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REVIEW ARTICLE

Hair as a Biological Indicator of Drug Use, Drug Abuse or Chronic Exposure to Environmental Toxicants

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In recent years hair has become a fundamental biological specimen, alternative to the usual samples blood and urine, for drug testing in the fields of forensic toxicology, clinical toxicology and clinical chemistry. Moreover, hair-testing is now extensively used in workplace testing, as well as, on legal cases, historical research etc. This article reviews methodological and practical issues related to the application of hair as a biological indicator of drug use/abuse or of chronic exposure to environmental toxicants. Hair structure and the mechanisms of drug incorporation into it are commented. The usual preparation and extraction methods as well as the analytical techniques of hair samples are presented and commented on. The outcomes of hair analysis have been reviewed for the following categories: drugs of abuse (opiates, cocaine and related, amphetamines, cannabinoids), benzodiazepines, prescribed drugs, pesticides and organic pollutants, doping agents and other drugs or substances. Finally, the specific purpose of the hair testing is discussed along with the interpretation of hair analysis results regarding the limitations of the applied procedures.

Keywords Hair, Drugs of Abuse, Prescribed Drugs, Alcohol, Toxicants, Doping Agents

The rationale for performing an analysis in a particular specimen differs depending upon the question(s) to be answered. Drug use, drug dependence, overdose situations of medication or abused drugs, chronic exposure, actively or passively performed, all are matters that could be answered through a specific analysis. But not all tissues or fluids are suitable for answering all the questions that could be imposed. The body matrices usually used for toxicological analysis of clinical and forensic interest are urine, blood, saliva, hair, sweat, breath, meconium and in postmortem cases any tissue or fluid is considered suitable for the particular analysis requested.

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Undoubtedly blood and urine are the routine samples of choice for drug analysis. Nowadays, besides blood and urine, hair is being recognized as an alternative and fundamental biological specimen for drug testing. The major practical advantages of hair testing are (i) larger detection windows (from 3 days to years), depending on the length of the hair shaft, compared to those of urine/blood (hours to 2–4 days for most drugs); (ii) evaluation of long term history to short term history (e.g. if urine is analyzed); (iii) the sample collection is non-invasive, it is easy to be performed under conditions that prevent adulteration and substitution. Furthermore, hair analysis has found even broader applications in evaluating environmental exposure to toxicants even from the intrauterine period of life (incorporated to human body from any source), in doping controls and in drug abuse studies in the fields of forensic toxicology, clinical toxicology and clinical chemistry.

This contribution describes the application of hair analysis in the detection of drugs and other substances, and reviews all the substances (except metals) that have been detected in human hair. All the basic aspects and limitations in the analysis of every substance category are discussed along with the pitfalls in evaluating the results.

HAIR STRUCTURE AND DRUG INCORPORATION

Hair Formation and Hair Structure

Hair although appears to be a fairly uniform structure, in fact is very complex and its biology is only partially understood (Robbins 1988). Hair has two separate domains the hair shafts (external domain) which are cylindrical structures made up of tightly compacted cells that grow from the follicles (internal domain) which are small sac-like organs in the skin (Figure 1).

Hair follicles are embedded in the epidermal epithelium of the skin and are associated with the sebaceous glands. In the axillary and pubic areas hair follicles are also associated with the apocrine gland. Both sebaceous and apocrine glands empty their ducts into the follicle. The eccrine sweat glands are located near

