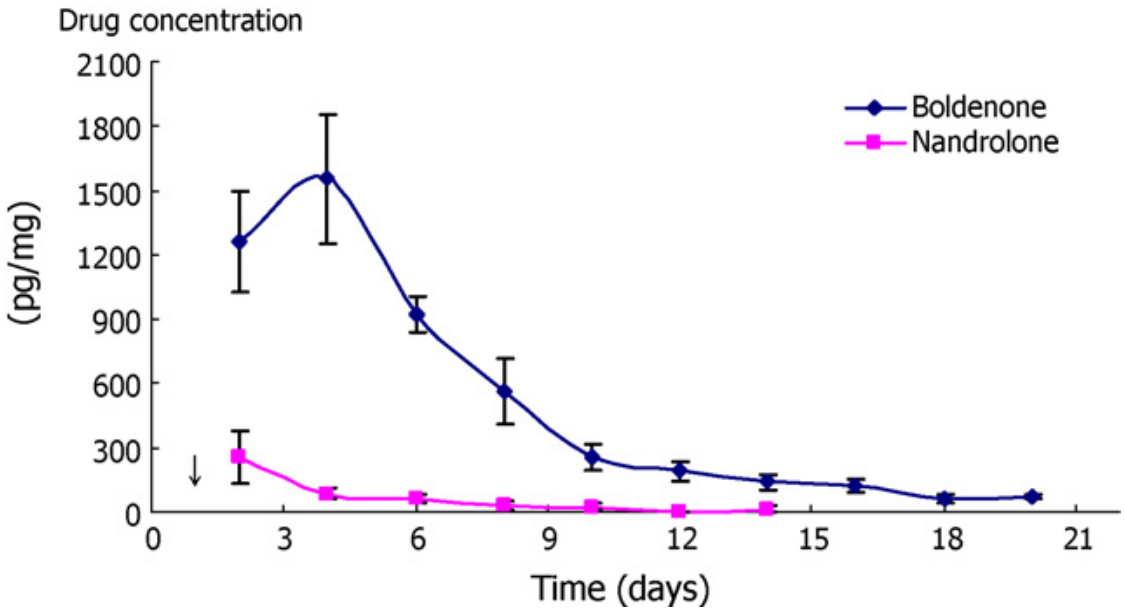


Fig. 16. Concentration- time curve of boldenone and nandrolone in guinea pig hair after a single intraperitoneal injection of 60 mg kg^{-1} dosage (n = 6).

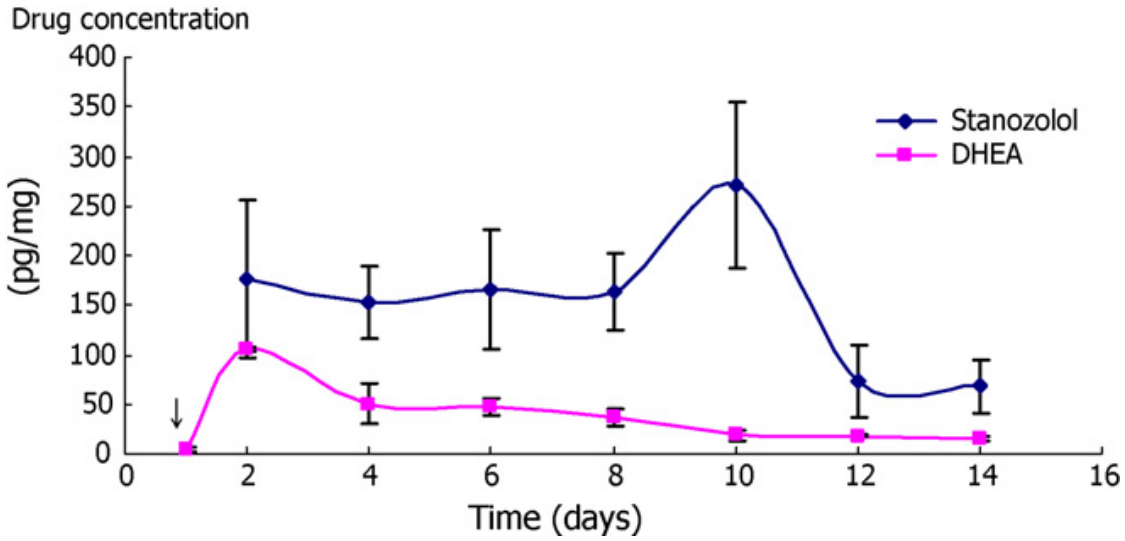


Fig. 17. The concentration- time curve of stanozolol and DHEA in guinea pig hair after receiving a single intraperitoneal injection of 60 mg kg^{-1} dosage (n = 6).

(g) Segmental hair analysis to determine the time of drug exposure

Publications 2 and 7 show that segmental hair analysis can provide important information with respect to the length of drug exposure by the individual. The scalp hair samples (8-24 cm) of 11 meperidine abusers were collected together with information about their drug histories and hairs were sectionally analyzed. Meperidine concentrations in each section of hair cut from the root end were evaluated with respect to each subject's drug history. As shown in Fig. 18, the results revealed that distribution of meperidine in the hair agreed with the drug use history. The growth rate of hair is such that the distribution of meperidine along the hair shaft at 1.2 cm per month was indicative of the length of time since the person was exposed to that drug (Publication 2).

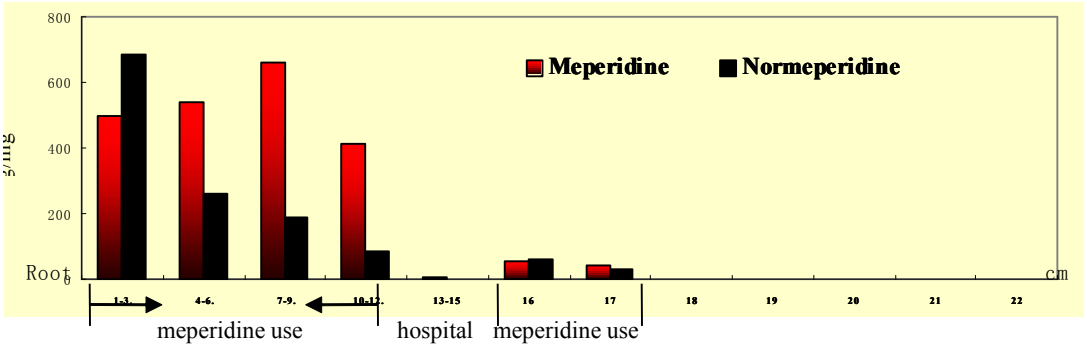


Fig. 18. Meperidine distribution in hair and meperidine use history.

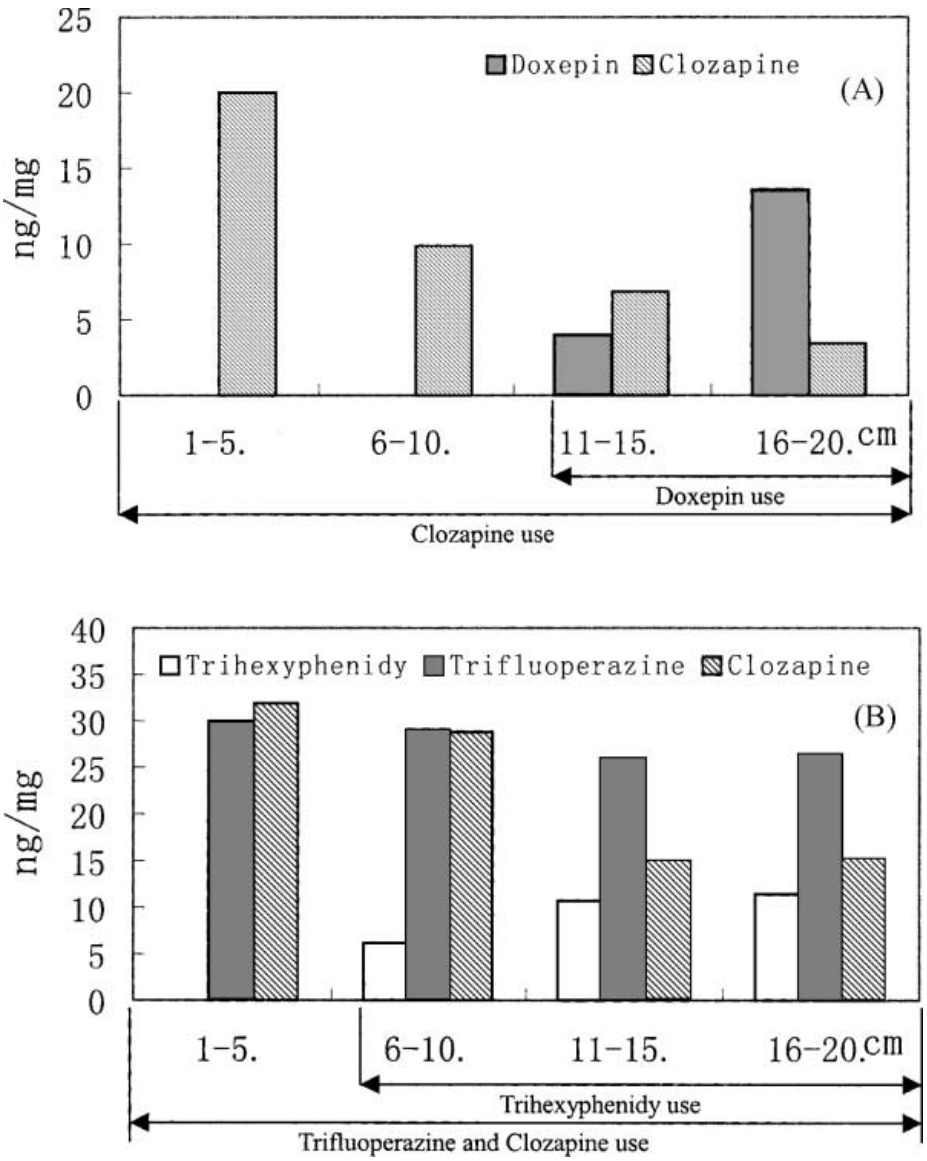


Fig. 19. Drug distribution in hair and drug use history. (A) Case 1; (B) Case 2.

The scalp hair samples of psychiatric patients were also collected together with information about their history of drug use and were sectionally analyzed, as shown in Fig. 19. Drug concentrations in each section of hair cut from the root end were again in accordance with the history of drug use (Publication 7).

In recent years, hair was suggested as a valuable specimen in drug-facilitated crimes (such as sexual assault or robbery) where, as a result of a delay in reporting the crime or in apprehending the suspect, natural processes have eliminated the drug from typical biological specimens. Benzodiazepines are the most frequently used drugs in drug-facilitated crimes. Estazolam is a benzodiazepine-type drug that is used mainly in China in treating insomnia. It possesses anxiolytic, anticonvulsant, sedative, and skeletal muscle relaxant properties. It has been shown in some cases to be more potent than diazepam or nitrazepam. Most of studies on estazolam were from Asian countries with a few reports from Western countries.

Volunteer experiments were designed to investigate the time course of estazolam in hair. 14 volunteers had ingested a single 1-6 mg estazolam tablet. Hair was collected one month after administration. The results are shown in Table 3. All the proximal segments (0-2 cm) were positive for estazolam. There was a good correlation between the dosage and 0-2 cm segment concentration with narrow variations for subjects at the same dosage. With increased dosage, estazolam can be detected in the 2-4 cm segments in some subject's hair. Even some of 4-6 cm segments were positive (Publication 26).

On the other hand, hair analysis was applied to authentic criminal cases with victim 1 (V#1) and victim 2 (V#2). Full-length hair samples collected 5 weeks after the offense were cut into segments of 2 cm from the root, analyzed and quantified. The clonazepam concentrations measured in the first two segments for V#1 and V#2 was 15.47 and 11.93 pg mg^{-1}, respectively. However, both the 4-6 cm and the 6-8 cm segment of V1# remained positive, while those of V#2 were negative (Publication 26).

There was a significant difference between some findings (Kintz, 2007; Villain, 2005; Villain, 2004) and our results. Although experimental error due to collecting and sectioning the hair samples might have been a factor, the results of successive 0.5 cm segments was consistent with that of 2 cm segments (Fig. 13). In addition, there was an increase of clonazepam and 7-aminoclonazepam concentrations at the corresponding time according to normal hair growth rate. Therefore, we believe that the segment data presented above is creditable on the whole.

Table 3 Concentration of Estazolam in Hair One Month after a Single Dose

No.	Age	Sex	Weight (kg)	Hair Length (cm)	Dosage (mg)	Concentration (pg mg^{-1})			
						0-2 cm	2-4 cm	4-6 cm	distal 2 cm
1	26	Female	50	20cm	1	0.56	+*	-**	-
2	26	Female	44	12cm	1	0.61	-	-	-
3	24	Male	58	4cm	1	+	-	/***	
4	27	Male	57.5	2cm	2	0.67	/	/	
5	24	Female	80	20cm	2	1.45	-	-	-
6	27	Male	60	4cm	2	1.11	-	/	
7	27	Male	57.5	4cm	4	1.52	0.77	/	
8	26	Female	44	12cm	4	1.12	0.83	+	-
9	24	Male	58	4cm	4	2.45	0.71	/	
10	26	Female	47	15cm	4	0.94	+	+	-
11	23	Female	42	22cm	4	1.49	+	-	-
12	25	Male	59	4cm	5	2.60	+	/	
13	26	Male	60	4cm	5	2.28	+	/	
14	27	Male	59	4cm	6	1.94	+	/	

*: + Detected, but below LOQ; **: - Not detected; ***: / Sample is absent.

The difference results may be based on inter-individual differences in dosage, blood concentrations, drug incorporation rates, hair pigmentation, physical state of the hair, age, gender, body weight, etc (Musshoff, 2007; Wennig, 2000). Another explanation for broadening the band of positive hair from a single dose is that drugs and metabolites are incorporated into hair during formation of the hair shaft via diffusion from sweat or other secretions (Kintz, 2007; Cone, 1996; Henderson, 1996; Raul, 2004). Until these mechanisms of drug incorporation into hair are better understood and the reasons for the intersubject variability clarified, more substantial guidelines to use segmental hair analysis in drug-facilitated crime will be needed.

(h) Physiological concentrations of anabolic steroids in human hair

Abuse of endogenous (i.e. naturally occurring) steroids is one of the most important issues in sport. Testosterone, epitestosterone, androsterone, etiocholanolone, and dehydroepiandrosterone (DHEA) are the major endogenous steroids. The measurement of levels of anabolic steroids in human hair is necessary in order to distinguish between pharmaceutical steroids and natural steroids. Currently, there is little data on the physiological concentrations of endogenous anabolic steroids in human hair, and there is no data on these concentrations in Asians.

A gas chromatography–tandem mass spectrometry (GC-MS/MS) method was developed for the simultaneous identification and quantification of five endogenous anabolic steroids (testosterone, epitestosterone, androsterone, etiocholanolone, and dehydroepiandrosterone) in hair. After basic hydrolysis, hair samples were extracted with

diethyl ether, derivatized, and then detected using GC-MS/MS in the multiple-reaction monitoring mode (MRM). The limits of detection for the five endogenous anabolic steroids were in the 0.1-0.2 pg mg^{-1} range. This method was applied to the analysis of testosterone, epitestosterone, androsterone, etiocholanolone, and dehydroepiandrosterone in human hair. Full-length hair samples were taken at the skin surface from the vertex of 39 males, 30 females and 11 children from China. None of the subjects were professional athletes. Testosterone and dehydroepiandrosterone were detected in all the hair segments. The physiological concentrations of testosterone were in the range 0.8-24.2 pg mg^{-1}, 0.1-16.8 pg mg^{-1} and 0.2-11.5 pg mg^{-1} in males, females and children, respectively, but the mean values of dehydroepiandrosterone were much higher than the concentrations of testosterone.

Table 4 Level of Testosterone in Hair

	Subject	Age(years）	n	Mean(pg mg^{-1})	Range(pg mg^{-1})
Our research	male	18－61	39	7.4	0.8-24.2
	female	19－80	30	5.3	0.1-16.8
	children	2-17	11	2.6	0.2-11.5
Deveaux(2001)]	male	18-50	12	10.7	3.6-23.3
	female	23-54	9	3.6	1.7-6.4
	children	5-14	4	1.7	0.6-2.7
Scherer(1998)]	male		6	2.7	2.5-4.2
	female		6	1.7	1.0-3.4
Kintz(1999)	male	16-63	26	3.8	1.2-11.4
Kintz(1999)	male	17-42	15	2.7	0.5-9.8
	female	17-42	12		ND-2.4
Wheeler(1998)	male	21-65	22	14.8	5.6-33.6
	female	17-55	19	1.8	0.4-4.7
	children	5-10	6	2.1	

The level of testosterone in the hair of Chinese subjects was similar to the level of testosterone in people of different races at the pg mg^{-1} level. A comparison of the physiological values found in this study and those found in other scientific literature is summarized in Table 4. Kintz *et al.* (1999) reported that hair from two bodybuilders were positive for testosterone (46 and 71 pg mg^{-1}). It seems that the concentrations of testosterone in the hair of steroid abusers are higher than physiological concentrations.

The levels of epitestosterone, androsterone, and etiocholanolone in hair were comparatively low. With the exception of a few samples, the concentrations of epitestosterone, androsterone, and etiocholanolone in most of the hair samples were 0 – 8.7 pg mg^{-1}, 0 – 20.2 pg mg^{-1} and 0 – 23.5 pg mg^{-1}, respectively. There have been no reports on the physiological concentrations of epitestosterone, androsterone, and etiocholanolone in human hair. However, the levels of androsterone and etiocholanolone were found to be higher than that of testosterone in urine. Based on the fact that the degree of drug disposition in hair closely relates to its lipophilicity, the difference between urine and hair relates to the fact that androsterone and etiocholanolone are the final metabolites of endogenous anabolic steroids in the body and are not easily incorporated into the hair because of potent polarity.

The values for testosterone in males were higher than those in females, while the concentrations between males and children were proved to be statistically significant ($p = 0.006$) (Fig. 20). Our results also show that testosterone and DHEA concentrations were significantly lower below the age of 20 (Figs. 21 and 22, $p < 0.05$).

