Indian Internet Journal of Forensic Medicine & Toxicology
Vol. 10, No. 2 April-June 2012, 41-48
DOI : 10.5958/j.0973-1970.10.2.013

Review Article

Hair: An Evidence for Drug of Abuse

Shruti Gupta*, Dr. Vinod Dhingra**

*Research Student, **Scientific Officer, Regional Forensic Science Laboratory Gwalior (Madhya Pradesh)
Regional Forensic Science Laboratory, Gwalior, Madhya Pradesh-474 011

**E-mail id: vdhigraso@hotmail.com

ABSTRACT

Hair analysis for drug abuse has been gaining increasing significance in forensic sciences. Hair is a special evidence used for the investigation of chronic drug of abuse or poisoning in criminal cases. It provides results with sensitive methods even if the drug is present in low amount. Segmental hair analysis can yield information about the time course of the substance used. Forensic hair analysis generally involves cases where the drug history of an individual is required. The hair analysis has to be undertaken by a specialist forensic toxicologist and the test is sometimes referred to as a 'hair follicle test'. Apart from collecting samples of hair for analysis, the forensic group involves an internationally recognized specialist forensic hair toxicologist who reviews work already completed by other forensic hair toxicologists. The hair sampling, sample preparation, and analytical performance are very important for final results. The outcomes of hair analysis have been reviewed by dividing the drugs into six groups: opiates, cocaine, amphetamines, cannabinoids, abused therapeutic drugs, and the markers of chronic alcohol consumption.

INTRODUCTION

In the early 1960s and 1970s, hair analysis was done to evaluate exposure to toxic heavy metals, such as arsenic, lead or mercury using atomic absorption spectrum. Ten years later, drugs could be extracted from the hair by means of radio immune assay. In this, the differences in the concentration of morphine along the hair shaft correlated with the time of drug use for hair analysis. Nowadays, hair is being recognized as a third fundamental biological specimen for drug testing besides urine and blood. Due to the progress of separation techniques and detection sensitivity and selectivity, drugs in the hair can be detected and determined at the levels of pico-mole/mg. Human hair has also been successfully used to assess drug and substance abuse since drugs of abuse cannot be often detected in body fluids. Using very sophisticated analytical techniques such as immunoassay and gas chromatography, investigators are now extensively using human hair to solve cases of poisoning and drug and substance abuse.

Using hair as a medium to analyse drug use has been receiving increased attention during the recent years. This is because they are a lesser embarrassing evidence to collect and also due to the fact that hair does not decompose like other body fluids or tissues.

Hair testing also offers a wider detection window after drug exposure than urine testing. The major practical advantage of hair testing compared to urine or blood testing for drugs is that it has a larger surveillance time. That is, the drug remains in the hair for weeks and months whereas it remains in the urine and blood for hardly 2-4 days. Thus, long-term history of drug usage can be understood through hair analysis of the abuser.

BIOLOGY OF HAIR

Hair may be defined as slender filamentous outgrowths of the skin and is primarily composed of keratin. It differs from one animal species to another in length, colour, shape, root appearance, and morphological characteristics. There is also a considerable deal of variability in the type of hair found on the body of a particular animal. In humans, they are distributed on the head, pubic region, arms, legs, and

other body areas. Structurally, hair consists of: a) an inner cortex comprising spindle-shaped cells, and b) an outer sheath called the cuticle. Each cortical cell consists of many fibrils. In between the fibrils, a softer material called the matrix is present which grows from a hair follicle. The fibrils run to form the fibre axis. The cuticle consists of scale-shaped layers and is responsible for much of the mechanical strength of the hair fibre. The cuticle is made up of a number of layers, which varies from one species to another. Typical human hair has six to eight layers of cuticle.