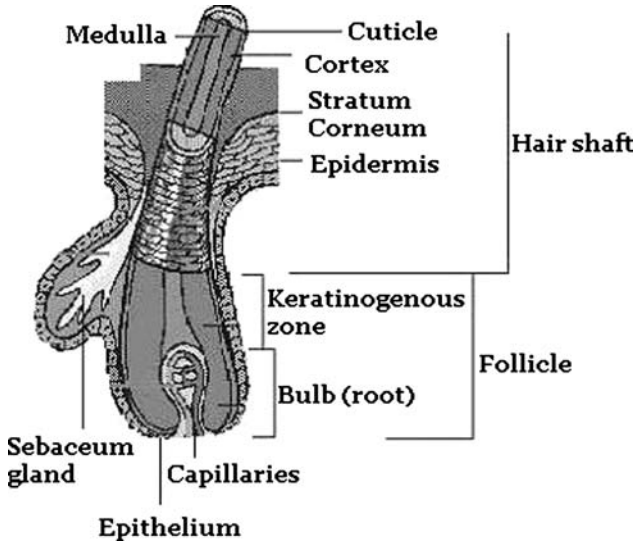

FIGURE 1
Simplified diagram of the hair structure, the associated sebaceous gland and the surrounding tissues.

the follicles but do not empty their ducts into them. The innermost zone of the follicle, called bulb, is the site of biosynthesis of hair cells and it is in contact with capillaries. The cells in the bulb divide every 23 to 72 hours, faster than every other cell type in the body. Directly above the bulb is located the keratinogenous zone where the hair undergoes hardening and solidification. The final zone is the permanent hair shaft.

As the cell division proceeds, the cells increase in volume, they elongate and they move up the follicle into the keratinogenous zone. There, the cells synthesize pigment (melanin) and begin to keratinize (enrich in keratins, which are sulfur-rich proteins). Then, the hair cells gradually die and decompose by eliminating the nucleus and releasing water, coalescing into a dense mass. Meanwhile, keratins form long fibers, which bound together through the formation of disulfide bonds (-S-S-) and through cross-linking with other proteins.

Each hair shaft consists of three distinct types of dead, keratinized cells arranged in three layers. (a) The outer layer is the cuticle which consists of elongated, overlapping (like roof shingles) individual cuticle cells, each having 0.5 to 1.0 μm thickness and about 45 μm length. The function of the cuticle is to protect the interior fibers. Chemicals, heat, light or mechanical injury can damage or even destroy the cuticle. It is worth mentioning that most hair conditioning products affect the cuticle and as a result it becomes less intact and may be frayed and fell apart. (b) The second layer is the cortex, which forms the built of the hair shaft and is composed of long keratinized cortical cells, which form long fibers, about 100 μm in length. Between the cortex cells, very small spaces are located called fusi. Fusi are filled initially with fluid but as the hair grows and dries out the fluid is replaced by air. In cortical cells are also found pigment granules containing mainly melanin. Melanin is synthesized in specialized cells, the melanocytes, located in the hair bulb. The amount, the density and the type of melanin in melanocytes determines the exact color of hair. (c) The central layer of the hair shaft is the medulla, which consists of medullar cells. In human hair, medulla comprises a small percentage of the hair mass—continuous along the central axis or discontinuous—or it is completely absent. In general, the size of medulla increases as the hair fiber's diameter increases. Individual human hair shaft ranges in diameter from 15 to 120 μm depending upon the hair type and the body region the follicle is located.

It could be stated that hair is an oriented polymeric network, partially crystalline, containing different functional chemical groups (e.g. acidic, basic etc.) which have the potential to bind small molecules. The composition of human hair (depending on its moisture content) is 65–95% protein, 15–35% water and 1–9% lipids. Mineral hair content varies from 0.25 to 0.95% (dry weight basis). The lipids found in hair are derived from sebum and the secretions of apocrine glands. They consist of free fatty acids, mono- di- and triglycerides, wax esters, hydrocarbons and alcohols. Hair proteins are rich in the amino acids glycine, threonine, aspartic and glutamic acid, lysine and cysteine.

The lifetime of the human hair consists of three phases: anagen, catagen and telogen. Anagen is the active growth phase of hair where the cells in the bulb of the follicle are divided rapidly. A new hair is formed as new cells elongate and form a thin filament. Then the hair cells push their way upwards into the follicular canal, differentiate into cuticle, cortex or medulla cells and keratinization is initiated. During this phase the hair grows about 1 cm every 28 days. Scalp hair stays in this phase for 2–6 years. The hair on the arms, legs, eyelashes and eyebrows have an anagen phase of about 30–45 days.