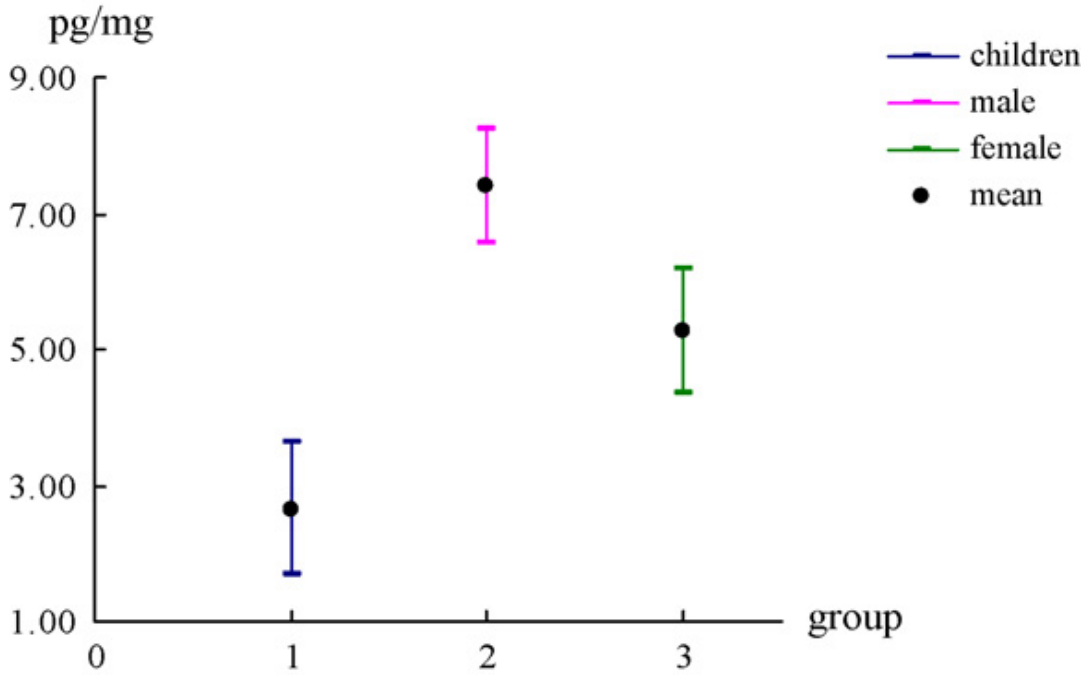


Fig. 20. Mean and standard error of testosterone concentration in human hair for the three groups (1 =children; 2=male; 3 = female).

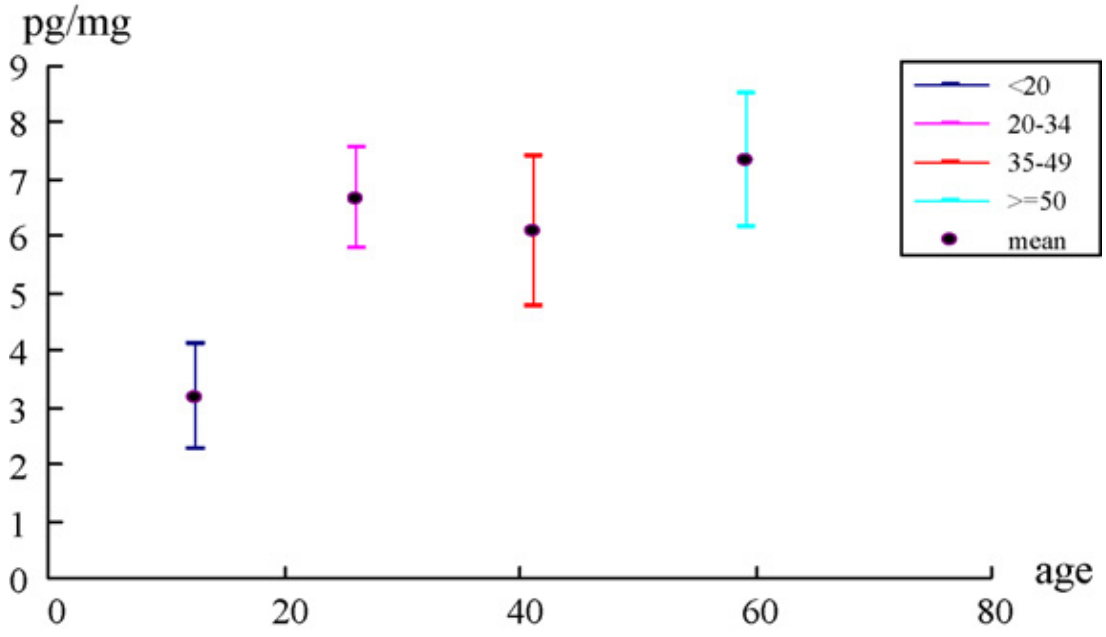


Fig. 21. Mean and standard error of testosterone concentration in human hair for different age group.

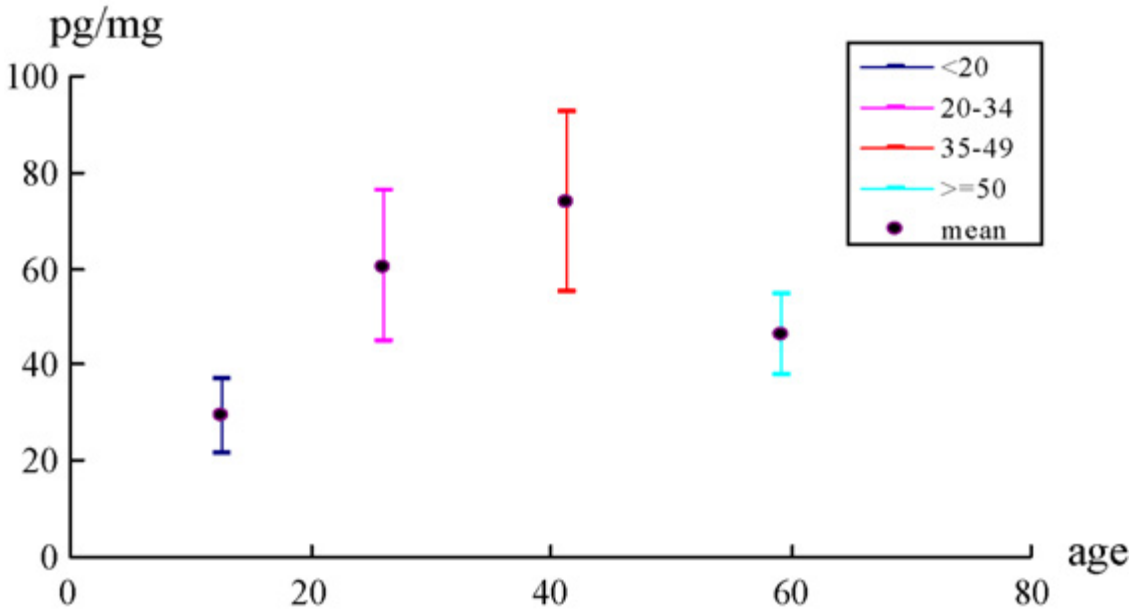


Fig. 22. Mean and standard error of DHEA concentration in human hair for different age group.

These data are suitable reference values and can form the basis for the interpretation of results from investigations into the abuse of endogenous anabolic steroids.

General Discussion

The publications present in this thesis are related mainly to drugs of abuse and hair analysis. In this general discussion, I will consider in more detail aspects arising from these investigations that relate to drug abuse, the pharmacokinetics of abused drugs, the choice of specimen for analysis, analytical methods used to determine drugs levels in samples, and interpretation of the results.

Drug abuse

Drug abuse is either the use of illicit drugs, or the abuse of prescription or over-the-counter drugs. Drug abuse is an intense desire to obtain increasing amounts of a particular substance or substances to the exclusion of all other activities and can hence lead to drug dependence or addiction. Drug dependence is the body's physical need, or addiction, to a specific agent. Over the long term, this dependence results in physical harm, behavior problems, and association with people who also abuse drugs. Stopping the use of the drug can result in a specific withdrawal syndrome.

Drug abuse is a common societal problem that plagues all ethnic groups and social classes worldwide. Drug abuse not only affects the abuser directly, but also affects his or her family, and can very well affect the greater society. Some abused substances are not ordinarily thought of as ‘drugs’. Alcohol is the best example. The fact is, however, that alcohol abuse costs society an enormous amount in various ways such as through traffic accidents, broken families, crime, lost productivity, and so on (Gaensslen, 2008).

Commonly abused classes of drugs include opioids and morphine derivatives (heroin, morphine, codeine, fentanyl, etc), stimulants (amphetamines, cocaine, nicotine, etc) depressants (benzodiazepines, GHB, barbiturates, etc), dissociative anesthetics (ketamine, PCP, etc), hallucinogens (LSD, mescaline, psilocybin, etc), cannabinoids, other compounds (anabolic steroids, etc), and alcohol (NIDA, 2010).

Heroin, amphetamines and ketamine are the most major popular drugs of abuse in China. According to the World Drug Report 2010 from the United Nations Office on Drugs and Crime (UNODC) (UNODC, 2010), Chinas 2.2 million heroin users, the largest population in absolute terms, were estimated to consume some 45 mt of heroin in 2008. There is also an increase in 'ecstasy' use, amphetamines-group substance use and cocaine use in China.

Based on the prevalence and developing trends of drugs in China, the studies in this thesis were designed to focus on opiates (Publications 1, 2, 8, 10, 12, 23), amphetamines (Publications 3, 4, 8, 11, 12, 23), ketamine (Publication 8, 9, 11, 13, 27), cocaine (Publication 18), cannabinoids (Publications 6, 23), antidepressant and antipsychotic drugs (Publications 5, 7, 20, 25, 26) and anabolic steroids (Publications 14, 15, 16, 17, 19, 21, 22, 24).

The pharmacokinetics of abused drugs

Study of the pharmacokinetics of abused drugs aids investigators in addiction medicine,

forensic toxicology, and clinical pharmacology in understanding why particular drugs are abused, identifying factors that affect their potential for abuse, and how their use can be detected and monitored over time. It also provides a rational, scientific basis for treatment therapies (Karch, 2008). Table 5 provides a summary of the volume of distribution for selected drugs of abuse (Drummer, 2004).

Heroin and other opioids

Heroin is converted within minutes in the body to morphine through the intermediate acetylmorphine (6-MAM) (Drummer, 2004). All species are active pharmacologically, although both heroin and 6-MAM are only present in blood and tissues for a relatively short period. Morphine is often the dominant active species in cases and is removed from the body by metabolism to 3- and 6-glucuronides and subsequent excretion in urine and bile. Morphine is rapidly excreted in urine as glucuronides, with up to 85% of the dose recovered in urine within 24 hr. Only small amounts of morphine are excreted unchanged (2-10%). Up to 80% of a dose is excreted in the urine within 24 hr, mainly as morphine-3-glucuronide, with about 5 to 7% of the dose as free morphine, 1% as 6-MAM, 0.1% as unchanged drug, and trace amounts of other metabolites; after inhalation, 14 to 20% of the dose appears in the urine; morphine metabolites are excreted in the bile (Karch, 2008). The presence of 6-MAM in urine distinguishes heroin use from morphine. Small amounts of codeine are also present in the urine of heroin users because of the presence of acetylcodeine in the heroin.

Table 5 Pharmacokinetic Properties for Selected Drugs of Abuse

Drug/drug class	Common dose(mg)	Usual blood levels*	Main active metabolite or bio-marker**	Vd(l kg^{-1})	T1/2(h)
Amphetamine	10-100	0.2	None	3-5	4-30
Methamphetamine	50-2000	0.2	Amphetamine (~%)	3-4	10-30
MDMA	50-250	0.3	MDA	Moderate	~8
MDA	50-250	0.4	None	Unknown	-
Heroin	10-100	-	Morphine, 6-MAM	See morphine	<0.1
Morphine	10-100	0.5	None, but bio-conversion from glucuronides	2-4	2-4
Codeine	8-60	0.2	Morphine (10%)	4	2-4
Meperidine	50-200	1.0			
Flunitrazepam	1-2	0.05	7-Amino-flunitrazepam	3-6	11-25
cocaine	10-100	0.5	Benzoylecgonine,EME	1-3	0.6
THC	5-25	50	11-Carboxy-THC	9-11	19-96

* Maximum postmortem blood concentrations following usual doses, although higher concentrations can be achieved in particular situations. ** Bio-marker of parent drug.

The Society of Hair Testing (SOHT) proposed that heroin consumption has to be assumed when the ratio of 6-MAM and morphine is greater than 1.3. Heroin use has to be

differentiated from codeine or morphine use by the presence of 6-MAM in hair, with a recommended limit of quantification of 0.2 ng mg^{-1} hair using chromatographic techniques (Kintz, 2007). Usually, the concentrations of basic drugs and metabolites were much higher in pigmented hair than in non-pigmented hair (Rollins, 2003; Scheidweiler, 2005). As shown in Fig 23, there will be electrostatic interactions between 6-MAM and melanin with strong polyanionic properties of melanin.

In our previous study (Publication 1), which analyzed seven hair specimens of individuals with presumed drug abuse, 6-MAM was determined at a concentration of 0.44- 4.8 ng mg^{-1}, whereas morphine was at 4.8-35.5 ng mg^{-1}. Overnight acid hydrolysis using HCl 0.1M was responsible for the difference, with that an unstable compound 6-MAM changed into morphine. Now pulverization in a freeze mill and ultra sound bath are applied in hair analysis to prevent this (Publications 26, 27).

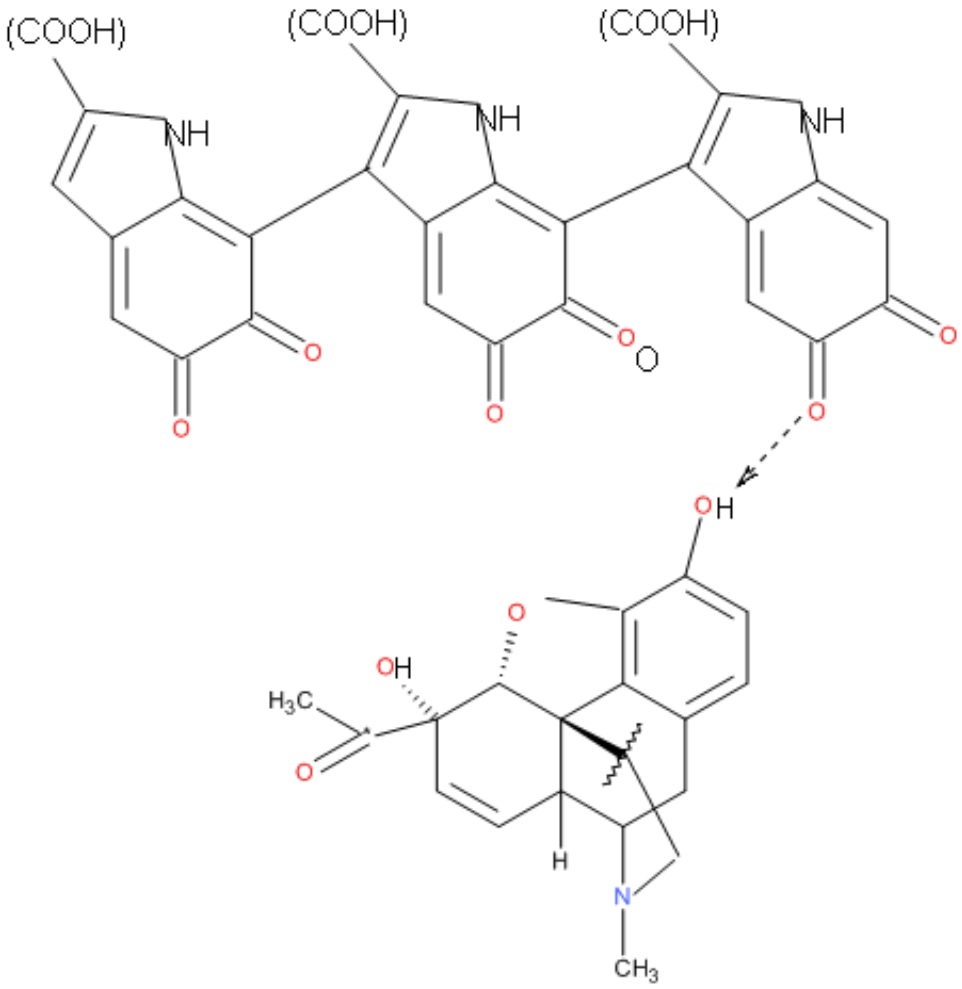


Fig. 23. Hypothesised binding between 6-MAM and a sub unit of melanin.

Amphetamines

This group of strong stimulants is based on the dexamphetamine nucleus, and includes methamphetamine, MDMA (ecstasy), methylenedioxy-amphetamine (MDA), methylenedioxyethylamphetamine (MDEA), and other designer forms. Their pharmacokinetics and metabolism are diverse, as suggested from their chemical names (Drummer, 2004).

Oral use of methamphetamine typically produces postmortem blood concentrations of up to about 0.2 mg L^{-1}. Corresponding blood concentrations of its active metabolite amphetamine are similar or slightly higher than methamphetamine (Drummer, 2004). Oyler et al. (2002) described the appearance of methamphetamine and amphetamine in urine after volunteers (n=8) ingested 4×10 mg doses of methamphetamine hydrochloride daily for 7 days followed by 4×20 mg daily several weeks later. Parent and metabolite were generally detected in the first or second void (0.7–11.3 hr) collected after drug administration, with concentrations of 82–1827 ng ml^{-1} and 12–180 ng ml^{-1}, respectively. Peak methamphetamine urine concentrations (1871 to 6004 ng ml^{-1}) occurred within 1.5 to 60 hr after a single dose.

MDMA produces peak levels of 0.4 mg L^{-1} at 2 hr following an approx. 100 mg dose. Under conditions of a single oral dose of MDMA, little MDA is detectable in blood (Helmlin, 1996).

Villamor *et al.* (2005) developed a new GC-MS method for the simultaneous identification and quantitation of amphetamines. 24 hair specimens tested positive for one

or more amphetamines, with average concentrations of 0.88 ng mg^{-1} for amphetamine, 10.14 ng mg^{-1} for methamphetamine, 1.30 ng mg^{-1} for MDA, and 8.87 ng mg^{-1} for MDMA.

Publication 4 was designed to determine the time courses of deposit of methamphetamine in guinea pig hair. Methamphetamine and amphetamine concentrations reached their peaks on the 2nd day after administration and then decreased rapidly. The concentration of methamphetamine was significantly greater than that of amphetamine in the same segments.

Amphetamines and related designer drugs are weak bases with a relatively low molecular weight, allowing them to easily diffuse across the membranes before incorporation into hair (Kintz, 2007). On the other hand, organic amines have high melanin affinity. These substances are positively charged at physiological pH and interact through the melanin polymer by electrostatic forces between their cationic groups and the anionic carboxylic groups on the surface of the melanin polymer, as shown in Fig 24. The electrostatic binding of the substances is strengthened by van der Waals forces between aromatic indole rings in the melanin polymers and aromatic rings of the organic amines.