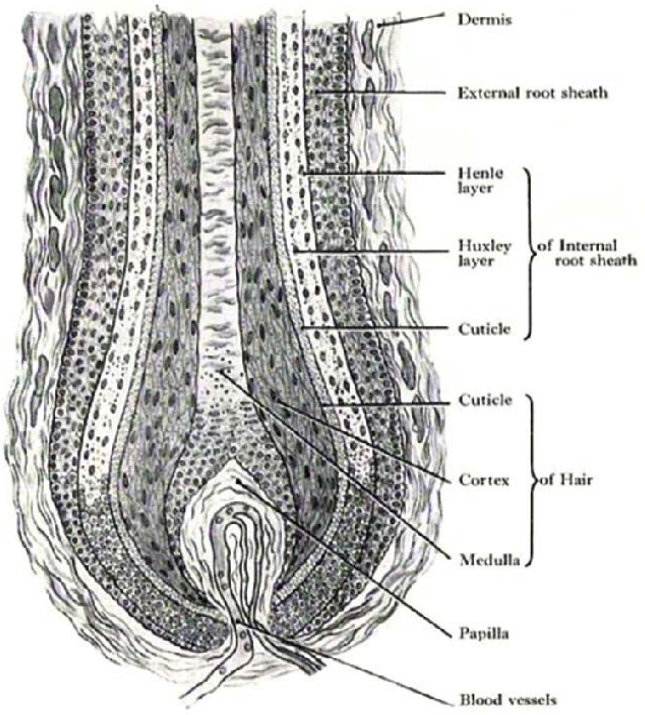

Diagram of a longitudinal section of a hair in a follicle.

HAIR GROWTH AND FORMATION

Hair formation begins in the third month of foetal life. Each hair grows through a follicle and is made up of epidermal cells that grow under the dermis. Initially, the epidermis thickens and cells begin to grow down into the dermis. This down growth forms a cap over some of the connective tissue to create papillae whose cells multiply to form the hair. As these cells are pushed up the central canal of the hair shaft and away from their source of nourishment, they become impregnated with keratin. The morphogenesis of most hair follow a cyclic pattern of cell proliferation and differentiation initiated in mid to late embryonic development and repeated throughout life (Orwin 1979). As hair undergoes a cyclical growth, intermediate and resting phases, the visible morphological characteristics under the microscope are sufficient to determine the phases of growth of the hair.

INCORPORATION MECHANISM OF DRUG IN TO HAIR

Drugs can penetrate into the hair by through two processes: adsorption from the external environment and through penetration into the growing hair shaft from blood that supplies the hair follicle. Drugs can be transported from blood and also from deep skin compartments not only into hair growing cells but with some time delay also into the keratogenous zone during hair shaft formation. The other mechanisms are diffusion from sweat or sebum secretions. A contamination from external environment need not be excluded on the hair surface exiting the skin the three key factors which influence the drug incorporation into hair are melanin content in a hair, lipophilicity and basicity of a drug substance. The physicochemical properties of drugs, lipophilicity and basicity related to molecular structure clearly affect the drug incorporation into hair and on the other side, hair structure and its colour play a very important role too. so, it can be concluded briefly that drugs appeared to be incorporated into hair by at least three mechanisms:

1. From the blood during hair formation.
2. From sweat and sebum.
3. From the external environment.

SPECIMEN COLLECTION PROCEDURE

Sample collection doesn't necessarily require a physician but can be done by a responsible authority who respects ethical and legal principles. Hair samples are collected from random locations on the scalp. Hair is best collected from the area at the back of the head, which is called the vertex posterior. This is because the hair in this area has less variability in hair constant and is less subject to age and sex-related influences. Hair strands are cut as close as possible from the scalp. During collection, hair specimen orientation must be marked so that the root or