The average rate of hair growth it is usually stated to be 0.44 mm per day (range 0.38–0.48) for men and 0.45 mm per day (range 0.40–0.55) for women in the vertex region of the scalp (Nakahara 1999). The hair growth rate depends on the anatomical location, race, gender and age. Scalp hair grows faster than pubic or axillary hair (about 0.3 mm/day), which in turn grows faster than beard hair (approximately 0.27 mm/day). In general, the longer the hair type is the longer the growing phase lasts. During this phase, the capillary blood supply around the follicle provides nutrients and delivers any extraneous substances that might be in the blood stream such as trace metals, drugs etc. These substances become incorporated into the hair shaft and as it grows they are carried along. Moreover, chemicals could become incorporated into hair at the level of keratinogenous zone from surrounding tissues, lymph or intercellular fluids.

The catagen phase is the short transitional phase that enters the hair, following the anagen phase. During this phase, the cell division stops and the hair shaft becomes fully keratinized. The follicle becomes considerably shorter. This phase lasts for about 2–3 weeks.

Telogen is the resting or quiescent phase, in which the growth of the hair shaft stops completely. During the telogen phase the hair is just anchored in the follicle by the root. The germ cells below the root will give rise to the next anagen hair while the old hair will be forced out and lost. The resting phase lasts for

about 10 weeks for scalp hair while for the rest body surface hair lasts for about 2–6 years.

Individual hair shafts do not have synchronous growth cycles. On a healthy head, 80–90% of the hair follicles are in the anagen phase, 2% in the catagen phase and 10–18% in the telogen phase (Harkey 1993).

Mechanisms of Drug Incorporation into Hair

The simplest model proposed for drug incorporation into hair is the simple passive transfer. In this model chemicals move by passive diffusion from the bloodstream into the growing hair cells at the base of the follicle and then during subsequent keratogenesis they become tightly bound in the interior of the hair shaft. The incorporation is dependent on the drug concentration in blood, which depends on the ingested drug dose. Since hair is assumed to be growing at a constant rate, this model forms the scientific base for determining the time-course of drug use by performing segmental hair analysis. This means that the position the drugs are found along the hair shaft can be correlated with the time the drugs were present in the bloodstream. Therefore, segmental analysis can provide a "calendar" of drug use for an individual.

Several studies have shown a positive correlation between dose and amount of drug in the hair (Baumgartner, Hill, and Blahd 1989; Nakahara, Ochiai, and Kikura 1992; Cone 1990). However, in other studies, has been stated that the distribution of substances along the hair shaft does not always correlate well with the time of exposure (Cone 1990; Nakahara, Shimamine, and Takahashi 1992).

The first dispute on the passive diffusion model came from data concerning incorporation of elements into hair and the poor correlation that was observed between the body burden in elements and their resulting levels in hair (Chittleborough and Steel 1980). Later, other studies concerning morphine and cocaine intake reported also poor correlation between dose and hair drug concentration, since the observed variability could not be explained by differences in the plasma pharmacokinetics of the subjects (Henderson 1993). To date, there has not been established a clear correlation between drug intake and drug concentration in hair for all drugs.

The passive transfer model has been further questioned by the finding of very high parent drug to metabolite ratios in hair (Harkey et al. 1991; Goldberger et al. 1991; Moeller, Fey, and Rimbach 1992). For example the ratio of nicotine/cotinine is 10 (range 5–30) in the hair of smokers (Kintz, Ludes, and Mangin 1992b). The ratio of 6-monoacetylomorphine 6-MACM/morphine is 3 in hair of heroin users (Cone 1990) while in the hair of cocaine users the ratio of cocaine/benzoylecgonine (BEG) averages 10 (range 1–50) (Henderson et al. 1992). Other studies have reported that the parent drug and the lipophilic metabolites are primarily found in hair (Cone et al. 1991; Henderson et al. 1992; Kintz, Ludes, and Mangin 1992a).