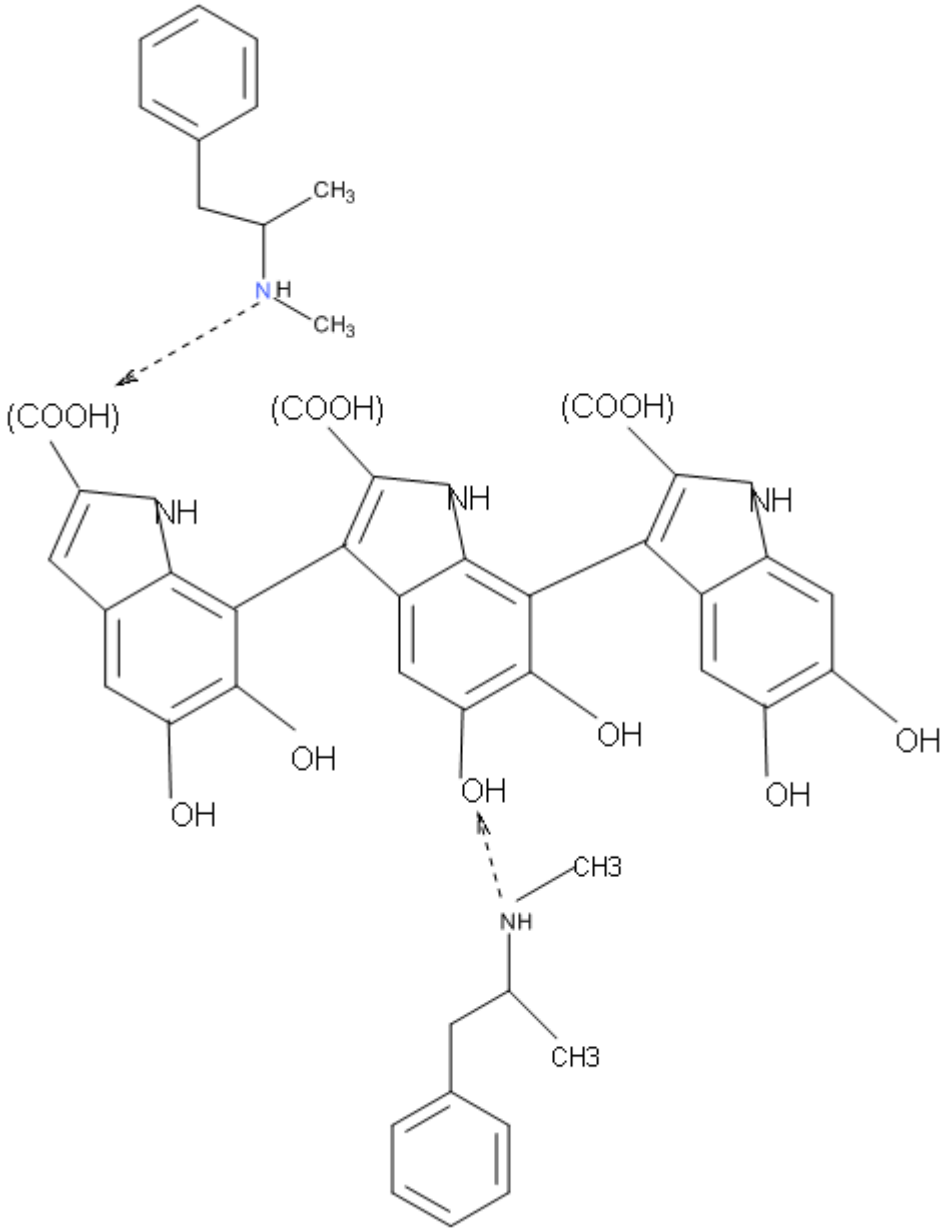


Fig. 24. Hypothesised binding between methamphetamine and a sub unit of melanin.

Ketamine

After intravenous administration of 175 mg/70 kg to five individuals, the average peak serum concentration was 1.0 mg L^{-1} achieved at 12 minutes after administration (Wieber, 1975). The concentration declined by 50% within 30 minutes. The plasma half-life is reported to be 3 to 4 hr with a volume of distribution of 3 to 5 L kg^{-1} (Baselt, 1995). Ketamine undergoes an extensive liver metabolism by CYP-450 N-demethylation to norketamine (NK). The cyclohexanone ring also undergoes oxidative metabolism to form the second metabolite dehydronorketamine (DHNK) (Reich, 1989). Approximately 2% of a single dose of ketamine is excreted in the 72-hr urine as unchanged drug.

In Publication 13, the concentration of ketamine deposited in black hair was found to be the highest while the lowest in white hair samples. Ketamine is basic compound that contain the amino group. The basic compound is protonated to a great extent. As melanin is a polyanionic polymer, melanin and ketamine interact strongly and facilitate its incorporation in hair through an electrostatic interaction, as shown in Fig 25.

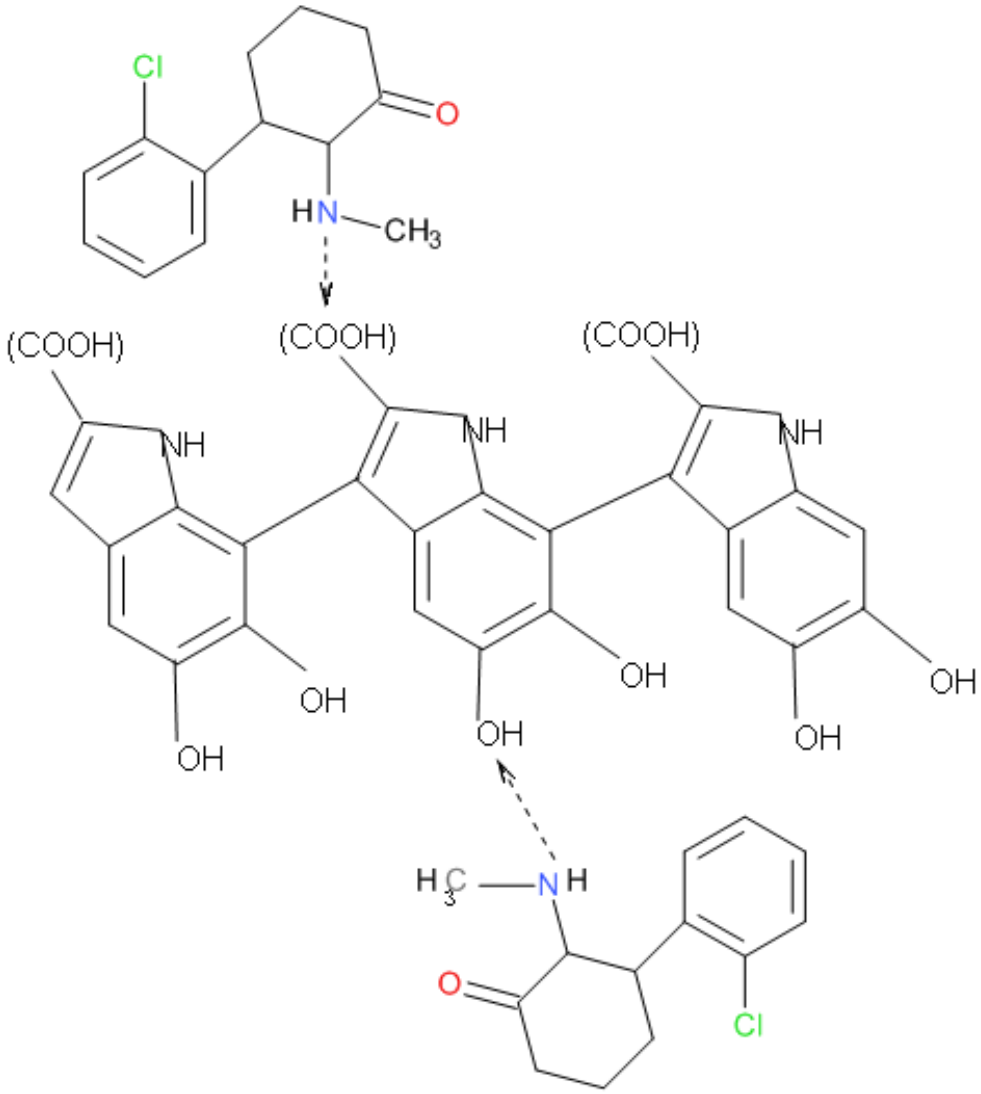


Fig. 25. Hypothesised binding between ketamine and a sub unit of melanin.

Cocaine

The terminal elimination half life of cocaine ranges from about 40 minute to 4 hr, depending on dose (Cone, 1995). Cocaine is rapidly metabolized to a range of hydrolytic substances of which benzoylecogine (BZ) and ecgonine methyl ester (EME) are most significant. The biologically active ethyl trans-esterification analog cocaethylene (CE) is found in significant amounts in tissues of persons co-consuming ethanol. BE and EME are commonly used to identify past use of cocaine when the parent drug is no longer present in blood. The predominant species in urine are BE and EME, although about 1–9% is cocaine.

Ropero-Miller *et al.* (2000) demonstrated dose-related concentrations of cocaine and metabolites in human hair after controlled subcutaneous cocaine administration. The study was performed with eight volunteers enrolled in a 10-week inpatient clinical study. Drugs were not administered during the first three weeks. Beginning in week 4, subjects were administered a low dose of cocaine hydrochloride (75 mg/70 kg) by subcutaneous injections on Monday, Wednesday, and Friday. Following low dosing, subjects were administered placebo doses subcutaneously during weeks 6 and 7. Beginning in week 8, subjects were administered a high dose of cocaine hydrochloride (150 mg/70 kg) on alternative days. Hair specimen collection was continued for two weeks after high dosing. There was considerable intersubject variability in the maximum concentration for low doses (ranging from 1.7 to 15 ng mg^{-1}) and for high doses (ranging from 5.1 to 27 ng mg^{-1}); nevertheless, in the same person, the mean peak concentration in hair after low dosing was around half the concentration observed after high-dose administration.

After a single dose of cocaine (0.4 mg kg^{-1} or 10 mg kg^{-1}) administration for guinea pigs, the newly grown hair segments were collected in two weeks. Cocaine and BZE appeared in hair in the first week after drug administration. Compared with the second week's results, the concentrations in the first week were much higher, which indicated that the majority of cocaine and BZE was incorporated into hair in the first week. When it came to the second week, BZE could no longer be detected, while cocaine still had a lower concentration in hair samples (Publication 18).

Interpretation of hair drug analyses is complicated by the extent of hair pigmentation. Cocaine, as a basic drug, has a greater affinity for melanin, as shown in Fig 26. Stout (1999) examined cocaine, which was administered to pigmented and nonpigmented animals and then the distribution of cocaine into the hair was examined by autoradiography of skin sections containing developing hairs. This study demonstrates association of cocaine with melanosomes in the forming hair shaft after systemic administration. This relationship is confirmed by analysis of mature hair, which shows significantly more incorporation of cocaine in pigmented hair than nonpigmented hair.

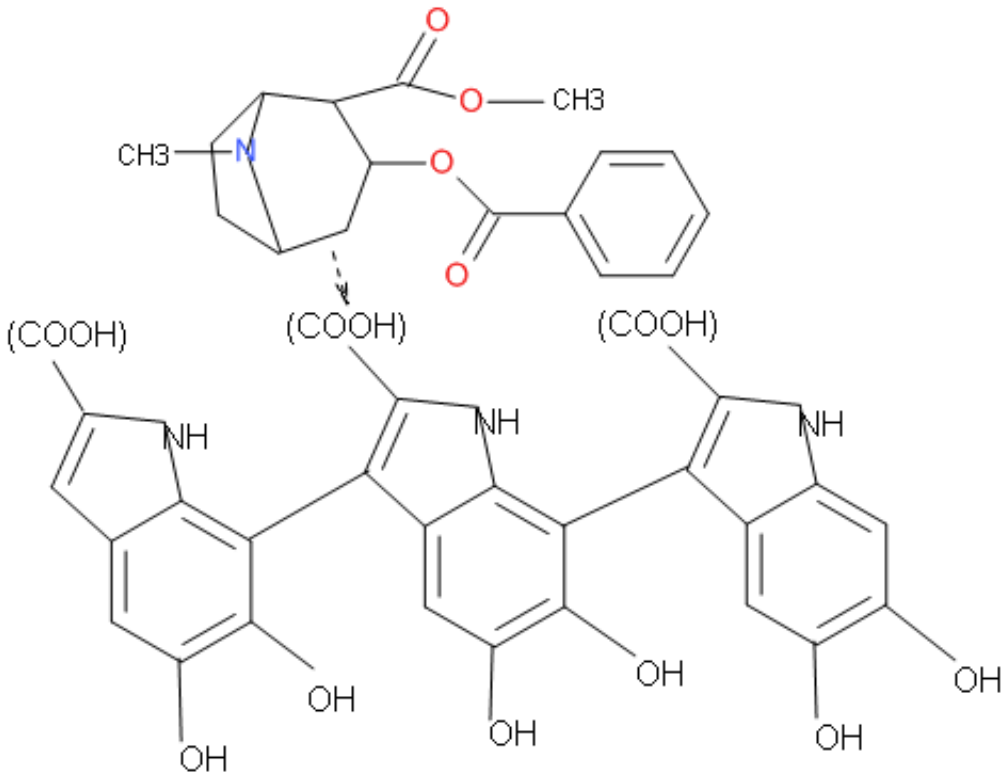


Fig. 26. Hypothesised binding between cocaine and a sub unit of melanin.

Cannabis

The major psychoactive constituent of marijuana is delta-9-tetrahydrocannabinol (THC). Peak THC plasma concentrations in blood rapidly exceed 50 ng ml^{-1} within 15 minutes of smoking and can reach 200 ng ml^{-1} with higher THC-content cigarettes (Huestis, 1992; Perez-Reyes, 1982). THC is rapidly distributed to fat and muscle due its low water solubility, resulting in a rapid decline in plasma THC concentrations. The half-life of this distribution phase is less than 1 hr and plasma THC concentrations greater than 10 ng ml^{-1} are uncommon after 1 hr even after moderate to high doses of cannabis (Huestis, 1992).

The two monohydroxy metabolites, 11-hydroxy (OH)-THC and 8-beta-hydroxy THC, are also active, with the former exhibiting similar activity and disposition to THC, while the latter is less potent. Oxidation of 11-OH-THC produces the inactive metabolite, 11-nor-9-carboxy-THC, or THC-COOH. This metabolite may be conjugated with glucuronic acid and is excreted in substantial amounts in the urine. Approximately 70% of a dose of THC is excreted in the urine (30%) and feces (40%) within 72 hr (Widman, 1975). Huestis *et al.* (1996) reported a mean (± SEM) urinary excretion half-life for THC-COOH of 31.5 ± 1 hr and 28.6 ± 1.5 hr for six healthy volunteers after administration of a single marijuana cigarette containing 1.75 or 3.55% THC, respectively.

Racial bias has been reported for basic drugs such as cocaine in hair, supported by studies that found a higher concentration of cocaine in hair from black males than Caucasian males (Kintz, 2007). Cannabinoids were not among the compounds studied. Smeal (2006) concluded that pigmentation did not appear to be a factor in the incorporation of THC and THCCOOH in hair. Huestis (2007) also indicated no difference in detection rates and no statistical difference in THC or THCCOOH concentrations between African-American and Caucasian subjects).

The choice of specimen

The choice of specimen is often dictated by the case being investigated. Modern forensic toxicology can be divided into three major categories: post-mortem forensic toxicology, human performance forensic toxicology and forensic urine drug testing. According to the

Society of Forensic Toxicologists (SOFT) (www.swgtox.org) , post-mortem forensic toxicology determines the “absence or presence of drugs and their metabolites, chemicals such as ethanol and other volatile substances, carbon monoxide and other gases, metals and other toxic chemicals in human fluids and tissues, and evaluates their role as a determinant or contributory factor in the cause and manner of death.” Human performance forensic toxicology, also known as behavioral toxicology: “Determines the absence or presence of ethanol and other drugs and chemicals in blood, breath or other appropriate specimen(s) and evaluates their role in modifying human performance or behavior.” Forensic urine drug testing: “determines the absence or presence of drugs and their metabolites in urine to demonstrate prior use or abuse.”

Blood is the sample of choice for quantification of drugs, because pharmacological or toxic affects correlate best with their concentrations in this body fluid, if equilibrium distribution with all organs and tissues has been reached. Generally, blood is the most useful sample, and the most readily available specimen. A positive finding may indicate very recent drug use or exposure, for many substances disappear from blood circulation fairly rapidly. Determination of the drug blood concentration is sufficient in cases where a drug concentration is clearly in the fatal range and poisoning is evident from the circumstances surrounding death. Blood is also the appropriate specimen in cases of some prescription medications to verify whether or not the deceased was taking any such medication. In cases of driving under the influence of drugs (DUID), blood analysis is even mandatory in some countries (Maurer, 2005).

Urine has a great potential to provide information on antemortem drug exposure since the accumulation of drugs and metabolites in urine usually results in high concentrations facilitating detection of drug use or exposure. Urine is usually the specimen of choice in forensic urine drug testing. Generally, there is no correlation between urine drug concentration and pharmacological effects, and urine may not always be indicative for acute poisoning. Negative urine findings may be obtained when death had occurred very rapidly.

Hair is a valid specimen and is accepted in most courts of justice. Specimens can be more easily obtained with less embarrassment. Hair analysis has been used extensively for the analysis of drugs of abuse to provide evidence of longer term exposure (or abstinence) of drugs and can provide important information as to the time course of drug use to establish a history of drug exposure (Publications 1, 2, 3, 5, 6, 7, 13).

In fact, blood, urine and hair specimens complement each other. Urinalysis and blood analysis provide short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis. For example, in drug-facilitated crime cases, because of amnesia caused by drugs, there will be a 24-72 hr or longer delay between a victim's report and the drug's ingestion. In addition, drugs used can be difficult to detect because low does were administered, or the active metabolite is chemically unstable. Some drugs are quickly cleared from the body fluids. Therefore, in such circumstances, blood, and even urine samples are often of limited usefulness in detecting a drug's presence. To prolong the window of detection, hair analysis has been proven to be a solution. Actually, many publications, including Publications 26 and 27, on

drug-facilitated crime cases, have demonstrated the usefulness of hair analysis in documenting the involvement of drugs.

Testing methodologies

Efficient toxicological analysis is the basis of competent toxicological judgment and consultation in clinical and forensic toxicology and for drugs of abuse too. As the drugs which have to be analyzed are mostly unknown, the first step, before quantification, is the screening and the identification of compounds of interest. In laboratories with the capacity for throughput of large sample numbers, immunoassay drug testing kits are used as screening devices. Because of the serious clinical or forensic consequences, high selectivity and reliability are demanded.

Gas chromatography-mass spectrometry (GC-MS) is still the most widely used reference method in analytical toxicology, but liquid chromatography coupled with single-stage or tandem mass spectrometry (LC-MS, LC-MS/MS) is becoming increasingly important in routine analysis, especially for quantification of the analytes identified (Maurer, 2005). LC-MS/MS allows for analysis of biological specimens with minimal sample preparation, where high sensitivity, specificity, precision, and high throughput are required.

With the development of analytical techniques, the work described in this thesis on hair analysis was able to make quick progress. The limits of detection for drugs in hair were decreased from nanograms per milligram using GC-MS methods (Publications 1, 2, 3, 6, 7, 9, 13) to picograms per milligram range with LC-MS/MS methods (Publications 15, 16,

18, 19, 21, 24, 25, 26, 27). With sensitive LC-MS/MS methods, it is possible to track a single drug exposure using segmental hair analysis (Publications 18, 24, 25, 26, 27).

Recommendations

Although hair analysis has been proved to be a useful adjunct to conventional (e.g. urine and blood sample based) drug testing in forensic science and in clinical applications, there is still a need to further define the interpretation of results, particularly concerning external contamination, cosmetic treatments, ethnical bias or drug incorporation. Research in the candidate's team is currently involved with the following:

(a) Developments in analytical techniques;

(b) Quality assurance in hair analysis;

(c) Drug incorporation and retention in hair;

(d) Strategies in segmental hair analysis.

General Conclusions

The main conclusions from the publications presented in this thesis can be summarised as follows:

1. A series of sensitive, specific, and reproducible methods for drugs of abuse in hair have been developed using LC-MS/MS and GC-MS/MS. These methods are able to screen and quantify drugs of abuse in hair, especially useful applications in the monitoring of a single dose or to document exposure in forensic cases involving drug-facilitated crimes. These sets of conclusions are justified fully in publications 1, 5, 8, 10-12, 14, 15, 17, 21, 23, and are listed on page 24 and 25.