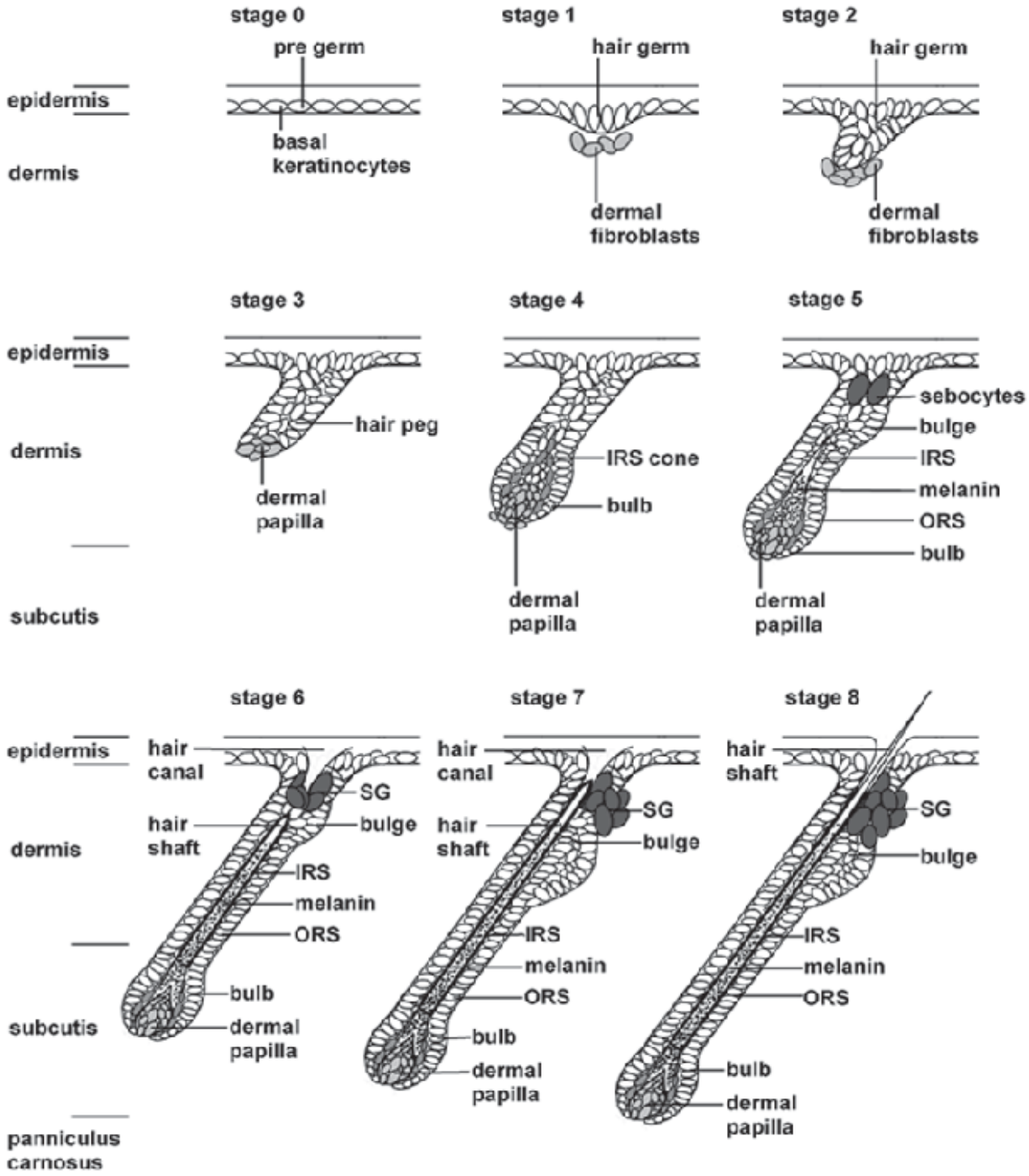

Figure 2: Stages of hair growth

Table 1: Drugs of abuse found in the hair

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

Table 2: Expected concentration ranges in hair of drug users

Compound	Concentration (ng/mg hair)
Cocaine	0.1–28.9
Cocaethylene	0 –2.6
Benzoylecgonine	0 –4.4
Ecgonine methylester	0 –4.4
Heroin	0 –16
6-Monoacetylmorphine	0.1– 67
Morphine	0.1–10
Codeine	0 –4.2
Methamphetamine	3.1–126
Amphetamine	0.8 –12