In the view of the aforementioned experimental data, a more complex multi-compartment model is now acceptable in order to explain how drugs get into hair. In this model, drugs are suggested to be incorporated into hair via: (i) the blood circulation during formation; (ii) sweat and sebum after formation; and (iii) the external environment after hair formation and after the hair has emerged from the skin. Substances may also be transferred from multiple body compartments that surround the hair follicle as well (Henderson 1993).

Evidence supporting that sweat and sebum contribute to drug deposition into hair came from several studies. Alcohol (Brown 1985), amphetamine (Vree, Muskens, and van Rossum 1972), cocaine (Smith and Liu 1986), phencyclidine (Perez-Reyes et al. 1982) and methadone (Henderson and Wilson 1973) have been found in sweat, often in concentrations greater than in blood. Individual variability in these secretions could partly explain the variability in hair drug concentration in subjects receiving the same dose, as well as the fact that the drug is sometimes dispersed over a large area of the hair shaft. Moreover, the inter-laboratory variability in washing and extraction procedures could be explained by this model, since drugs can be transferred, through these secretions, to hair after its formation and thus may be bound less tightly and be removed by washing more easily (Henderson 1993).

External contamination of hair is divided into two categories: deposition of drugs on the surface of the hair and passive inhalation. External deposition of substances on the keratinized mature hair fiber from air, water and cosmetic hair treatments has been suggested as a source for some of the trace elements present in hair, as well as, the reason for the difficulty in establishing cut off concentrations for trace elements in hair. Deposition from air could also be a potential route of entry into hair of substances that are smoked, such as amphetamine, cocaine, heroin, marijuana and nicotine (Cone 1990; Koren et al. 1992; Henderson 1993). The interpretation of false positive hair tests resulting from external contamination still remains a subject of debate. On the other hand, passive inhalation of substances that are smoked would result in the incorporation of the substances with the same mechanisms as the active use of substances.

Another possible mechanism of drug entrance into hair is the intradermal transfer of very lipid-soluble drugs like tetrahydrocannbinol (THC) to hair (Touitou et al. 1988). The accumulation of certain drugs into skin layers has been considered the reason for the drug transfer into hair via "a deep compartment model" and a possible explanation for the unusual elimination of cocaine and its metabolites in hair. Another possible mechanism is the drug binding to melanin-related sites in skin, which would result in inter-individual differences in drug uptake into hair, reflecting the differences in melanin levels (Henderson 1993).

In conclusion, the incorporation of drugs and other chemicals into hair is proposed to occur from multiple sites, via multiple mechanisms, and at various periods during the hair growth cycle. The multi-compartment model seems to be more possible for explaining drug incorporation into hair. However, more has to be known about the mechanisms and the factors influencing this procedure, in order a precise correlation to be extracted,

regarding concentration and location of drug in hair and the drug use history of an individual.

HAIR COLLECTION AND PREPARATION

The site of choice for hair collection has been the vertex of the head for most investigators. Its analysis can reveal drug use during time intervals ranging from a week to years prior to sampling. The vertex hair compared to hair from other areas of the head has less variability in the hair growth rate, the number of hairs in the growing phase is more constant and the hair is less subjected to age and sex related influences. Vertex hair appears to be the most uniform in growth and the most consistent in the growth phase. On the other hand, it is exposed to sweat secretions as well as contaminants from air, water or dust and may be modified by cosmetic treatments.

Beard hair is produced from male sexual hair follicles, which are unique to males and are responsible for the high androgen levels found after puberty (Harkey 1993). Beard hair may also be subjected to contamination although is less possible to be contaminated by sebaceous secretions. Pubic hair could be always available even in the absence of other hair and may be less contaminated through environmental exposure or cosmetic treatment. On the other hand, it is exposed to sebum, sweat and other excretions and is rather curved making segmental analysis difficult.