2. From experiments using guinea pigs, the concentrations of drugs and their metabolites in hair were found to be related to dosage, melanin affinity, and lipophilicity. In our experiments, the presence of hair with different colours on the same guinea pig effectively minimized variability due to individual differences and other inter- and intra-factors such as living condition, age and health condition. These sets of conclusions are justified fully in publications 4, 9, 13, 16, 18, 19, 24, 25, and are listed on page 24 and 25.

3. Segmental hair analysis was found to be appropriate for investigations into drug-facilitated crimes (DFC), and effectively increased the window of detection. Our research subjects were all Chinese subjects with black hair. Hair was collected 1 month after administration. All the proximal segments were positive for benzodiazepines. With

increased dosage, drugs can be detected in the 2-4 cm segments in some subjects' hair. Even some of 4-6 cm segments were positive. It needs more substantial guidelines to use segmental hair analysis in drug-facilitated crime investigations. Thess sets of conclusions are justified fully in publications 20, 26, 27, and are listed on page 25.

4. For anabolic steroids, the major components in hair are the parent drugs. The time courses of the concentrations of the steroids in hair (except methenolone, which does not deposit in hair) demonstrated that the peak concentrations were reached on days 2-4, except stanozolol, which peaked on day 10 after administration. The concentrations in hair appeared to be related to the physicochemical properties of the drug compound and to the dosage. This is the first investigation into the physiological concentrations of anabolic steroids in human hair in Chinese subjects. These sets of conclusions are justified fully in publications 22, 24, and are listed on page 25.

5. Studies on the distribution of drugs in the hair shaft and the time course of distribution will have important implications for developing the criteria for the appropriate time and method of collecting hair samples. The degree of disposition of drug in hair closely relates to its affinity with melanin, physicochemical properties of drugs, and dosage. Such studies also provide basic data that will be useful in the application of hair analysis for drugs of abuse and in the interpretation of results. These sets of conclusions are justified fully in publications 2-4, 6, 7, 10, 11, 13, 16, 25-27, and are listed on page 24 and 25.

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Estimated Contribution Made by Candidate on Each Publication

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Determination of heroin matabolites in biological fluids, tissues and hair of addicts using GC/MS-SIM

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Abstract:

Introduction: Heroin abuse is undoubtedly the most serious current drug problem in China. In forensic practise the simultaneous quantification of opiates in biological human samples is important to elucidate the cause of death and to evaluate their role in modifying human behaviour.

Aims: The purpose of the current study was to determine the concentrations of morphine in body fluids, tissues and hair from heroin delayed death.

Methods: Body fluids, minced tissue was mixed with 5 μl 0.2mg/ml ethylmorphine (IS) and hydrolyzed with strong acid. After hydrolysis, the pH of the solution was adjusted to pH9.2 with NaOH. The sample was then mixed with 2 ml chloroform: isopropanol (9:1), vortexed, centrifuged (3000rpm), evaporated to dryness. The residue was derivatized with 50ul acetic anhydride and 50ul pyridine for 30min at 60°C. After evaporation with a nitrogen stream at 60°C, the residue was dissolved in 25 ul methanol for GC-MS.

Hair samples were washed with 0.1% sodium dodecyl sulfate (SDS), and then rinsed with deionized water and acetone. After being air-dried, the segments were cut into 1 mm. One milliliter of HCl (0.1 M) was added to 50 mg of segments and incubated overnight at 45°C. After cooling to room temperature, the resulting digests were adjusted to pH 9.2 using 0.4% NaOH and mixed with 2 ml chloroform: isopropanol (9:1), vortexed, centrifuged (3000rpm), evaporated to dryness. The residue was derivatized with 25ul BSTFA for 30min at 70°C. After cooling down, the residue was injected to GC-MS.

Results: 7 hair samples obtained from heroin addictions were determined using developed method. The analytical results are presented in Table 1. The concentration of morphine ranged from 4.8 to 35.5 ng/mg, while negative to 4.8 ng/mg for 6-acetylmorphine. The concentration of morphine was much higher than that of 6-acetylmorphine. Dr. Kintz found higher concentrations for 6-MAM than for morphine. The differences may be due to different incubation methods of hair specimens before extraction as a result of the conversion of 6-MAM to morphine.

As shown in table 1, morphine concentrations in case 1 were 35.5ng/mg in head, 12.11ng/mg in axillary, and 21.28ng/mg in pubic hair. In case 2, morphine concentrations were similar in head and pubic hair.

Table1 results from human hair samples

No.	hair	morphine (ng/mg)	6-acetylmorphine (ng/mg)
1	head	35.5	2.24
	pubic	21.28	1.36
	axillary	12.11	-

2	head	18.11	-
	pubic	16.8	-
3	head(0-5cm)	16.51	4.8
	head(5-9cm)	-	-
4	head	10.16	1.92
5	pubic	10.0	3.28
6	head(0-2cm)	9.95	0.44
	head(2-4cm)	8.89	2.32
	head(4-6cm)	13.02	3.52
	head(6-8cm)	13.17	3.40
	head(8-10cm)	8.25	1.44
7	head(0-3cm)	14.52	4.10
	head(3-6cm)	11.67	2.76
	head(6-12cm)	5.62	1.96
	head(12-18cm)	5.65	2.44
	head(18-32cm)	4.8	2.16

Conclusion: The developed method was sensitivity and selectivity for opiates in hair. 6-acetylmorphine in hair was a suitable biomarker for illicit heroin use. Hair collected from different parts of the body may be used as a substitute for head hair in case they had no head hair.

Key words: GC/MS-SIM; heroin matabolites; biological fluids; tissues; hair

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Detection of meperidine and its metabolites in the hair of meperidine addicts

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Abstract

The presence of meperidine and its metabolites in the hair of meperidine addicts was investigated using GC–MS (EI, PCI). Meperidine and its three metabolites – normeperidine, *N*-methoxy meperidine and acetyl normeperidine, were found in hair samples from addicted subjects. Methods for the simultaneous determination of meperidine and its metabolites by GC–MS-SIM were also established for human hair samples. After the addition of d_4-meperidine as an internal standard, hair samples weighing 5 mg were incubated in 0.1 M HCl at 45°C overnight, and the resulting digests were extracted with ether. The recoveries were greater than 80%, with coefficients of variation (CVs) between 4.48 and 8.31%. The calibration curves for meperidine and normeperidine in hair were linear over a concentration range of 1 to 500 ng per mg of hair, with correlation coefficients of $r=0.9990$ and $r=0.9992$, respectively. Values less than 0.25 ng/mg of hair were cut off. Hair samples obtained from 60 drug addicts were analyzed using this method, and the content of meperidine and normeperidine was determined to be 103 ± 130 and 117 ± 143 ng/mg, respectively. Sectional analysis revealed that meperidine was present and stable in hair for at least 20 months, but normeperidine content at the level of the hair root was higher compared to the tip of the hair shaft. The results also revealed that there was a correlation between the subject's drug abuse history and the distribution of drug along the hair shaft, and between the doses of meperidine and drug content presented in hair.

Keywords: Hair; Sectional analysis; Meperidine; Normeperidine; Drug abuse; GC–MS

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1. Introduction

In the last decade, the analysis of hair for drugs of abuse gained widespread attention in the forensic and clinical communities, and was also gaining acceptance in the criminal court system as supportive evidence in forensic and drug-related cases. Until recently, the main prevalent drugs dealt with in hair analysis were cocaine, morphine, amphetamines, cannabinoids, nicotine and their respective metabolites [1]. However, an accurate interpretation of analytical results continues to be complex and difficult because of the many factors that still remain unclear. These include the following: dose–response relationship; influence of external contamination or diffusion along the hair shaft; mechanism of drug incorporation into hair and stability of the drug in hair. Although there are published studies of drug levels in hair, their results have not been consistent. The inconsistencies may be due to different drugs of abuse or different methods used in each study [2–5].

Meperidine is an anaesthetic and analgesic drug. The attention given to meperidine by toxicologists arises from its widespread abuse by addicts who become addicted after receiving this drug for medicinal purposes. Analysis of hair samples for meperidine affords a non-intrusive method for detecting and monitoring drugs over a long time period. However, there are currently no published methods for detection of meperidine or its metabolites in hair. Therefore, the goal of the present study was to develop a sensitive and accurate procedure for measurement of meperidine in human hair. Through gas chromatography–mass spectrometry (GC–MS) with selected ion monitoring (SIM), identification of meperidine metabolites in hair and simultaneous determination of meperidine and normeperidne concentrations were performed using hair samples from subjects known to be addicted. The drug levels in human hair samples from head, axillary and pubic regions were compared, and the stability and distribution of meperidine and normeperidine in hair were evaluated. The dose and subject's abuse history was seen to be associated with meperidine levels in hair. This new analytical method provides a useful tool for toxicological and forensic screening.

2. Experimental

2.1. GC–MS method

GC–MS analyses were performed using a GC–MS (Hewlett-Packard, HP 5988A) equipped with a 15 m×0.25 mm HP-1 capillary column. The instrument was operated under electron ionization (EI) and chemical ionization (CI) conditions using CH_4 as reagent gas. The column temperature was programmed from an initial temperature of 100°C (2 min) to 280°C (8 min hold) at 25°C/min. Splitless injection was employed with an off time of 1 min. The injection and interface temperatures were 250°C, and the source temperature was 200°C.

Analytes were identified and quantitated by their retention time and the relative abundance of two confirming ions to deuterated internal standards. For each drug, the following retention times (t_R values) and ions were used: meperidine (t_R 7.1 min; m/z

247, 218), normeperidine (t_R 7.3 min, m/z 233, 158), acetyl normeperidine (t_R 9.3 min, m/z 275, 158) and d_4-meperidine (t_R 7.1 min, m/z 251). The underlined ions were used for quantitation.

2.2. Hair sample collection

Drug-free hair for preparation of standards and controls was obtained from laboratory volunteers with black hair.

Meperidine hair samples, cut as close as possible from the vertex posterior of head and with root and tip ends carefully identified, were collected from 60 meperidine addicts (52 males and eight females) aged 19 to 42 years. Specimens were stored in plastic bag at ambient temperature until analysis. All special features of the hair samples were noted. Urine analysis and drug abuse histories were obtained at the time of hair sample collection.

2.3. Hair sample preparation

Root ends of the hair samples were aligned carefully cut into short sections (1–5 cm), washed with 0.1% sodium dodecyl sulfate (SDS), and then rinsed with deionized water and acetone. Samples were allowed to drain completely between each rinse and dried in a fume hood overnight before weighing.

Dried hair samples were cut into 2~5 mm segments, collected into 5-mg aliquots and incubated in 0.1 M HCl at 45°C for 18 h. After 1 μg d_4-meperidine was added, the resulting digest was adjusted to pH 11 with NaOH and extracted with ether. Extracts were evaporated under N_2 at 40°C, and then derivatized with acetic anhydride and pyridine or with trifluoroacetic anhydride by microwave irradiation for 2 to 3 min [6]. The solution was evaporated to dryness again under N_2 and then reconstituted in 25 μl methanol. A 1-μl aliquot of the solution was injected into the GC–MS system.

2.4. Calibration curves, linearity and reproducibility

The calibration curves for the measurement of meperidine and normeperidine were constructed by analysis of extracted and derivatized 5-mg samples of control hair, to which the standard solutions of meperidine and normeperidine had been added (1–500 ng per mg of hair), together with d_4-meperidine (I.S.), as mentioned above. The calibration curves for meperidine ($y=0.0113+0.0198x$) and normeperidine ($y=0.0151+0.0134x$) were linear over a concentration range 1 to 500 ng/mg of hair, with correlation coefficients $r=0.9990$ and $r=0.9992$, respectively. Values less than 0.25 ng/mg of hair were chosen as cut-off values.

The precision of the method was determined using hair of healthy volunteers spiked with meperidine and normeperidine at three concentrations with respect to the calibration curves, and three hairs positive for meperidine (six samples were analyzed for each concentration). The coefficients of variation (CVs) for meperidine and normeperidine were between 4.48 and 8.31%.

3. Results and discussion

3.1. Comparison of the extraction methods

Three main methods of hair sample preparation – ultrasonic extraction with methanol after milling, ether extraction following acid hydrolysis and following basic hydrolysis, were evaluated. The extraction efficiencies of the methods for meperidine in two spiked hairs (10 ng/mg and 100 ng/mg) and three positive hairs (Nos. 1, 2 and 3) were compared. As shown in Fig. 1, the best method was ether extraction after acid hydrolysis, which produced the highest yield of meperidine from the hairs (about 85%) and the cleanest background. Although ultrasonic extraction with methanol after milling also produced high recovery, it created more background noise with some interfering peaks and high CV values (6.5–13.7%). However, the basic hydrolysis led to the lowest recovery (about 30%). From these results, ether extraction after acid hydrolysis was selected for the extraction of meperidine from hair.

To investigate the effect of hair length on quantitative analysis, six washed hair samples of meperidine addicts (A, B, C, D, E, F) were cut into 1-, 2-, 4- and 8-mm segments. The samples were then hydrolyzed, extracted, derivatized and analyzed as described previously. The results are summarized in Table 1. These data demonstrated there was no difference in the amount of meperidine detected in hair ranging in length between 1 and 8 mm.

Theoretically, the shorter hair segments had a larger surface area, and solvent extraction should be more efficient compared to longer hair segments. However, this was not observed in the actual samples, suggesting that meperidine was readily released from all regions of the hair shaft including the medulla, cortex and cuticle.

3.2. Identification of meperidine metabolites in hair from addicted subjects

Meperidine and its three metabolites – normeperidine, *N*-methoxy normeperidine and acetyl normeperidine, which had been identified in addict urine [7,8], were found in hair samples from addicts. Fig. 2 and Table 2 show the total ion chromatogram and EI, CI mass spectra characteristics derived from the extracted hair samples.

N-Demethylation is a major metabolic pathway of meperidine, and thus normeperidine was detected in almost all hair samples of addicts, EI MS of the metabolite shows a molecular ion at m/z 233 with an abundant ion at m/z 158 ($M–C_2H_5OH–CO$). *N*-Methoxy normeperidine [m/z 261 (M), m/z 187 ($M–C_2H_5OH–CO$)] and acetyl normeperidine [m/z 275 (M), m/z 187 ($M–C_2H_5OH–CO–CH_3$)] were formed through future metabolism of normeperidine, which were not in all hair samples from addicts.

Fig. 3 shows the area ratios of meperidine and its metabolites (normeperidine, *N*-methoxy normeperidine and acetyl normeperidine in the hair samples from four subjects. There was a clear correlation between meperidine and normeperidine. In contrast, for the other two metabolites, a large inter-subject variation in meperidine metabolism was observed.

From the results, it is clear that meperidine, normeperidine, *N*-methoxy normeperidine and acetyl normeperidine all can be incorporated into hair.

Metabolism must be considered in order to prevent false-positive results by external

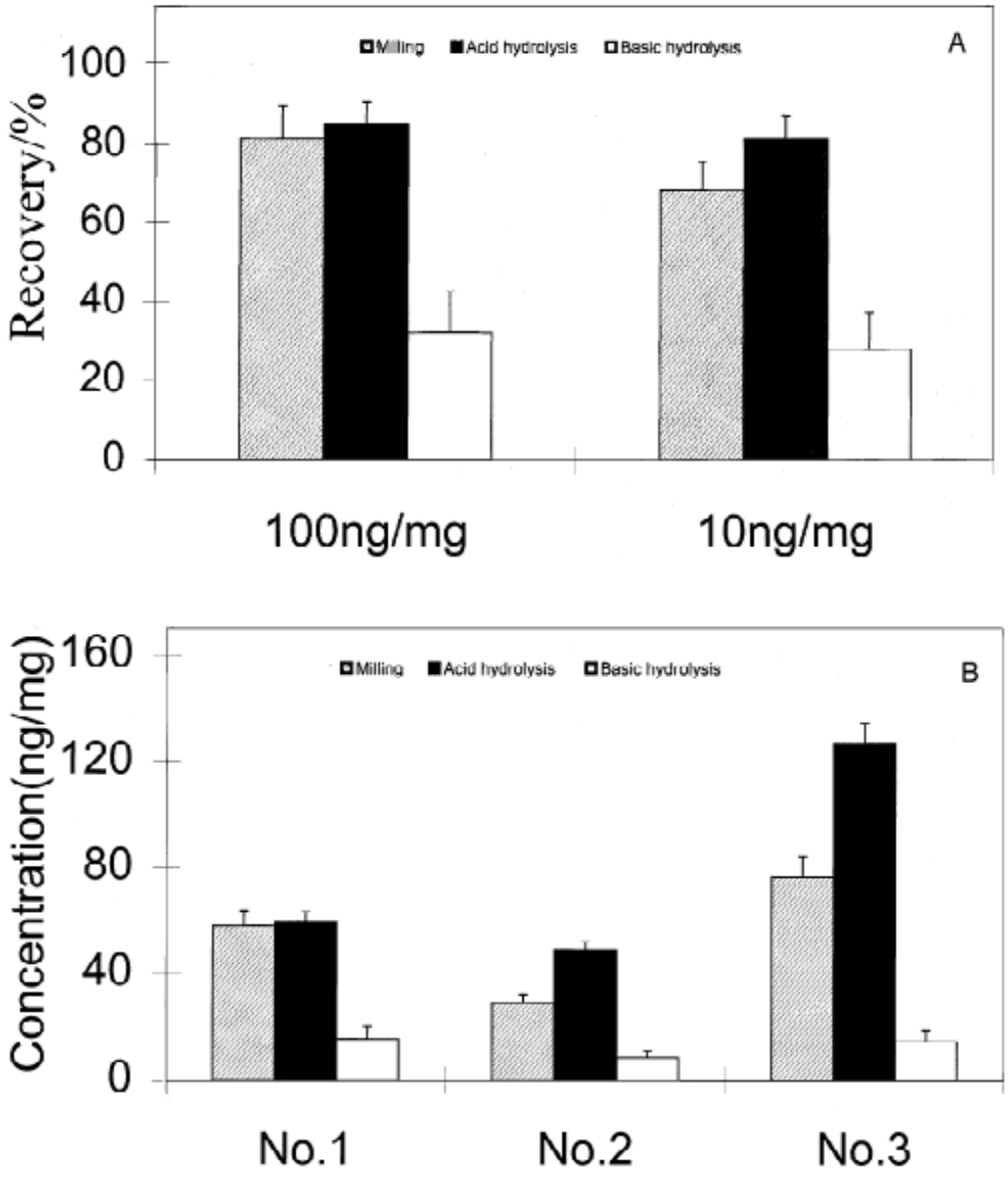

Fig. 1. The results of milling, acid hydrolysis, and basic hydrolysis. (A) Blank hair spiked with standard; (B) hair positive for meperidine.

Table 1
Effect of hair length on detected level (ng/mg)

Length (mm)	A	B	C	D	E	F
1	56.4	2.02	36.2	38.5	75.2	64.3
2	56.3	2.13	32.4	37.1	75.8	63.2
4	50.9	2.13	32.7	39.1	78.5	61.5
8	54.7	2.03	31.9	37.8	75.3	62.4

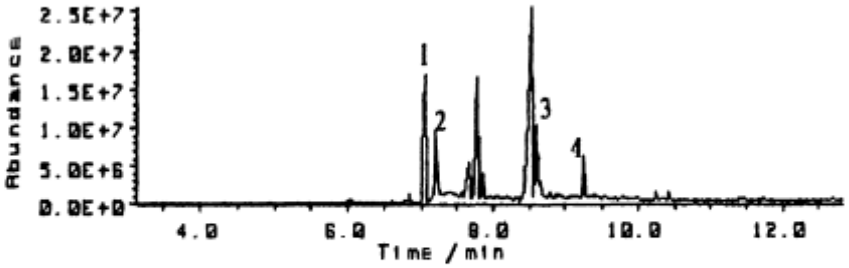

Fig. 2. The total ion chromatogram of the hair extracts. Peaks: 1=meperidine; 2=normeperidine; 3=*N*-methoxy normeperidine; 4=acetyl normeperidine.