tip end could be clearly identified. Analysis of hair involves a series of steps generally starting with documenting weight, length, colour and potential chemical treatment of hair and the anatomical part of the body from where the sample was collected. Prior to analysis, the hair sample is decontaminated by washing with a variety of solvents. Once collected, the hair samples are stored at ambient temperature in aluminium foil, an envelope or a plastic tube. The sample size taken varies considerably between laboratories and depends on the drug to be analysed and the test methodology. For example, when fentanyl or buprenorphine are investigated, a 100 mg sample is recommended. When sectional analysis is performed the hair is cut into segments of about 1, 2, or 3 cm, which corresponds to about 1,2, or 3 months growth.

DECONTAMINATION OF HAIR SAMPLE

The collected hair sample may be contaminated due to the drugs of abuse or their metabolites thus interfering with the analysis and interpretation of the test results. It is unlikely that anyone would intentionally or accidently apply anything to their hair that contained a drug of abuse. The most crucial issue facing hair analysis is to avoid technical and evidentiary false-positives. Technical false positives are caused by errors in the collection processing and analysis of specimens, while evidentiary false positives are caused by passive exposure to the drug.

Washing is done to decontaminate the hair sample. In this paper, washing of hair samples investigated mostly for the analysis of cocaine has been described. Washing solvents for hair samples containing cocaine are generally divided into three categories; MeOH or EtOH, 0.1% sodium dodecyl sulfate (SDS) or other detergents. The hair samples are incubated, briefly washed or stirred in these solvents at room temperature-additionally used as a rinse. The purpose of washing is to remove only the external contamination or unnecessary dirt and grease from the surface of hair. Therefore, over-washing should be avoided so that drugs inside the hair shaft remain unaffected. When control hair was soaked in an aqueous solution can of 10 mg/ml of methamphitamine (MA) hydrochloride for 24 hours, MA contaminated on the hair surface could be removed easily by washing with 0.1% SDS.

EXTRACTION METHOD OF DRUGS FROM HAIR

Drug extraction procedures are mainly divided into three categories: digestion with alkali, acid extraction, and enzymatic treatment.

1. Digestion (alkaline agent)

The general alkaline digestion method involves incubation of the hair sample in 0.1|2.5 *M* NaOH at378 ^{0}C b overnight. After adjustment to pH 9 with acid, the aqueous solution is extracted with solid phase extraction. Such alkaline methods are applicable to alkaline stable compounds such as morphine, amphetamines, and cannabinoids, but generally cannot be used for the analysis of cocaine, heroin /6MAM and other ester compounds in hair.

2. Enzymatic treatment

Moeller reported a hydrolysis method with b-glucuronidase / arylsulfatase (glusulase) for the destruction of the hair structure. Hair (10|30mg) was hydrolysed with 75 ml glusulase solution for 2 h at 408 C. After centrifugation, the supernatant was extracted by SPE. The demerit of this method is that it is relatively expensive.

3. Acid extraction and acidic methanol

Acidic extraction of drugs from hair has been described at many places. The extraction in 0.1~0.6*M* HCl or 0.05M sulphuric acid is generally carried out at room temperature or 378 ^{0}C overnight. After acid treatment, the solution is neutralized and extracted with SPE. An effective method for the direct extraction of hair is with methanol–5N hydrochloric acid (20:1) under ultra-sonification for 1 hour with storage over night. Methanol–trifluoro-acetic acid (9:1) is good for extraction of 6MAMfrom heroin users' hair. The method using methanol–trifluoroacetic acid (9:1) minimizes hydrolysis of acetylmorphine and maximizes its extraction efficiency.