Sample size usually ranges from 100–200 mg of hair, cut as close to the scalp as possible in order to obtain the most suitable sample for the more resent drug use, especially if sectional analysis is to be performed. Hair samples should be stored in a dry place at ambient temperature in aluminum foil, an envelope or a plastic bag. Special attention should be paid to align all hairs in the same direction and to properly label the root end and the tip end of each hair.

Hair collection offers the advantages that could be performed under close supervision without embarrassment and the specimen and analytes are stable even under adverse environmental conditions. Moreover, a second sample could be collected and matched to the first one by macroscopic or/and microscopic examination, or DNA/RNA procedures. Furthermore, hair has a wide window of detection, for identifying drug users, compared to other specimens (Magura et al. 1992; Callahan et al. 1992).

Next to hair collection is the hair preparation for analysis. Hair is an external to the body sample and is subjected to external contamination. Any substance that might be introduced into the body by inhalation (smoking, or gas/vapor inhalation) must be considered as a possible source of producing positive results in hair analysis through passive absorption by hair shaft. In the same manner any substance, liquid or solid, which is handled by an individual could be transferred to his hair. Thus, prior to analysis sufficient decontamination of hair is needed in order to avoid false positives due to passive environmental exposure (Blank and Kidwell 1995). Hair decontamination procedures rest on the assumption that drugs, transferred into hair by passive environmental exposure, are loosely bound to the surface of the hair or to the hair matrix and therefore can be removed by appropriate washing procedures. However, today, it is considered that the amount of a drug deposited from the environment onto the hair is depended on the porosity of the hair shaft. Very porous specimen absorbs high amounts of substances, which are washed out as readily as they enter the hair (Cairns et al. 2004a).

Decontamination procedures, for hair samples, are divided into the following general categories: (1) washing with methanol, ethanol, acetone etc. (Cone et al. 1991; Koren et al. 1992; Goldberger et al. 1991; Fritch, Grioce, and Rieders 1992); (2) washing with sodium dodecyl sulfate solution or other detergents (Harkey et al. 1991; Nakahara, Ochiai, and Kikura 1992; Tagliaro et al. 1993; Welch et al. 1993); (3) washing with dichloromethane (Gaillard and Pepin 1997; Clauwaert et al. 1998) and (4) combined procedures using organic solvents and repetitive washings with phosphate buffers (Cairns et al. 2004a; Blank and Kidwell 1995).

The goal of washing is to remove only the external contaminants, dirt and grease from the surface of the hair as well as drugs that have penetrated the epidermal layers while drugs, inside the hair shaft, should remain unaffected. If the drug found on a hair segment does not correspond to the time of ingestion then is externally deposited drug that has to be largely removed by aggressive washing procedures. However, during the washing process, both (i) removal of incorporated drugs (Wilkins et al. 1997; Koren et al. 1992; Schaffer, Wang, and Irving 2002) and (ii) remaining of externally deposited drugs (Wang and Cone 1995; Goldberger et al. 1991; Koren et al. 1992; Welch et al. 1993) have been reported to take place.

In order not to rely entirely on the removal or not of the contaminants, various washing methods have been suggested, which combine analysis of the washes and comparison of the drug concentration found in the washing solution with that found in hair after washing (wash ratios) (Baumgartner, Hill, and Blahd 1989). Wash ratios have been proposed as a criterion to distinguish passive exposure from active use by several authors (Cairns et al. 2004a; Baumgartner and Hill 1993).

In experiments involving differently colored hair, it was found that the incorporation of drugs into hair was variable depending on the hair type. The most absorbent was the thick black hair and thus it was the most resistant to decontamination, whereas thin brown hair was the less absorbent (DeLauder and Kidwell 2000). However, new experimental evidence, as well, as large population studies, have shown that hair color effects are non-existent or are insignificant provided the performed analytical methodology includes effective decontamination and digestion of the hair samples (Cairns et al. 2004a; b; c; Hoffmann 1999; Kelly 2000). An effective decontamination procedure should include a short (e.g., 15 min) wash in an organic solvent (isopropanol) to remove water insoluble substances and a minimum of three 30-minute-washes in aqueous medium (usually phosphate buffer) to allow swelling of the hair and diffusion of contaminating drugs into

the wash solution. If the wash criterion fails then five additional 1-hour-washes should be applied (Cairns et al. 2004a).