Table 2
EI and CI MS characteristics of peaks observed in the hair extracts

No.	Compound	t_R (min)	EI (relative abundance)		CI (relative abundance)	
			Base peak	M^+	$[M+1]^+$	Second peak
1	Meperidine	7.06	71	247 (45)	248 (100)	
2	Normeperidine	7.21	158	233 (30)	234 (100)	160 (10)
3	*N*-Methoxy normeperidine	8.69	187	261 (30)	262 (100)	188 (10)
4	Acetyl normeperidine	8.88	187	275 (35)	276 (100)	202 (8)

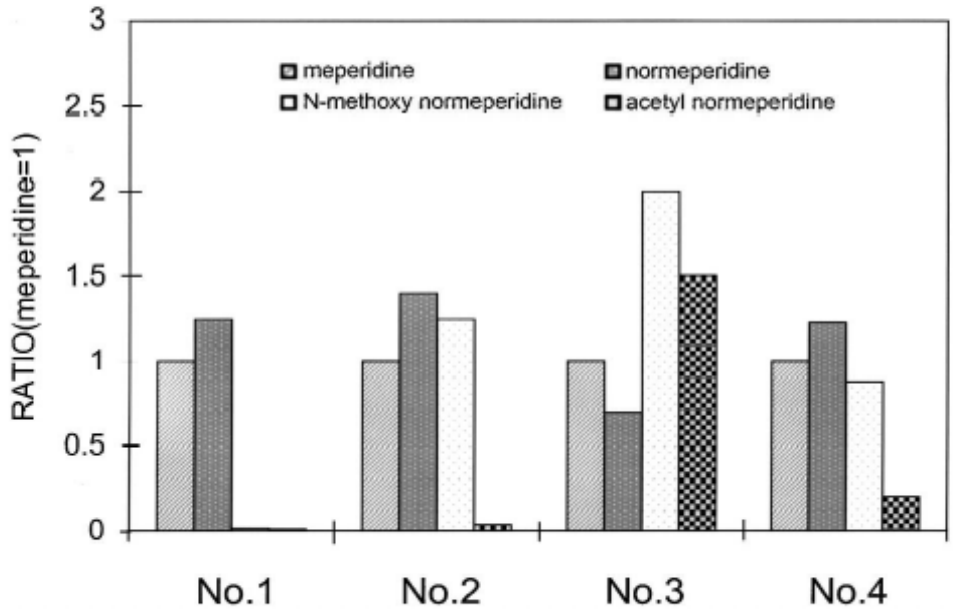

Fig. 3. The ratios of meperidine and its metabolites in hair samples from four meperidine addicts (the relative values are given when the area of meperidine is 1).

contamination. Therefore, the active abuse of meperidine by addicts must be confirmed by simultaneous detection of meperidine and its metabolite, normeperidine.

3.3. Concentrations of meperidine and normeperidine in hair samples from addicts

The hair root samples (taken 2 cm from the root) of 60 meperidine addicts were evaluated and the results summarized in Table 3. The highest values for meperidine and normeperidine were found at 496 ng/mg and 685 ng/mg, respectively, which were much higher than those reported for morphine and cocaine. The concentrations of metabolites in hair were usually larger than the parent drug, but no metabolite was found in samples where the concentration of the parent drug was below 10 ng/mg. From the high drug levels in hair and the large variation present in the results, it can be postulated that drug levels in hair are correlated with the amount of meperidine injected.

3.4. Stability of meperidine and normeperidine in hair

It is well known that the hair shaft is gradually damaged by environmental influences during hair growth. The question therefore arises as to whether the drug is present and stable in hair for a long period. For the practical purpose of hair analysis, the stability of meperidine and normeperidine in hair was investigated with the goal of being able to confirm meperidine addiction. From the results of sectional analysis, it was found that meperidine was stably present in hair.

Fig. 4 shows two typical samples. Scalp hairs from subject A and B were cut into 3-cm sections and analyzed. The meperidine concentration was seen to be constant in all hair sections was found. Therefore, it is suggested that meperidine was present and stable in hair at least for 20 months. The result is different from that reported by Nakahaka et al. [3], who found that methamphetain level in hair decreased approximately 50% five months later. However, this was not the case for normeperidine as shown in Fig. 4. In cases of equivalent meperidine dosage, normeperidine levels in the root-side sections were higher relative to the tip-side of the hair. This phenomenon could be due to further degradation of normeperidine, or to the failure of the polar metabolite to bind strongly and thereby washing out more easily than the parent compound. The results also demonstrated that the hair root is an appropriate specimen for the demonstration of acute poisoning.

Table 3
The statistics of positive results

No.	Meperidine (ng/mg)			Normeperidine (ng/mg)			Normeperidine/meperidine	
	Mean±SD	Median	Range	Mean±SD	Median	Range	Mean±SD	Median
60	107±130	582	4.8~496	117±143	53.4	17~685	1.40±1.07	1.21

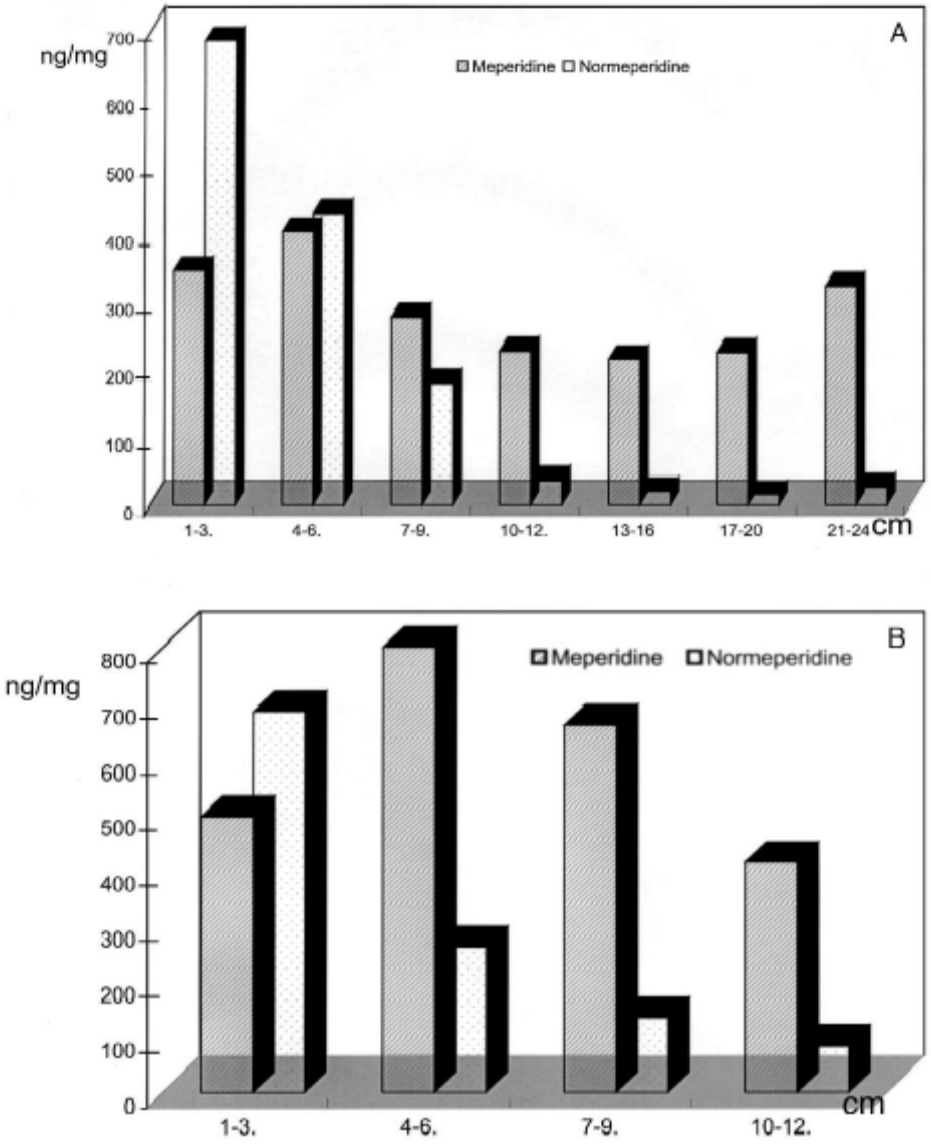

Fig. 4. Stability of meperidine and normeperidine in hair.

3.5. Meperidine distribution in hair and meperidine use history

The scalp hair samples (8~24 cm) of 11 meperidine abusers were collected together with information about their drug histories and were sectionally analyzed using the described method. Meperidine concentrations in each section of hair cut from the root end were evaluated with respect to each subject's drug history. The results revealed that distribution of meperidine in the hair agreed with the drug use history. The growth rate

of hair is such that the distribution of meperidine along the hair shaft at 1.2 cm per month could be indicative of the length of time since the person was exposed to that drug. Fig. 5 shows three examples of hair sectional analysis of meperidine addicts, in which the horizontal axis showed the length of hair from root to tip.

3.5.1. Case 1

A 26-year-old man Z had used meperidine for three months. From his 7-cm-long hair was cut 1-cm sections from the root end shown in Fig. 5. The sectional hair analysis showed that meperidine was found in sections 1 to 4, and normeperidine was found only in the first section.

3.5.2. Case 2

A 24-year-old woman Y was prosecuted for drug dealing. She claimed that she was addicted to meperidine for six months prior to the last five months. Her 15-cm-long hair was cut into 5-cm sections from the root end. No meperidine and normeperidine was detected in the first 5-cm section, but a significant amount of meperidine and normeperidine was present in the other two sections. Her statement was in accordance with the analytical results and could therefore be confirmed.

3.5.3. Case 3

A 34-year-old woman G had 22-cm-long hair, which indicates exposure to the drug during an 18–20 month period. G was heavily addicted to meperidine in the last 10 months. Before that time, she had been hospitalized for three months and she also had a two-month meperidine use history prior to hospitalization. Her 22-cm-long hair was cut into 3-cm sections from root end for five sections, followed by 1-cm sections for seven sections. The sectional hair analysis showed very high meperidine and normeperidine values for sections 1 to 4 (1–12 cm), trace meperidine for the next five sections (13–15 cm), low concentrations of meperidine and normeperidine for sections 6 and 7 (16–17 cm) and no drug in the remaining hair sections (18–22 cm).

These examples demonstrate the utility of hair analysis, especially sectional analysis, in disclosing the past meperidine history. More research is needed for detailed interpretation, specifically of drug diffusion and of the influence of variation in the rate of hair growth.

3.6. Meperidine level in hair and dosage

Table 4 shows the drug levels in hair samples from 20 meperidine addicts, divided into two groups according to drug dosage taken. In group 1, 10 subjects (1–10) used lower doses and had shorter drug histories, whereas the 10 subjects (11–20) in group 2 used higher doses and longer drug histories. As shown in Table 4, there was a significant difference in drug levels measured in the two groups. Although the data did not allow us to conclude definitively on the existence of a linear correlation between dose and concentration in hair, they revealed an apparent dose–concentration relationship for meperidine in human subjects. In the future, the correlation between dosage and

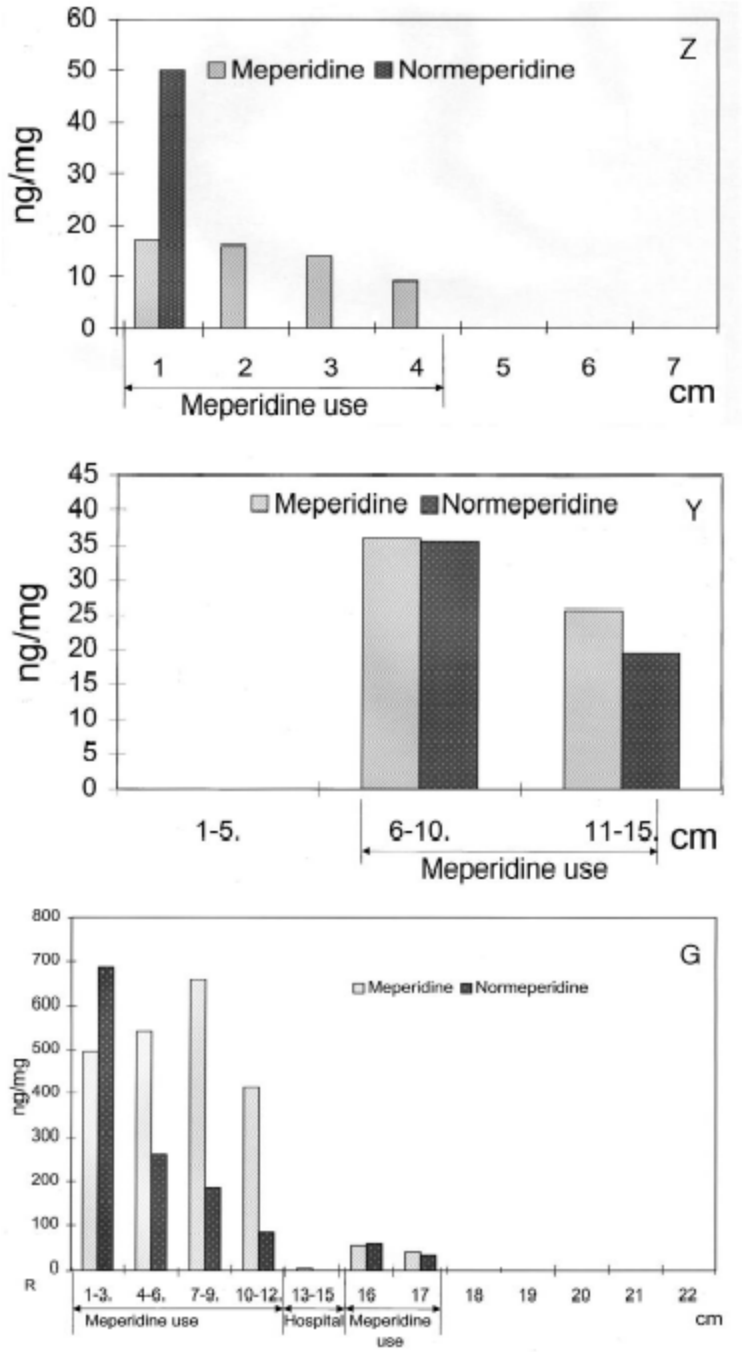

Fig. 5. Meperidine distribution in hair and meperidine use history. Z: Case 1; Y: case 2; G: case 3.

Table 4
The drug level in hair and dosage

Group 1	Concentration in hair (ng/mg)		Dosage (mg/month)	Group 2	Concentration in hair (ng/mg)		Dosage (mg/day)
	Meperidine	Normeperidine			Meperidine	Normeperidine	
1	2.8	–	300	11	346	431	300
2	16.7	19.1	1500	12	250	292	200
3	15.5	20.8	1500	13	442	288	300
4	2.4	–	300	14	496	685	400
5	2.4	–	400	15	289	196	200
6	17.4	50	2000	16	136	189	100
7	9.9	–	1000	17	404	631	400
8	3.5	–	500	18	138	408	200
9	14.3	–	1000	19	95.7	154	100
10	8.7	–	1000	20	242	196	200
Mean	9.36			Mean	284	347	

meperidine levels can be clarified by administration meperidine under controlled conditions.

3.7. Meperidine and normeperidine concentrations in human head, axillary and pubic hair

The presence of meperidine and normeperidine were investigated in hair samples collected from the head, axillary and pubic regions obtained from a fatal meperidine overdose case. Different hair samples (5 cm), cut as close as possible to root, were analyzed following the described method. The results are listed in Table 5. Meperidine was detected in all hair samples, with the highest concentration in hair of the head, followed by pubic hair and axillary hair, whereas normeperidine was only detected in hair of head and pubis.

Taken together, literature reports of drug levels in hair samples have given rise to a complex and sometimes contradictory picture. Offidiani et al. [9] found that cocaine and morphine levels are highest in pubic hair samples, followed by head and axillary hair. In contrast, according to Balabanova and Wolf [10], the highest values are in the axillary hair, followed by pubic and head hair. Our findings are not in accordance with either of these reports. The discrepancies are most likely due to differences in length and growth rate of the hair that was analyzed. Generally, the slower growth rate of pubic hair and axillary hair, 0.37 mm/day, could have concentrated more drug than head hair, which

Table 5
Meperidine and normeperidine concentrations in hair of the head, axillary and pubic regions

Sample	Meperidine (ng/mg)	Normeperidine (ng/mg)
Head	198	256
Pubis	175	218
Axillary	49	–

has a growth rate of 0.39–0.44 mm/day. Lack of equivalent matching growth periods in the different hair samples is another confounding variable. Head hair samples probably represented drug use over the last four to five months, whereas pubic hair and axillary hair samples could have represented a longer time period. Despite the lack of equivalent growth periods, the result indicated that analysis of pubic hair generally provided similar information to that obtained from head hair and suggested that pubic hair may be a useful alternative when head hair is not available for the detection of meperidine.

4. Conclusions

This paper describes a GC–MS (EI, CI) procedure for the analysis of meperidine and its metabolites in hair. The method has been successfully applied to the analysis of meperidine in the hair of addicted subjects. The data supported the following conclusions: (1) meperidine and its metabolites – normeperidine, *N*-methoxy meperidine and acetyl normeperidine all are readily incorporated into hair. The mean levels of meperidine and normeperidine in hair from addicts were 103±130 ng/mg and 117±143ng/mg, respectively. The concentrations of metabolite in hair were usually larger compared to the parent drug; (2) meperidine was stably present in hair for at least 20 months, but at same drug dose, normeperidine levels in the root-side sections were higher relative to the tip-side; (3) when normalized to the growth rate of hair (1.2 cm/month), the distribution of meperidine along the hair shaft agreed with drug abuse history; (4) there was a relationship between dose and the meperidine concentration present in hair samples from human subjects; (5) analysis of pubic hair generally provided similar drug level information to that obtained from head hair. The results suggest that hair analysis could serve as a valuable tool in the determination of drug abuse history.

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Study on metabolism of illegal amphetamines in the hair of addicts

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Abstract:

Introduction: Amphetamines are CNS (central nervous system) stimulants used in therapy for the treatment of obesity, narcolepsy, and hypotension. Abuse of the powerful psychostimulants amphetamines is an increasing problem in our country. Urine and hair are generally chosen as good samples for analysis of most drugs including amphetamines. Between them, hair analysis is a reliable tool for verification of an individual's past history of medication, compliance, or drug abuse. The metabolites of drugs are the potential tool in distinguishing ingestion from external sources and help to interprete the results.

Aims: To investigate the presence of amphetamines and their metabolites in hair of addicts.