Drug solubilization

To determine the amount of drug that remains in hair after washing, it is necessary to solubilize the drugs in the hair. Solubilization must be such that the analytes are not altered or lost. Care must be taken to prevent the conversion of cocaine to benzoylecgonine or 6-mono acetyl morphine(6-MAM) to morphine for example.

The hair sample can be pulverized in a ball-mill prior to testing, cut into segments or the entire hair dissolved. The preparation techniques are generally based on one of the following procedures.

- Incubation in an aqueous buffer and analysis using immunological techniques, mostly RIA.
- Incubation in an acidic or basic solution followed by liquid-liquid extraction or solid-phase extraction and analysis with chromatographic techniques, mostly GC-MS.
- Incubation in an organic solvent (generally methanol with or without hydrochloric acid), liquid-liquid extraction or solid phase extraction and analysis with chromatographic techniques mostly GC-MS.
- Digestion in an enzymatic solution, liquid-liquid extraction or solid-phase extraction and analysis with chromatographic techniques, mostly GC-MS.

DRUG ANALYSIS METHODS

1. Immunological methods

Immunoassays are used as screening tests because of their sensitivity, speed, and convenience. The procedure must be compatible with the screening test used (that is, detergents or hair -digestion products must not interfere with the assay). Neutralization in case of chemical hydrolysis is always necessary. Destruction of the organic protein matrix of hair must be done under conditions sufficiently mild so as to not damage the entrapped analyte or the protein antibodies subsequently added for the immunoassay.

Radioimmunoassay (RIA) is the most common screening test for hair. Calibration curves are obtained from the controlled samples in the kit extracts of drug free hair samples, spiked with the drugs. The RIA results should be confirmed by GC-MS. However, even the high sensitivity of GC-MS is sometimes not sufficient to detect drugs, especially when they are present in small quantities. For these reasons, it may be necessary to carry out immunological analysis of drugs in hair using RIA reagents that are specific for the selective estimation of drugs like fentanyl, lysergic acid diethylamide or buprenorphine.

2. Chromatographic methods

Chromatographic methods have been used as screening and confirming tests. They allow quantification of the drugs and drug metabolites.

- Thin layer chromatography (TLC) is used for the detection and quantification of morphine in human hair.
- High–performance liquid chromatography (HPLC) is a method used to detect morphine, haloperidol, beta-blockers and buprenorphine. Different kinds of detectors are used, including ultraviolet (UV), fluorimetry, and coulometer.
- Gas chromatography (GC) is coupled to flame ionization or nitrogen detection is less useful for the analysis of drugs in hair. GC-MS is the most powerful tool for drug analysis in hair.

DRUG IDENTIFICATION

1. Cocaine

The procedure to detect cocaine is given below.

Decontamination: take hair strand (approx. 100 mg)

- Wash with 5 ml methylene chloride for 2 min.
- Dry with adsorbent paper.
- Secondly, wash in 5 ml methylene chloride for 2 min.

Homogenization: pulverize the hair in a ball-mill for 10 min at 100 cycles/min.

Solubilisation:

- To 30 to 50 mg of powdered hair add 1 ml 0.1 M HCl and 200 ng of deuterated cocaine derivatives.
- Incubate for 16 h at 56°.

Extraction:

- Homogenate with 10 ml chloroform:propan-1-ol:hept-1-ane(50:17:33)
- Agitation, 20 min at 95 cycles/min.
- Centrifuge for 15 min at 3000 rpm.
- Purify the organic phase by acid extraction (5 ml 0.2M HCl) then alkaline back extraction (1 ml 1M NaOH+ 2 ml phosphate buffer pH 8.4 in chloroform).
- Collect organic phase and evaporate to dryness.

Derivatization:

- To the dry extract add 30µL N O-bis (trimethylsilyl)-trifluoroacetamide (BSTFA)+ 1% trimethy-lchlorosilane (TMCS).
- Incubate for 30 min at 70°.