Another criterion, used to distinguish active use from environmental contamination, has been the presence of drug metabolites in hair. However, for some substances, like cocaine and heroin, has been shown that metabolites could be produced by other means than drug ingestion (hydrolysis of the parent drug onto the hair shaft) (Baumgartner and Hill 1993). The relevant studies have shown that the metabolites produced via environmental sources (that could be also produced via metabolic processes) should be removed during the washing procedures in order for their presence to be adding to the certainty of active use. Furthermore, some drugs may not have metabolite available at all. In cases where a metabolite, which could only be formed physiologically, like cocaethylene, is detected, washing may be less critical.

Therefore, the combination of the metabolite criterion along with the washing criterion and the application of cutoff levels can achieve the differentiation between environmental contamination and actual use. The proposed cutoff levels from SAMSHA for drugs of abuse are listed in Table 1 (SAMHSA, 1994).

TABLE 1
Cutoff concentrations of drugs of abuse in hair during screening and confirmatory tests as suggested by SAMHSA

Drug	Screening Test Cutoff Concentration (pg/mg)	Confirmatory Test Cutoff Concentration (pg/mg)
Marijuana metabolites	1.0	0.05[(1)]
Cocaine metabolites	500	100[(2)]
Cocaine parent drug		1000
Opiate metabolites	200[(3)]	
Morphine		200
Codeine		200
6-monoacetylomorphine		200
Phencyclidine	300	300
Amphetamines	500[(4)]	
d-amphetamine		300
d-methamphetamine		300[(5)]
MDMA		300
MDA		300
MDEA		300

[(1)]Delta-9-tetrahydrocannabinol-9-carboxylic acid.

[(2)]Benzoylecgonine (BE/Cocaine ratio ≥ 0.1).

[(3)]Screening test could be performed for 6-MACM at a 200 pg/mg concentration.

[(4)]Screening must significantly detect d-methamphetamine, d-amphetamine, MDMA, MDA and MDEA (75 to 125% cross-reactivity).

[(5)]Specimen must also contain d-amphetamine at a concentration >50 pg/mg.

DIGESTION PROCEDURES

After the extending washing of the hair samples and after a plateau has been reached in the drug concentration of the washing solvents, any drugs remaining in the hair define the drug fraction in the inaccessible domain of hair. These drugs are recovered from the hair matrix by extraction/digestion procedures and represent the drugs that were incorporated from the interior.

Hair extraction procedures for drugs are divided into three main categories:

1. alkaline digestion (Wilkins et al. 1995; Suzuki et al. 1989),
2. acidic extraction (Cone 1990; Nakahara, Kikura, and Takahashi 1994; Suzuki et al. 1989) and
3. enzymatic digestion (Nakahara, Ochiai, and Kikura 1992; Henderson et al. 1992; Moeller, Fey, and Wennig 1993; Potsch, Skopp, and Becker 1995; Hold et al. 1998).

Alkaline Digestion

Digestion with alkali should be applied when alkaline stable compounds, such as morphine, amphetamines and cannabinoids have to be analyzed. In general, it involves incubation of the hair sample in $0.1 \sim 2.5$ M NaOH, at 37°C overnight. Adjustment at pH = 9 follows and the procedure continues with solid phase extraction (SPE).

Acidic Extraction

Acidic extraction of drugs from hair is usually carried out with 0.1–0.6 M HCl or 0.005 H_2SO_4 at room temperarure or 37°C overnight. After neutralization of the solution SPE follows (Cone 1990; Suzuki et al. 1989).

Extraction with acidified methanol, under ultra-sonication has been proven as an another effective method. The mixture of methanol-trifluoroacetic acid-acetic anhydride has been considered a good mean for the extraction of 6-MACM from heroin users' hair (Nakahara, Kikura, and Takahashi 1994).