Methods: Hair samples were washed with 0.1% sodium dodecyl sulfate (SDS), and then rinsed with deionized water and acetone. After being air-dried, the segments were cut into 1 mm. One milliliter of HCl (0.1 M) was added to 50 mg of segments and incubated overnight at 45°C. After cooling to room temperature, the resulting digests were adjusted to pH 9.2 using 0.4% NaOH and mixed with 2 ml ethyl acetate, vortexed, centrifuged(3000rpm), evaporated to dryness after adding 1 drop of 2% acid methanol. The residue was derivatized with trifluoroacetic acid anhydride for 30min at 70°C. After evaporation with a nitrogen stream at 60°C, the residue was injected to GC/MS.

After shaving back hair (6cm×4 cm), guinea pigs were administered intraperitoneally once a day for 7 successive days, at 10 mg/kg, respectively. After the first dosing, hair segments with different colors were collected at 5,10,14,18 days.

Hair samples were collected from amphetamines abusers in entertainment places with their urine samples tested positive.

Results: The metabolites amphetamine, MDA, and norfenfluramine were confirmed in methamphetamine, MDMA, and fenfluramine positive hair using GC/MS, respectively. No other metabolites were found.

The concentrations of amphetamines and their metabolites in white hair were lower than that in black hair from the same guinea pig (shown in table 1).

Table1 The concentrations of amphetamines and their metabolites in guinea pigs

compounds	black hair (ng/mg)		white hair (ng/mg)	
	parent	metabolite	parent	metabolite
fenfluramine	1.3	2.3	1.2	0.95
Methamphetamine1	4.5	9.1	-	7.6
Methamphetamine2	11.0	26.9	5.1	9.4

Methamphetamine3	6.8	27.8	2.6	14.0
MDMA1	8.0	36.7	1.2	3.7
MDMA2	7.5	14.3	1.0	0.53

As shown in table 2, the concentration of MDMA was hair than that of its metabolite MDA in 7 human hair samples. The ratio of the concentrations of MDMA to MDA was between 8 and 15.4.

Table 2 the concentrations of MDMA and MDA in hair from 7 MDMA addicts

No.	MDMA (ng/mg)	MDA(ng/mg)	MDMA/MDA
1	21.9	2.3	9.5
2	7.7	0.5	15.4
3	15.9	2.0	8
4	2.4	0.2	12
5	-	-	
6	13.2	0.9	14.7
7	19.6	1.7	11.9

Conclusion: Methods for the determination of amphetamines and their metabolites in hair were established using GC-MS/SIM, which had the following features: a small amount of hair (10 mg) needed, low limits of detection (0.2ng/mg), and high specificity. The methods have been successfully applied to routine analysis for confirmation of amphetamines-abuse cases.

Key words: hair analysis; methamphetamine; MDMA; fenfluramine; metabolites

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Study of distribution and metabolism of methamphetamine in hair of guinea pig

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Abstract:

Introduction: Hair provides both an alternative and a complementary approach for drug abuse detection. Hair differs from other biological specimens, such as blood or urine, used for toxicological analysis due to its long detection window (months to years), enabling retrospective investigation of chronic and past consumption. In addition, hair gives the additional advantages that it can be easily obtained, it is not easily adulterated, and it can be stored and transported without specific precautions due to its stability. There are also, however, some limitations of hair testing. The difficulty in interpreting hair testing results is that the mechanism of drugs incorporation into hair is not clear and the concentration of drugs in hair can be influenced by factors such as ethnic differences in hair pigmentation.

Aims: To study the distribution and metabolism of methamphetamine in the hair of guinea pig.

Methods: Determination of methamphetamine and its metabolite amphetamine in hair was performed by GC/MS and GC/NPD. Concentration-time course of methamphetamine and amphetamine in hair of guinea pig were recorded. Relationship between hair color, administrated dose and drug concentration in hair were also discussed.

Results: The concentration of amphetamine is higher than that of methamphetamine in the hair of guinea pig administrated a single dose or seven doses of methamphetamine. The concentration of methamphetamine and amphetamine were significantly related with administrated dose (Fig 1 and Fig 2) and the incorporation rate into white and brown hair is much poorer than that of black hair (Fig 3 and Fig 4).

Conclusion: administration dose and the color of hair affect the concentration of methamphetamine and amphetamine in the hair of guinea pig.

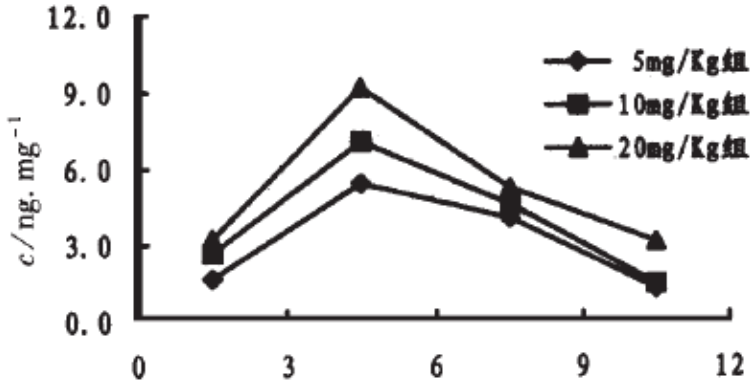

Fig 1 concentrations of amphetamine after different dose

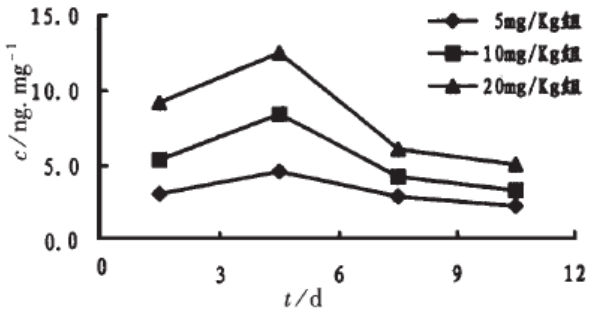

Fig 2 concentrations of methamphetamine after different dose

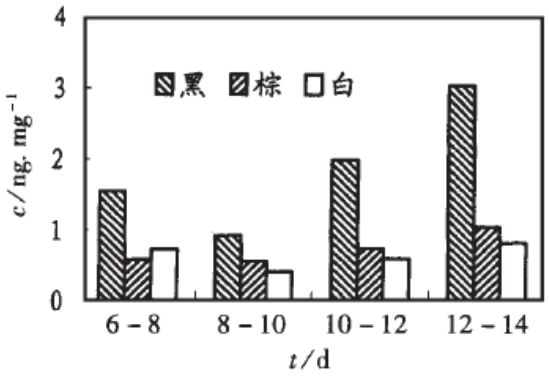

Fig 3 Concentrations of amphetamine found in different color hair samples from the same guinea pig

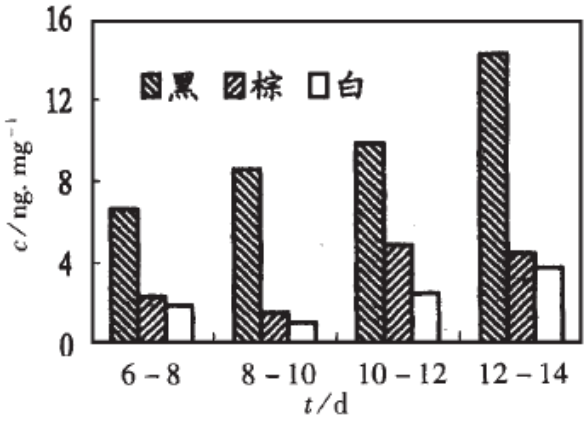

Fig 4 Concentrations of methamphetamine found in different color hair samples from the same guinea pig

Key words: distribution; metabolism; methamphetamine; amphetamine; hair; guinea pig

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Detection of psychotropic drugs and their metabolites in human hair

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Abstract:

Introduction: Psychoactive drugs are chemical substances that acts primarily upon the central nervous system. Many psychoactive substances are abused because of subjective changes in consciousness and mood that the user may find pleasant (e.g. euphoria) or advantageous (e.g. increased alertness). The analysis of hair for drugs is now recognised as an important detection tool for toxicologists. Once incorporated into the growing hair the drug can be detected long after it has been eliminated from more conventional samples such as blood and urine. Accordingly, hair analysis has found applications in clinical toxicology. But until now, there were few reports about psychotropic drugs in hair.

Aims: The study was intended to investigate psychotropic drug and their metabolites in hair.

Methods: After contamination, 10-20mg of hair was digested in 1 ml of 0.1M NaOH at 80°C for 30min. The pH of the digested samples was adjusted to 10 with HCl and extracted with ethyl acetate. Extracts were evaporated to dryness and then reconstituted in 25 ul methanol and 1 ul was injected to GC-MS.

Results: Using EI and PCI mode, more than 20 psychotropic drugs and their metabolites were confirmed in hair from paychiatric patients as follows: nicotine and C-oxidation metabolite, carbamazepine and D-dealkylation metabolite, amitriptyline and N-demethylation metabolite and ring-hydroxylation metabolite, doxepin and N-demethylation metabolite and ring-hydroxylation metabolite, trihexyphenidyl and ring-hydroxylation metabolite, chlorpromazine and N-dealkylation metabolite and S-oxidation metabolite, chlorprothixene and ring-oxydation metabolite, trifluoperazine, clozapine and N-dealkylation metabolite and haloperidol.

Conclusion: Nicotine, carbamazepine, amitriptyline, doxepin, trihexyphenidyl, chlorpromazine, chlorprothixene, trifluoperazine, clozapine, haloperidol and their metabolites were found in hair from paychiatric patients. Chlorpromazine and clozapine concentrations in the hair were found to be dependent on the dosage.

Key words: hair analysis; psychotropic drugs; clinical toxicology; GC-MS

Published work: Shen Min, Wu Hejian, Xiang Ping, et al. Detection of psychotropic drugs and their metabolites in human hair. Journal of Chinese Mass Spectrometry. 2001, 22(1): 32-39

Simultaneous Quantification of Cannabinoids and the Major Metabolite, THC-COOH in Human Hair

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Abstract

Introduction: The derivates of Cannabis sativa are one of the most widely abused illicit drugs in the world. Like Δ9-tetrahydrocannabinol (THC), the primary psychoactive analyte, cannabidiol (CBD) and cannabinol (CBN) are constituents that can be isolated from Cannabis. 11-nor- Δ9-tetrahydrocannabinol-carboxylic acid (THC-COOH) is the main metabolite of THC in body. Hair analysis for drugs has been gaining recognition in various fields and is being promoted in many countries. The major advantage of hair is its larger detection window, ranging from weeks to months, depending on the length of hair shaft analyzed, urine detect only for a few days. The analytical procedures described in the literature for the analysis of cannabinoids in human hair by GC/MS are mainly targeted for the simultaneous detection of THC, CBD and CBN or THC and THCCOOH separately or simultaneously.

Aim: To establish and validate a screening procedure for simultaneous identification and quantification of the cannabinoids (THC, CBD, and CBN) and the metabolite THC-COOH, in human hair, by GC/MS. The analytical method was applied to 10 human hair samples, obtained from individuals who were cannabis addicts.

Methods: Hair sample was decontaminated either with dichloromethane or 0.1%SDS, water and acetone successfully. The washed hair was cut into 1mm length. 250ng of chlofenamic acid was added to 50mg of hair sample as internal standard. Then it was hydrolyzed with 1mL of 1mol/L sodium hydroxide at 80℃ for 20min. After the sample was cooled, 0.5mL of acetic acid was added. The mixed sample was extracted with 2mL of n-hexane: ethyl acetate (9:1, vol) twice. The organic layer was collected and dried under air flow at 60℃. The residue was derivatized with either 100μL of PFPA and PFPOH (1:1, vol) or 40μL of BSTFA and acetonitrile(1:1,vol). Finally, the sample was analyzed by GC/MS-SIM.

Results: Derivatization method with PFPA didn't produce interference peaks at the retention times of THC and THC-COOH, while BSTFA did. Dichloromethane had more significant decontamination effect than the combination of 0.1%SDS-water-acetone. With established method, the limits of detection for THC, CBN and THC-COOH were 0.01, 0.05 and 0.01ng/mg, respectively. The calibration curves for those analytes were 0.05-5ng/mg, 0.2-5ng/mg and 0.05-5ng/mg, respectively. The extraction recovery ranged from 95.0% to 111.5%, and the intra-day precision was less than 15%. Ten hair samples of cannabis addicts were analyzed, as shown in table 1. THC was detected in all the ten hair samples with the range from 0.11 to 8.84ng/mg. THC-COOH was not detected in three samples out of ten, and the concentration of the rest seven samples were too low to be quantified. CBN was not detected in two samples out of ten, and the concentration of the rest eight samples ranged from 0.2 to 6.5ng/mg. The concentration of CBN had no correlation with those of THC and THC-COOH in hair.

Table 1 Results of 10 hair samples of cannabis addicts

No.	Hair length(cm)	THC(ng/mg)	CBN(ng/mg)	THC-COOH(ng/mg)
1	0.5	+*	+	+
2	0.5	0.19	+	-**
3	0.5	0.29	0.82	+
4	5	0.35	+	+
5white	0.5	8.84	4.49	+
5black		4.45	4.60	+
6white	2	0.22	-	-
6black		0.16	-	+
7	0.5	0.23	0.40	+
8	0.5	0.11	0.34	-
9	10	1.25	6.5	+
10	2	3.48	2.03	+

* Not detected; ** below quantification limit.

Conclusion: The established method is sensitive and effective to detect THC, CBN and THC-COOH simultaneously in human hair. The derivatizing reagent BSTFA is the best choice for both cannabinoids and THC-COOH. THC-COOH was difficult to incorporate into hair.

Key words: cannabinoids, THC-COOH, hair, GC-MS.

Published work: Xiang Ping, Shen Min, Shen Bao-hua, et al. Simultaneous quantification of cannabinoids and the major metabolite, THC-COOH in human hair. Journal of Forensic Medicine. 2002, 18(4): 216-219

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Detection of antidepressant and antipsychotic drugs in human hair

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Abstract

The presence of therapeutic drugs and their metabolites in the hair of psychiatric patients was investigated using gas chromatography (GC)–mass spectroscopy (MS)–electron ionization (EI) and GC–MS–chemical ionization (CI). In hair samples tested from 35 subjects, carbamazepine, amitriptyline, doxepin, trihexyphenidyl, chlorpromazine, chlorprothixene, trifluoperazine, clozapine and haloperidol were detected, with maximal concentrations of 22.5, 57.7, 183.3, 15.6, 68.2, 30.0, 36.8, 59.2 and 20.1 ng/mg of hair sample, respectively. Chlorpromazine and clozapine concentrations in the hair were found to be dependent on the dosage used and their correlation coefficients were 0.8047 ($P < 0.001$, $n = 16$) and 0.7097 ($P < 0.001$, $n = 16$), respectively. Segmental analysis demonstrated that there was a correlation between the history of subject's drug exposure and the distribution of drug along the hair shaft. Our results also show that drug analysis in hair may provide useful information about drug treatment and the history of usage, and that drugs can be detected in normally kept hair for at least 16 months after intake.

Keywords: Hair analysis; Antidepressant; Antipsychotic drugs; Toxicology; History of drug usage

1. Introduction

Analysis of therapeutic drugs is usually performed using bodily fluids. However, concentration in such specimens only reflects the use of drugs within a few days prior to sampling, therefore it is difficult to distinguish between acute or chronic drug use. The application of hair analysis for the detection of drugs in the human body has become important because it can provide evidence of drug use over a much longer period than the conventional testing in blood and urine [1].

Antidepressant and antipsychotic drugs are one of the most widely used medicines, and similar to drugs of abuse, are usually administered for a long period of time. Due to the accumulation and storage of drugs, hair provides a useful indication of long-term exposures to drugs. However, in the past decade, only limited studies have been performed on antidepressant and antipsychotic drugs, and very few individual drugs have been targeted [2–8].

Our study reported here was designed to develop a sensitive method for simultaneously measuring the antidepressant and antipsychotic drugs in the human hair, and to determine whether a wide range of such drugs are incorporated in human scalp hair following the use and whether segmental analysis can assist in obtaining histories of drug use.

2. Experimental

2.1. Standards and reagents

Carbamazepine, amitriptyline, doxepin, trihexyphenidyl, chlorpromazine, chlorprothixene, trifluoperazine, clozapine, haloperidol and SKF_{525} were obtained either from Sigma (Shanghai, PR China or St. Louis, MO, USA) or from Institute of Shanghai Medicine (Shanghai, PR China). All chemicals were analytical reagent grade.

2.2. Hair sample collection

Drug-free hair for preparation of standards and controls were obtained from laboratory volunteers with black hair.

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The hair samples, cut as close as possible to skin from the vertex posterior of head, were collected from 35 psychiatric patients (29 males and 6 females) aged 32–57 years, who are chronically treated with the antidepressants and antipsychotic drugs. Tapes were placed around the root ends of the hair strands so the most recently grown region can be distinguished. In a questionnaire for each patient, the history of usage for at least the last 12 months and all special features of the hair samples were registered. Specimens were stored in plastic bags at room temperature (10–25 °C) until analysis.

2.3. Hair sample preparation

Root ends of the hair samples were aligned carefully, cut into short sections (1–5 cm), washed with 0.1% sodium dodecyl sulfate (SDS), and then rinsed with deionized water and acetone. Samples were allowed to drain completely between each rinse and dried in a fume hood overnight before weighing. Dried hair samples were weighted and cut into pieces of approximately 1–3 mm in length.

2.3.1. Alkaline digestion

Hair (10–20 mg) were digested in 1 ml of 0.1 M NaOH at 80 °C for 30 min. After 1 μg internal standard (SKF_{525}) was added, the pH of the digested samples was adjusted to 9.5–10 with HCl and extracted with ether. Extracts were evaporated to dryness under a gentle stream of nitrogen at 40 °C and then reconstituted in 25 μl methanol. An aliquot of 1 μl of the solution was injected into gas chromatography (GC)–mass spectroscopy (MS) or GC system.

2.3.2. Acid hydrolysis

Hair (10–20 mg) were incubated in 1 ml of 0.1 M HCl at 45 °C for 18 h. After 1 μg of SKF_{525} was added, the pH of the digested sample was adjusted to 9.5–10 with NaOH and extracted with ether. Further treatments were performed as described.

2.3.3. Methanol extraction

Two milliliter of methanol was added to 10–20 mg of hair, the mixture was ultrasonicated for 1 h and then incubated at room temperature overnight. After 1 μg of SKF_{525} was added, the solvent was separated and the extracts were treatment as described.

2.4. Mass spectral identification

GC–MS analysis were performed using a GC–MS (Saturn 2000) equipped with 30 m × 0.25 mm DB-5 capillary column. The instrument was operated under electron ionization (EI) and chemical ionization (CI) conditions using CH_4 as reagent gas. The temperature program was set at 100 °C for 2 min, increased at 10 °C/min to 150 °C, then at 25 °C/min to 280 °C. The injection and interface temperatures were 250 °C, and source temperature was 150 °C.

Peaks were identified using a full scan GC–MS analysis of samples, scanning over a mass range of m/z 50–500. The drugs were identified based on the comparison of retention time and the relative abundance of confirming ions. Confirmations were obtained based on the high percentage match of the spectral libraries, metabolite patterns and comparison with standards.

2.5. GC quantitation

After identification by GC–MS, the concentrations of the antidepressants and antipsychotic drugs in the hair were quantitatively measured by GC–NPD with SKF_{525} as an internal standard. Chromatography of drugs was achieved on AC-5 capillary column (l5 m × 0.25 mm i.d.). The temperature program was set at 180 °C, increased at 10 °C/min until 270 °C, then held for 10 min. Under the conditions used, there was no interference of the target drugs with any extractable endogenous material present in hair, and the simultaneous measurement of antidepressants and antipsychotic drugs was possible.