Analysis:

A 1.5µL portion is injected in splitless mode into an HP5-MS capillary column (30m×0.25mm). The GC parameters are:

- Flow rate helium N55, 1.0 ml/min.
- Injector temperature, 270°.
- Temperature programme, 60° for 1 min, 30° /min to 295°, 295° for 6 min.

2. Amfetamines

The procedure to detect cocaine is given below.

Decontamination: take hair strand (approx. 100mg)

- Wash with 5 ml methylene chloride for 2 min.
- Dry with adsorbent paper.
- Second wash in 5 ml methylene chloride for 2 min.

Homogenization: pulverize the hair in a ball-mill for 10 min at 100 cycles/min.

Solubilisation:

- To 30 to 50 mg of powdered hair add 1 ml 1 M NaOH and 200 ng of deuterated drugs.
- Incubate for 10min at 95°.

Extraction:

- Homogenate with 10 ml 5 ml ethyl acetate.
- Agitation, 20 min at 95 cycles/min.
- Centrifuge for 15 min at 3000 rpm.
- Collect organic phase, add 20 µL of methanol:HCl (99:1) and evaporate to dryness.

Derivatization:

- Dry extract with 150 µL ethyl acetate: heptafluoro-butyric anhydride (HFBA) (1.2).

- Incubate for 30 min at 60°.
- Re-evaporate to dryness.

Analysis:

The derivatised extract is dissolved in 25 µl ethyl acetate. A 1.5 µl portion is injected in split less mode into an HP5-MS capillary column (30m×0.25mm).

The GC parameters are:

- Flow rate heliumN 55, 1.0 ml/min.
- Injector temperature, 240°.
- Temperature programme , 60° for 1min,30° /min to 295°, 295° for 6 min.

3. Cannabis

The procedure to detect cocaine is given below.

Decontamination: take hair strand (approx. 100 mg)

- Wash with 5 ml methylene chloride for 2 min.
- Dry with adsorbent paper.
- Secondly, wash in 5 ml methylene chloride for 2 min.

Homogenization: pulverize the hair in a ball-mill for 10 min at 100 cycles/min.

Solubilisation:

- To 50 mg of powdered hair add 1 ml 1 M NaOH and 200 ng of deuterated drugs.
- Incubate for 10 min at 95°.

Extraction:

- Homogenate with 5 ml hex-1-ane:ethyl acetate(9:1).
- Agitation, 20 min at 95 cycles/min.
- Centrifuge for 15 min at 3000 rpm.
- Collect organic phase, and evaporate to dryness.

Analysis:

The extract is dissolved in 20 µl cyclo hexane. A 1.5 µl portion is injected in split less mode into an HP5-MS capillary column (30m×0.25mm).

The GC parameters are:

- Flow rate helium N55, 1.0 ml/min.
- Injector temperature, 250°.
- Temperature programme, 60° for 1min, 30°/min to 295°, 295° for 6 min.

Cannabinoid	Retention time (min)	Ions
THC	9.59	299,314,271
THC-d3	9.58	302,317,274
Cannabidiol	9.28	231,314,246
Cannabinol	9.84	295,310,238

Benzodiazepines

The procedure to detect cocaine is given below.

Decontamination: take hair strand (approx. 100mg)

- Wash with 5 ml methylene chloride for 2 min.
- Dry with adsorbent paper.
- Secondly, wash in 5 ml methylene chloride for 2 min.

Homogenization: pulverize the hair in a ball-mill for 10 min at 100 cycles/min.

Solubilisation:

- To 30 to 50 mg of powdered hair add 1 ml Sorensen Phosphate buffer and 5 µl of deuterated flunitrazepam and amino flunitrazepam.
- Incubate for 2 hours at 45°.

Extraction:

- Homogenate with 5 ml diethyl ether : chloroform (80:20)
- Agitation, 20 min at 95 cycles/min.
- Centrifuge for 15 min at 3000 rpm.
- Collect organic phase, and evaporate to dryness.

Derivatization :

- Dry extract with 150 µl ethyl acetate: heptafluorobutyric anhydride (HFBA)(1.2).
- Incubate for 30 min at 60°.
- Re-evaporate to dryness.