2.6. Calibration curves, linearity and reproducibility

The calibration curves for the measurement of antidepressants and antipsychotic drugs were constructed by the analysis of extracts from 20 mg of control hair samples in which the standard solutions of carbamazepine, amitriptyline, doxepin, trihexyphenidyl, chlorpromazine, chlorprothixene, trifluoperazine, clozapine and haloperidol had been added (0.2–100 ng/mg of hair), together with SKF_{525} (IS), as mentioned. The calibration curves for all drugs were linear over a concentration range of 0.2–100 ng/mg of hair, with correlation coefficients of $r > 0.998$, and detection limit of 0.1–0.5 ng/mg of hair.

The precision of the method was determined using hair from healthy volunteers spiked with nine antidepressants and antipsychotic drugs at two concentrations with respect to the calibration curves, and three pieces of hair positive for antidepressants and antipsychotic drugs (six samples were analyzed for each concentration). The coefficients of variations (CVs) for nine antidepressants and antipsychotic drugs were between 2.9 and 0.8.

3. Results and discussion

3.1. Comparison of the extraction methods

Three major methods of hair sample preparation—alkaline digestion, acid hydrolysis and methanol extraction, were evaluated. The extraction efficiencies of the methods for clozapine in five positive hair (A1, A2, A3, A4 and A5) and for chlorpromazine in six positive hair (B1, B2, B3, B4, B5

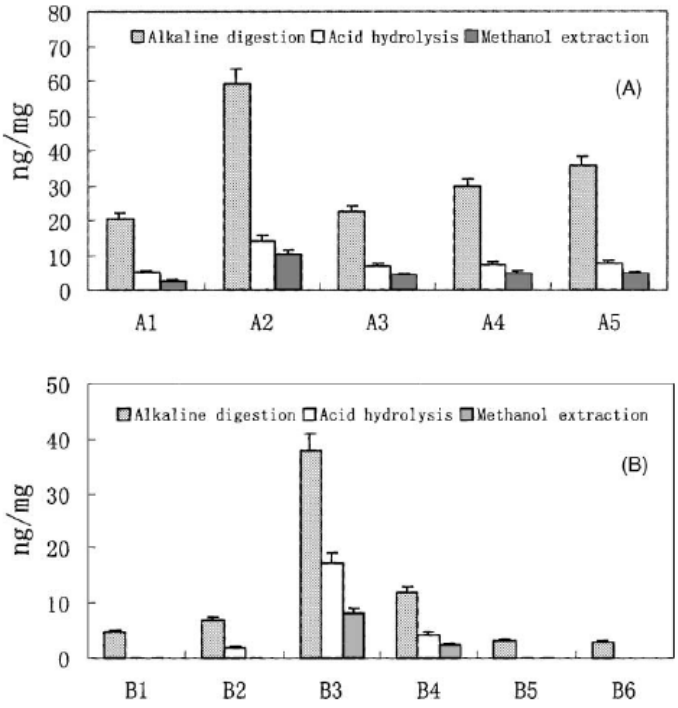


Fig. 1. The results of alkaline digestion, acid hydrolysis and methanol extraction. (A) Clozapine; (B) chlorpromazine.

and B6) were compared. As shown in Fig. 1, the best method was alkaline digestion, which produced the highest yield of drugs from all hair samples with the lowest background. This is possibly due to the complete dissolution of the hair components and the stability of tested drugs under the chemical conditions (basic digestion) used to dissolve hair. Lower yields were observed after acid hydrolysis and methanol extraction, especially the later led to the lowest yield together with heavily laden chromatograms, though this non-selective extraction method can be used in screening analysis.

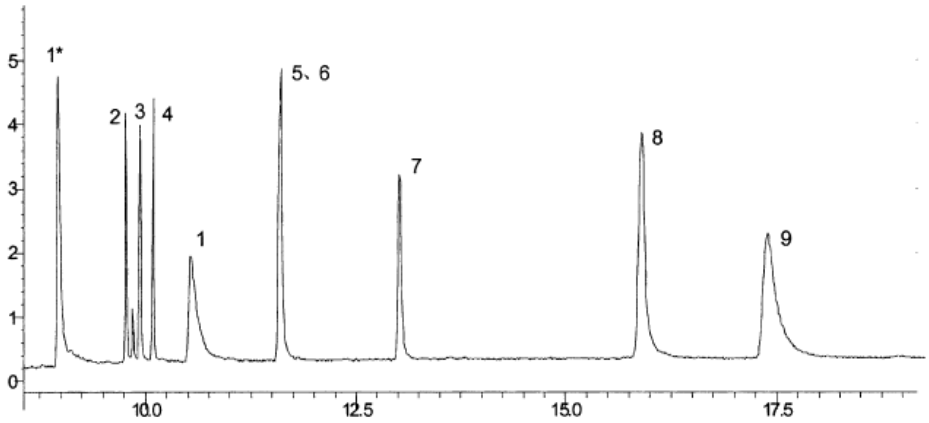
The question always remains whether drugs were quantitatively extracted from the real hair samples, although they were easily extracted from spiked control hair samples. Therefore, it is necessary to use positive hair instead of spiked ones for evaluation, and more important to develop a way to generate hair standards with precise concentration of drugs.

The recovery of extracted drugs from the spiked hair using ether, ethyl acetate and chloroform was also evaluated. Ether appeared to be a more effective solvent because it gives the highest recovery and the cleanest background (Fig. 2). From these results, ether extraction after alkaline digestion was selected for the extraction of antidepressants and antipsychotic drugs from the hair, and the recoveries of extraction of nine drugs ranged from 64.6 to 88.1% with CV 2.9–10.8.

3.2. Identification of antidepressant and antipsychotic drugs and their metabolites in hair samples from psychiatric patients

The presence of antidepressant and antipsychotic drugs and their metabolites in the hair of psychiatric patients was investigated using GC–MS–EI and GC–MS–PCI. In the hair of 35 subjects tested, 19 drugs and metabolites were detected as follows: carbamazepine and N-dealkylation metabolite (carbamazepine-M), amitriptyline and N-demethylation metabolite (amitriptyline-M1) and ring-hydroxylation metabolite (amitriptyline-M2), doxepin and N-demethylation metabolite (doxepin-M) and ring-hydroxylation metabolite (doxepin-M2), trihexyphenidyl and ring-hydroxylation metabolite (trihexyphenidyl-M), chlorpromazine and N-dealkylation metabolite (chlorpromazine-M1) and S-oxidation metabolite (chlorpromazine-M2), chlorprothixene and ring-oxidation metabolite (chlorprothixene-M), trifluoperazine, clozapine and N-dealkylation metabolite (clozapine-M) and haloperidol. Trihexyphenidyl and chloroprothixene were not detected previously in the hair sample. Table 1 shows the characteristics of EI and CI mass spectra for drugs and metabolites in the hair of psychiatric patients.

From the results, it is clear that oxidation is the major drug metabolic pathway in the body, which includes N-dealkylation, ring-hydroxylation, and N- or S-oxidation. From the metabolites of antidepressant and antipsychotic drug



1.Carbamazepine, 1*.Carbamazepine-M, 2. Amitriptyline, 3. Doxepin, 4.Trihexyphenidyl, 5.Chlorpromazine, 6.Chlorprothixene, 7.Trifluoperazine, 8.Clozapine, 9.Haloperidol

Fig. 2. The total ion chromatogram of the hair extracts.

identified from psychiatric patients the metabolic pathways are proposed (Fig. 3).

3.3. Concentrations of drugs in the hair from psychiatric patients

The hair root samples (taken 2 cm from the root) of 35 psychiatric patients were measured for antipsychotic and antidepressant drugs by GC–NPD after GC–MS identification and the results summarized in Table 2. Six cases were positive for carbamazepine, three cases for amitriptyline, five cases for doxepin, seven cases for trihexyphenidyl, sixteen cases for chlorpromazine and clozapine, respectively, and one case for chlorprothixene, trifluoperazine and haloperidol, respectively. No drugs were detected in all six control hair samples from laboratory volunteers.

Table 1
EI and CI MS characteristics of peaks observed in the hair extracts

Compound	MW	RT (min)	EI (relative abundance)		PCI (relative abundance)	
			M^+	Second peak	$[M+1]^+$	Second peak
Carbamazepine	236	10.6	236 (100)	193 (50)	237 (100)	473 (40)
Carbamazepine-M	193	8.9	193 (100)		194 (100)	395 (50)
Amitriptyline	277	9.9	277 (5)	58 (100)	278 (40)	58 (100)
Amitriptyline-M1	263	10.0	263 (35)	202 (100)	264 (100)	55 (80)
Amitriptyline-M2	293	10.8	293 (3)	58 (100)	294 (50)	59 (100)
Doxepin	279	10.0	279 (20)	58 (100)	280 (100)	58 (100)
Doxepin-M1	265	10.1	265 (100)	204 (100)	266 (100)	
Doxepin-M2	295	10.2	295 (3)	58 (100)	296 (35)	58 (100)
Trihexyphenidyl	301	10.2	301 (30)	218 (100)	302 (100)	98 (55)
SKF_{525}	353	10.4	86	99 (35)		
Trihexyphenidyl-M	317	11.8	317 (2)	98 (100)	318 (60)	98 (100)
Chlorpromazine	318	11.8	318 (70)	58 (100)	319 (60)	58 (100)
Chlorpromazine-M1	334	15.9	334 (30)	58 (100)	335 (70)	84 (100)
Chlorpromazine-M2	233	10.2	233 (100)	198 (70)	234 (100)	199 (25)
Chlorprothixene	315	11.9	315 (3)	58 (100)	316 (20)	58 (100)
Chlorprothixene-M	246	12.1	246 (100)	218 (40)	247 (100)	55 (30)
Trifluoperazine	407	12.9	407 (100)	70 (65)	408 (85)	113 (100)
Clozapine	326	16.4	326 (45)	256 (100)	327 (100)	99 (55)
Clozapine-M	312	16.9	312 (100)	243 (80)	313 (100)	
Haloperidol	375	17.6		237 (100)	376 (100)	165 (85)

MW: molecular weight; RT: retention time; EI: electron ionization; PCI: positive chemical ionization; M^+: molecular ion; $[M+1]^+$: molecular ion in PCI.

The concentration measured for chlorpromazine ranged from 2.9 to 68.2 ng/mg of hair with a dose of 100–500 mg per day. In the present study, its sulfoxidation and N-demethylation metabolites were also detected in all samples for chlorpromazine except in those that the level of the parent drug is less than 10 ng/mg. However, the concentration of the metabolites in hair were much lower than that of the parent, only representing about 5–10% of chlorpromazine.

Cloazpine was detected in 16 of 35 hair samples from psychiatric patients who had been treated in daily doses of

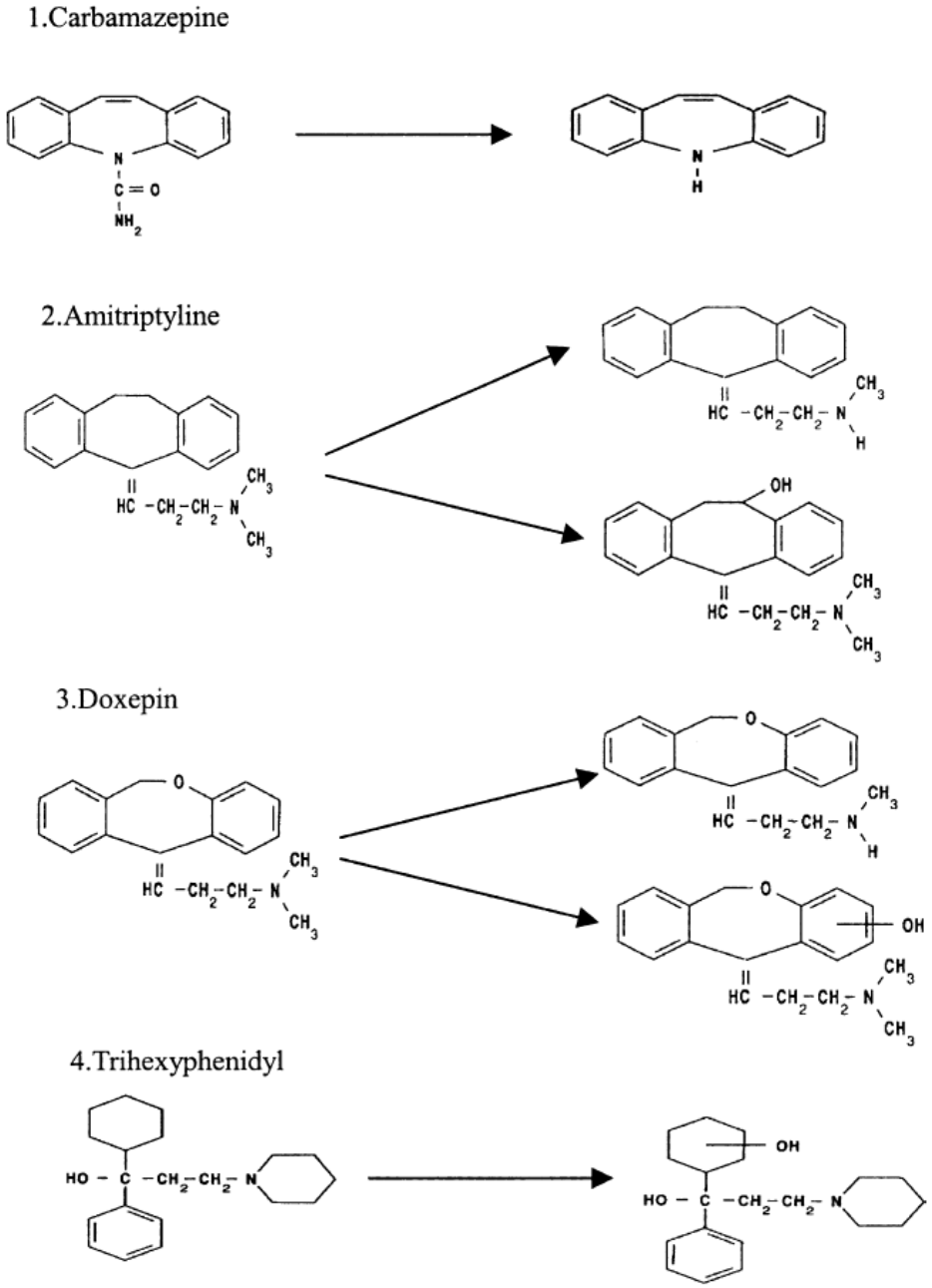


Fig. 3. Metabolism of antidepressant and antipsychotic drugs.

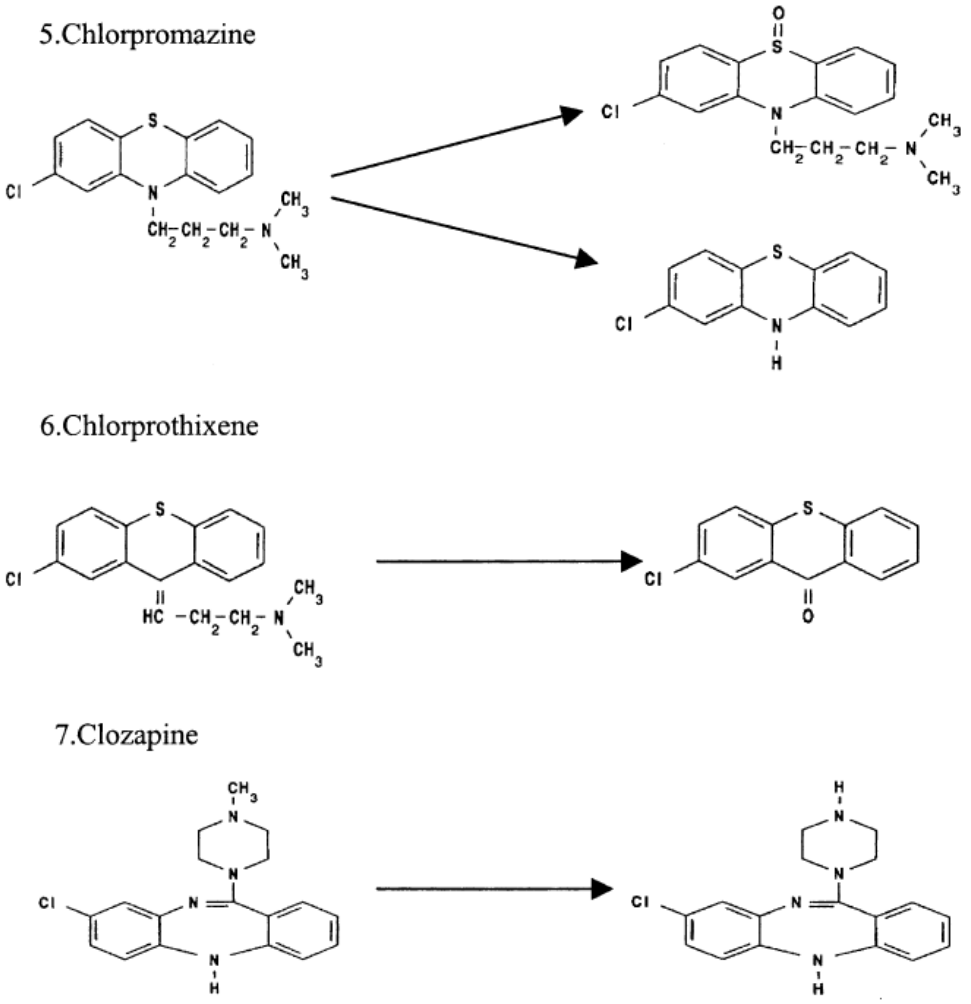


Fig. 3. (*Continued*).

Table 2
Antidepressant and antipsychotic drugs concentration in hair and dosage

Drug	Number of cases	Concentration in hair (ng/mg of hair)	Dosage (mg per day)
Carbamazepine	6	2.8–22.5	200–400
Amitriptyline	3	2.5–57.7	525
Doxepin	5	55.6–183.3	100–250
Trihexyphenidyl	7	3.0–15.6	2
Chlorpromazine	16	2.9–68.2	100–500
Chlorprothixene	1	30	50
Trifluoperazine	1	368	50
Clozapine	16	16.7–59.2	150–425
Haloperidol	1	20.1	28

150–425 mg, with concentration ranging from 16.7 to 59.2 ng/mg. In the sample with high concentration of parent drug, a metabolite of clozapine, *N*-desmethylclozapine was also detected. Cirimele et al. have reported the presence of clozapine in the hair samples from 26 subjects treated with clozapine at 200–700 ng per day, with concentration ranging from 0.17 to 34.24 ng/mg [3], which are much lower than those reported here. This may be due to the difference in the specimens tested, and the difference in extraction procedure because methanol extraction used by Cirimele et al. [3] has a poor recovery as shown by this study.

In two cases, positive for doxepin, the detected concentrations were 55.6 and 183.3 ng/mg in patients who had a fixed daily dose of 100 and 250 mg, respectively. The metabolites, nordoxepin and doxepin-*N*-oxide, were also presented in the hair samples, representing 20% of the parent

drug. The concentration of 183.3 ng/mg is the highest among the limited literature reported, reflecting the highest dose reported.

Three cases were positive for amitriptyline, the patient with concentration of 57.7 ng/mg has been treated with a daily dose of 25 mg. The result was similar to that of Yegles et al. Like doxepin, metabolites of amitriptyline, nortriptyline and amitriptyline-*N*-oxide, were also detected.

The concentration of trihexyphenidyl in seven cases, who had taken trihexyphenidyl in fixed daily dose (2 mg per day), ranged from 3.0 to 15.6 ng/mg, and data demonstrated the presence of variations among individuals. Trihexyphenidyl-ring-hydroxylation metabolite was also found in all hair samples analyzed for tribexyphenidyl, representing about 20% of the parent. To our knowledge there have been no data published on trihexyphenidyl in hair.

Carbamazepine were detected in 6 of 35 subjects who has been treated at 200–400 mg per day with concentration ranging from 2.8 to 22.5 ng/mg. Our results were in accordance with those of Psillakis et al. [6].

Trifluoperazine, chlorprothixene and haloperidol were detected in one case each, the concentration of the drugs was 30, 36.8 and 20.1 ng/mg, respectively. In addition, nicotine and its metabolite, cotinine were also detected in eight subjects with smoking habits.

The results showed:

1. Nine antidepressant and antipsychotic drugs and their metabolites can all be incorporated into the hair. The order of drug–hair incorporation tendency based on daily dose (concentration in hair–dosage) was trihexyphenidyl > amitriptyline > doxepin > haloperidol > trifluoperazine > chlorprothixene > chlorpromazine > clozapine > carbamazepine. The results suggest that lipophilicity, basicity and low molecular weight facilitate the hair incorporation tendency [9,10].
2. The deposition of the metabolites into hair is less effective than that of the parents. A possible reason for this phenomenon may be the increase in hydrophilicity of the metabolites. Similar observation, such as accumulation of non-polar parent drugs, has been reported for other compounds including cocaine and heroin [9].

3.4. Relationship between drug concentration in hair and dosage

Data of 16 hair samples for chlorpromazine was statistically analyzed. We have found that the accumulation of chlorpromazine in the hair was dependent on the dose, and the concentrations of chlorpromazine were significantly correlated ($P < 0.001$, $n = 16$) with the daily doses and the correlation coefficient was 0.8047 (Fig. 4). Sato et al. [2] have demonstrated that the concentration of chlorpromazine in black hair collected from 23 Japanese patients who had been taking chlorpromazine in fixed daily dose (30–300 mg per day) ranged from 1.6 to 27.5 ng/mg, and significantly correlated with the daily dose ($r = 0.788$, $P < 0.001$, $n = 23$), in agreement with our results.

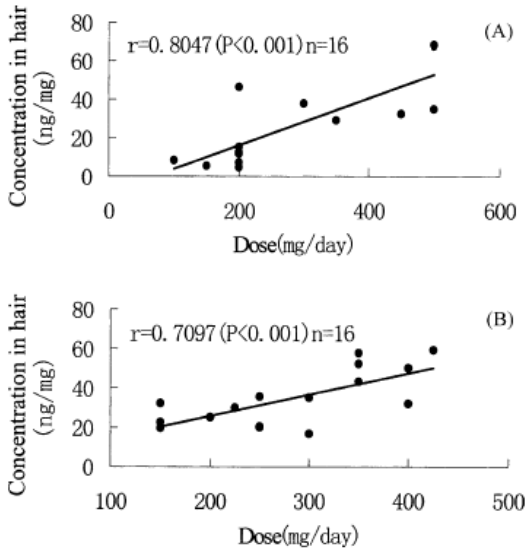


Fig. 4. Relationship between the daily dose of chlorpromazine and clozapine and their concentration in human scalp hair. (A) Chlorpromazine; (B) clozapine.

Statistical data of clozapine also showed a correlation between clozapine concentrations in the hair and the daily doses, with a correlation coefficient of 0.7097 ($P < 0.001$, $n = 16$). So far, no data has been published on clozapine except the one by Cirimele et al. [3] who had also observed a better dose–concentration relationship between daily dose and hair clozapine concentration ($r = 0.542$, $n = 23$).

3.5. Relationship between drug distribution in the hair and the history of drug use

The scalp hair samples of psychiatric patients were collected together with information about their history of drug use and were sectionally analyzed using the described method. Drug concentration in each section of hair cut from the root end were evaluated with respect to the history of drug use for each subject and the stability of drugs in the hair was investigated. Fig. 5 shows two examples of hair sectional analysis of the psychiatric patients, in which the horizontal axis showed the length of hair from root to tip.

3.5.1. Case 1

A 35-year-old woman (subject A) had 20 cm long hair, with an exposure to the drug during a 16 months period. She has taken a fixed daily dose of clozapine for more than 2 years and had also taken doxepin prior to the last 1 year. Her 20 cm long hair was cut into 5 cm sections from the root end shown in Fig. 5. The sectional hair analysis showed that clozapine was found in sections 1–4 and doxepin only in

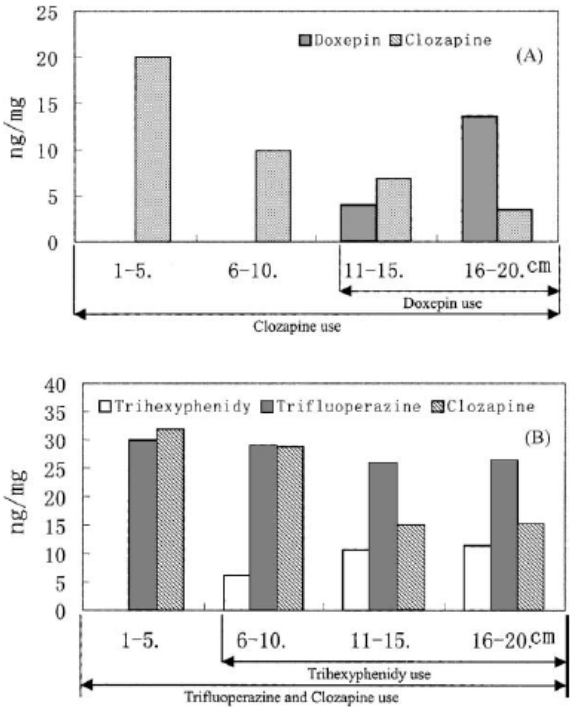


Fig. 5. Drug distribution in hair and drug use history. (A) Case 1; (B) case 2.

sections 3–4 (11–20 cm). Her history of drug use was in accordance with the results (Fig. 5).

3.5.2. Case 2

Subject B had been regularly treated with clozapine and trifluoperazine for more than 2 years, and also with trihexyphenidyl prior to the last 6 months for 2 years. Her 20 cm long hair was cut into 5 cm sections from the root end for 4 sections. Clozapine and trifluoperazine were detected in all sections, and trihexyphenidyl in sections 2–4 (6–20 cm).

These examples demonstrate that clozapine, doxepin, trifluoperazine and trihexyphenidyl can be detected in normally kept hair (no bleaching or coloring) for at least 16 months after intake, the longest period of time in the literature, and distribution of drugs along the hair shaft is in accordance with the history of drug use. The results are in agreement with previous investigations, most of them showed that hair might be a useful tool for monitoring individual dosage history [2–4,11].

The results also show that antidepressant and antipsychotic drugs in the hair have different stability data. Both subjects A and B had equivalent clozapine dosage, but its concentrations in the root-side section were higher than that in the tip-side of the hair. The result by Psillakis et al. [6] showed that carbamazepine has a similar behavior to clozapine. However, trifluoperazine is different, which has been observed to be of constant concentration in all hair sections. This phenomenon is possibly due to the differences in drug structure.

4. Conclusions

From the results we conclude:

1. The method developed for measuring simultaneously antidepressant and antipsychotic drugs and their major metabolites in the human hair by GC–NPD and GC–MS may be used as a screening analysis. This method provides valuable information to the clinical and forensic toxicologist regarding the degree of use of psychotropic drugs over a long period of time.
2. Nine antidepressant and antipsychotic drugs and their metabolites can all be incorporated in the hair, but the incorporation tendency may be related to molecular weight, lipophilicity and basicity of the drug.
3. Chlorpromazine and clozapine concentrations in the Chinese black hair were found to be dependent on the dosage and the correlation coefficients were 0.8047 ($P < 0.001$, $n = 6$) and 0.7097 ($P < 0.001$, $n = 6$), respectively.
4. Sectional hair analysis may provide useful information regarding antidepressant and antipsychotic drugs, treatment and history of administration, and they can be detected in the hair samples at least 16 months after intake.

In conclusion, hair analysis offers the potential for the detection of the use of antidepressant and antipsychotic drugs, and therefore provides a useful history of past drug use.

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The application of GC/MS/MS to screening for drugs of abused in hair

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Abstract:

Introduction: hair analysis has proved to be a useful and valuable indicator of medium/long term exposure to illegal and medicinal drugs. Blood and urine have traditionally been used as biological fluid for drug testing in most clinical and forensic toxicological situations. Hair has a longer surveillance window (months) compared to blood (hours to days) and urine (days to weeks), and can therefore be used as a complementary matrix. Immunoassay and gas chromatography–mass spectrometry (GC–MS) have previously been reported as screening methods in hair analysis. Immunoassay techniques are less specific than chromatographic analysis, and a relatively limited range of immunoassay reagents are commercially available for the determination of forensically relevant compounds. Gas chromatography–tandem mass spectrometry (GC/MS/MS) provide more sensitive than GC-MS, especially for targets in biosamples, and make it possible for hair analysis for drugs of abused.

Aims: To develop a sensitive and specific screening method for the simultaneous determination of 13 drugs of abused in human hair. The compounds included were amphetamine, methamphetamine, 3, 4- methylenedioxymethamphetamine (MDMA), MDA, morphine, 6-monoacetylmorphine (6-MAM), codeine, meperidine, normeperidine, cocaine, benzoylecgonine, methadone and EDDP.

Methods: Hair samples were washed with 0.1% sodium dodecyl sulfate (SDS), and then rinsed with deionized water and acetone. After being air-dried, the segments were cut into 1 mm. One milliliter of HCl (0.1 M) was added to 50 mg of segments and incubated overnight at 45°C. After cooling to room temperature, the resulting digests were adjusted to pH 9.2 using 0.4% NaOH and mixed with 2 ml ethyl acetate, vortexed, centrifuged(3000rpm), evaporated to dryness after adding 1 drop of 2% acid methanol. The residue was derivatized with 25ul MSTFA for 30min at 70°C. After cooling down, the residue was injected to GC/MS/MS.

Results: Ethyl acetate was the optional extraction solvent for all the 13 drugs to get good recovery and less matrix interferences. MSTFA is more active than BATFA for derivatization. As shown in table 1, GC/MS/MS in more sensitive than GC-MS to detect 13 drugs in hair.

Table1 LOD of GC-MS and GC/MS/MS methods

compounds	LOD(ng/mg)	
	GC-MS	GC/MS/MS
amphetamine	0.5	0.2
methamphetamine	0.5	0.2
MDA	0.02	0.01
MDMA	0.5	0.05
meperidine	0.5	0.02
normeperidine	2	2
EDDP	2	0.05
methadone	2	0.1
cocaine	0.5	0.5
benzoylecgonine	2	0.5
codeine	0.2	0.05
morphine	0.05	0.05
6-acetylmorphine	1	0.02

Conclusion: The developed GC/MS/MS method was sensitivity and selectivity for screen 13 drugs in hair, more suitable for detecting trace amounts of drugs in hair.

Key words: abused drugs; screening system; hair analysis; GC/MS/MS

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Analysis of Ketamine in Hair

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Abstract

Introduction: ketamine, called K powder in China, is classified as an NMDA receptor antagonist. Ketamine has a wide range of effects in humans, including analgesia, anesthesia, and hallucinations. It induces a state referred to as "dissociative anesthesia" and is used as a recreational drug. Ketamine has increasingly been abused as a ‘club-drug’ at dance and rave parties in recent years. Hair analysis has its unique advantages, such as, providing a complementary approach for drug abuse detection, having a long detection window, enabling retrospective investigation of chronic and past consumption. But there is a lack of information on the incorporation, accumulation, and pharmacokinetics of drugs in hair.

Aims: A rapid and sensitive method was developed for the simultaneous identification and quantitation of ketamine (K) and its two major metabolites, norketamine (NK) and dehydronorketamine (DHNK) in hair. The characteristics of K incorporation into the hair were elucidated.

Methods: Cavies were administrated intraperitoneally according to high, medium and low doses and hair segments with different colors were shaved. Human hair samples were collected from K abusers in the entertainment places. After decontamination, incubated and extracted, drugs were analyzed by GC/MS- SIM.

Results: The results of the analysis of all hair samples collected from cavies are reported in Table 1.There was an obvious correlation between the concentration of ketamine in experimental cavy hair and the dosage. The concentration of ketamine in hair increased with the melanin amount in hair in the order of white, brown and black. DHNK was only detected in high dose cavy hair; the ratio of ketamine to NK was between 2.33 to 12.94. Ketamine and NK were present in all the black hair samples, but DHNK was not detected. The concentration of metabolites in cavy hair was significantly higher than that of human.

Table 1 The results of all hair samples collected from cavies with high, medium and low doses

No.	K(ng/mg)	NK(ng/mg)	DHNK(ng/mg)	K/NK	K/DHNK	NK/DHNK
L1 white	-*	-	-			
L1 brown	0.60	-	-			
L1 black	1.30	0.14	-	9.29		
L2 brown	-	-	-			
L2 black	0.37	0.35	-	1.06		
L3 white	-	-	-			
L3 black	0.12	-	-			
L4 white	0.05	-	-			
L4 brown	0.09	-	-			
L4 black	0.15	-	-			

L5 white	0.26	-	-			
L5 brown	0.42	-	-			
L5 black	0.98	0.25	-	3.92		
M1 white	0.74	0.06	-	12.33		
M1 brown	1.61	0.33	-	4.88		
M1 black	3.63	0.45	-	8.07		
M2 brown	0.58	0.18	-	3.22		
M2 black	2.70	0.74	-	3.65		
M3 white	0.52	0.11	-	4.73		
M3 brown	0.42	0.27	-	1.56		
M3 black	1.55	0.55	-	2.82		
M4 brown	0.77	0.13	-	5.92		
M4 black	1.48	0.66	-	2.24		
M5 brown	0.31	0.22	-	1.41		
M5 black	1.62	0.81	+**	2.00		
H1 white	6.64	1.14	-	5.82		
H1 brown	9.11	1.73	-	5.27		
H1 black	9.92	2.38	2.13	4.17	4.66	1.12
H2 white	5.61	0.67	-	8.37		
H2 brown	6.59	1.71	0.90	3.85	7.32	1.90
H2 black	13.81	3.52	2.75	3.92	5.02	1.28
H3 white	10.97	1.46	-	7.51		
H3 brown	15.64	1.74	1.70	8.99	9.20	1.02
H3 black	18.11	2.00	2.59	9.06	6.99	0.77
H4 brown	5.00	1.15	1.02	4.35	4.90	1.13
H4 black	15.92	6.82	2.96	2.33	5.38	2.30
H5 white	6.08	0.47	-	12.94		
H5 brown	11.17	1.03	1.40	10.84	7.98	0.74
H5 black	20.44	1.69	3.80	12.09	5.38	0.44

* Not detected; ** Below quantification limit.

Conclusion: Our results suggest that there is a close correlation between the affinity of drug to melanin in hair and the lipophilicity of the drug. However, there is a difference in drug metabolism and the difficulty in drug incorporating into hair between human and animal. This method can be used in the analysis of ketamine in hair in forensic toxicology.

Key words: ketamine; metabolites; doses; hair; colors

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Determination of opiates in biological human samples by liquid chromatography-tandem mass spectrometry

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Abstract:

Introduction: Heroin abuse is undoubtedly the most serious current drug problem in China. In forensic practise the simultaneous quantification of opiates in biological human samples is important to elucidate the cause of death and to evaluate their role in modifying human behaviour. The major advantages of tandem mass spectrometry are highly specific and sensitive, especially simple preparation without derivatization for opiates.

Aims: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed for the determination of opiates in biological samples according to the emerging problem in drugs abuse.

Methods: Opiates such as heroin, 6-acetylmorphine, morphine, codeine, acetylcodeine, hydrocodone and hydromorphone were extracted with 2ml chloroform: isopropanol (9:1) from human blood, urine, oral fluid and hair. The sample was then separated on an Allure propyl PFP column, with a mobile phase of acetonitrile-20mM ammonium acetate (7:3, v/v). Detection was implemented with MRM mode by an API4000 triple quadrupole tandem mass spectrometer. The method was evaluated by real cases.

Results: The mobile phase gives the optimum separation for opiates. There was no interfering peak at the retention times for analytes in blank hairs. With the use of morphine-d3 as internal standard, good linear calibration curves were obtained for opiates, with correlation coefficients greater than 0.99 (Table 1).

Table1 Linearity and LOD

compounds	range (ng/mg)	Linearity	r	LOD(ng/mg)
morphine	0.04-100	y=2.52x+1.29	0.9993	0.01
6-acetylmorphine	0.1-10	y=3.93x-0.377	0.9993	0.04
codeine	0.01-100	y=1.99x+0.222	0.9999	0.004
acetylcodeine	0.01-0.4	y=11.9x+0.182	0.9967	0.004
hydrocodone	0.1-10	y=32.4x-4.75	0.9948	0.04
hydromorphone	0.4-10	y=3.07x-0.529	0.9948	0.1

10 hair samples obtained from heroin addictions were determined using developed method. The analytical results are presented in Table 2. The concentration of morphine was much higher than that of 6-acetylmorphine, which is similar with the result using GC-MS by the author.

Acetylcodeine (AC), which is an impurity of illicit heroin synthesis, is a specific marker of illicit heroin. As shown in Table 2, AC was hard to be found in hair. 3 specimens were negative for AC. The other 7 specimens were found positive but the concentration was lower than that of 6-acetylmorphine. This result is the same with Dr. Kintz P and Dr. O′ Neal‘s conclusion that

although AC is indicative of illicit heroin use, this substance would not make a suitable biomarker in place of 6-acetylmorphine because of its low concentration in hair compared with that of 6-acetylmorphine.

Table 2 results from human hair samples

No.	morphine (ng/mg)	6-acetylmorphine (ng/mg)	codeine (ng/mg)	acetylcodeine (ng/mg)	hydrocodone (ng/mg)	hydromorphone (ng/mg)
1	18.6	+	17.7	-	0.445	0.485
2	10.2	0.263	14.4	0.0449	0.371	+
3	0.565	0.112	2.19	-	0.181	-
4	30.2	0.385	29.4	0.0766	1.10	0.888
5	19.1	1.84	33.1	0.520	1.18	0.682
6	6.74	0.115	8.64	-	0.273	-
7	21.8	0.493	22.1	0.0767	0.788	0.624
8	7.37	1.65	10.6	0.314	0.303	+
9	19.3	2.64	30.7	0.804	0.822	0.504
10	2.74	1.03	4.01	0.203	0.231	+

Conclusion: The developed method was simple and rapid, offering superior sensitivity and selectivity for opiates, and was applied in real cases successfully. 6-acetylmorphine in hair was a suitable biomarker for illicit heroin use. Target compounds comprising hydrocodone and hydromorphone enlarge the applied area.

Key words: LC-MS/MS; opiates; hair; sensitivity

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