Analysis:

The derivatised extract is dissolved in 25 µl ethyl acetate. A 1.5 µl portion is injected in split less mode into an HP5-MS capillary column (30m×0.25mm).

The GC parameters are:

- Flow rate helium N55, 1.0 ml/min.
- Injector temperature, 240°.
- Temperature programme, 60° for 1 min, 30°/min to 295°, 295° for 6 min.

Flunitrazepam	Retention time (min)	Ions
Flunitrazepam	10.49	313,297,281
Flunitrazepam-d3	10.49	316,300,284
Aminoflunitrazepam	9.90	459,441,478
Aminoflunitrazepam-d3	9.90	462,444,481

CONCLUSION

Continuous advances in analytical technologies have resulted in a decrease in the detection time of analytical methods, improving their accuracy and thus allowing better scientific understanding and interpretation of test data. This will help in the acceptance of these techniques as being useful and an objective tool of evidence or important information for subsequent measures with impacts to an individual. So far, the target drugs in the analysis are mostly typical drugs of abuse. With modern laboratory facilities, lower and lower quantities will be detectable in hair and thus some other harmful substances will be of analytical interest .In the early stages of hair analysis, it was thought that it was very hard to detect a few ng or sub-ng amounts of drugs contained in hair. We can now detect and determine 0.01 ng/mg or less of drug in hair by GC–MS.

Now-a-days, the value of hair analysis in the identification of drug users is gaining recognition. This may be seen from its growing use in pre-employment screening, forensic sciences, and clinical applications. Hair analysis may be an useful adjunct to conventional drug testing in toxicology. Specimens can be obtained more easily with less embracement, and hair can provide a more accurate history of drug use.

However, the weaker side to hair analysis must also be mentioned.

- It is difficult to prepare reference hair standards containing accurate concentration of drugs which are necessary for calibration.
- The question of efficiency of drug extraction from solid matrix is very important and this parameter needs to be evaluated for each type of drug in every laboratory. The standardization of decontamination and extraction procedures is also desirable.
- Minimal performance standards should be kept in different laboratories to assure inters laboratory comparability of test results. A sufficient LOD values, comparable cut-off values will support correct identification of drugs and metabolites in hair.

A well-known weak point of hair analysis is the problem of making intact hair standards containing precise concentrations of drugs Therefore, it is necessary to develop a way to make hair standards containing precise concentrations of drugs. With the hair standards, a suitable method for each drug may be chosen.

REFERENCES

1. Sachs H. History of hair analysis. Forensic Sci International 1997; 84: 7-16.
2. Baumgartner AM, Jones PF, Baumgartner WA, Black CT. Radioimmunoassay of hair for determining opiate abuse histories. J Nucl Med 1979; 20: 748–752.
3. Saitoh MM, Uzuka M, Sakamoto M, Kobori T. Rate of hair growth. In: Montagna W, Dobson RL(Eds.), Advances in Biology of Skin. Vol. 9, Pergamon Press, Oxford; 1969.
4. Ramseier A, Siethoff J, Caslavska J, Thormann W. Electrophoresis 2000; 21: 380.
5. Moeller MR, Fey P, Wenig R. Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program. Forensic Sci International 1993; 63: 185–206.
6. Cirimele V, Kintz P, Doray S, Ludes B. Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis. Int J Legal Med 2002; 116: 54–57.
7. Pötsch L, Skopp G, Zörntlein S, Becher J. Zum Suchtmittelnachweis in Haaren. IV. Einfluss der Pigmentierung auf den Ofloxacingehalt in Haaren bei Meerschweinchen. Rechtsmedizin 1997; 7: 147–151.
8. Nakahara Z, Takahashi K, Kikura R. Hair analysis for drugs of abuse. X. Effect of physicochemical properties on incorporation rates into hair. Biol Pharm Bull 1995; 18: 1223–1227.