Enzymatic Digestion

The use of enzymes for hair analysis aims at the destruction of the hair structure and thus to the release of the incorporated drugs to the digestion buffer. For this purpose several enzymes like β-glucoronidase/arylsulfatase (glusulase) (Moeller, Fey, and Wennig 1993), proteinase K (Nakahara, Ochiai, and Kikura 1992; Henderson et al. 1992), protease E (Potsch, Skopp, and Becker 1995), protease VIII (Hold et al. 1998) and biopurase (Fujii, Higashi, and Nakano 1996) have been used.

It should be underlined that different digestion procedures recover from hair different concentrations of drugs and moreover, not all procedures are suitable for extracting all classes of drugs. For example, alkaline digestions can result in hydrolysis of compounds such as cocaine, heroin/6-MACM and other ester compounds in hair (Wilkins et al. 1995).

Solvent extraction procedures cannot guarantee the complete recovery of analytes, since extraction efficiency depends on the

physical properties of the hair (e.g. whether it is thin or thick, porous or not, the type and quantity of melanin present). Differences due to variable melanin content of hair are possible in cases where a major portion of the analyte is sequestered in the melanin granules. Thus, the method of choice, for extracting and measuring these residual drugs from the hair matrix, should be the enzymatic digestion of the hair specimen.

The enzymatic digestion, at neutral pH, has been proposed as a universal extraction procedure for all incorporated in hair substances, since the complete dissolution of the hair matrix produced the best recoveries (Baumgartner and Hill 1993). The procedure allows solubilization of the hair sample without degradation of unstable compounds like heroin/6-MACM and cocaine. Disadvantage of the enzymatic digestion of hair has been considered the fact that the resulting digest could denature, under certain conditions, the antibodies used for preliminary detection of drugs by immunoassays. The digest, nevertheless, could be used for the mass spectrometric detection of the analytes. However, in most reported cases so far, it is not the method of choice since it is rather expensive.

HAIR ANALYSIS TECHNIQUES

The procedures that have been used for the detection of drugs in hair specimen are either the same or slightly modified than the procedures used for the detection of drugs from urine, blood or other biological fluid specimen. The hair analysis methods are immunological, gas chromatography, liquid chromatography, and capillary electrophoresis. The use of infrared microscopy has been also reported in hair analysis of drugs of abuse (Kalasinsky, Magluilo, and Schaefer 1993). Analysis of drugs in hair has a minimum of analytical requirements, which are: sensitivity in the range of picograms per milligram of hair; specificity for parent drugs and lipophilic metabolites; and absence of matrix effects with hair digests.

Immunoassays

Immunoassays meet the aforementioned requirements although there are other limitations that should be kept in perspective. Radioimmunoassays (RIA) have been used since the early time of hair analysis (Baumgartner et al. 1979). RIA has been applied for the detection in hair of opiates (Baumgartner et al. 1979), cocaine/benzoylecgonine (Baumgartner, Black, and Jones 1982), phencyclidine (Baumgartner, Jones, and Black 1981) and methadone (Marsh and Evans 1994). Fluorescence polarization immunoassay (FPIA) with Abbott TDx has been reported for the detection of morphine in hair (Franceschin, Morosini, and Dell'Anna 1987). ELISA has been used for the detection of buprenorphine in hair specimen (Cirimelle et al. 2004). With immunoassays has been also detected fentanyl (Wang, Cone, and Zancy 1992).

The principal requirement for immunological hair analysis is that the hair digest mixtures should not denature the antibodies present in the reagent mixture. Chemical digest should be brought to neutral pH and the ionic strength of the final solution should not be too high. Positive and negative controls should be also made up of hair containing or not the drug and digested in the same manner as the hair samples. The sensitivity of the immunoassay should be in the range 10 pg/mg -10 ng/mg of hair which is the usually reported range of drugs of abuse found in hair (Cassani and Spiehler 1993).

The immunoassays applied should be highly sensitive for the parent drugs although antibodies usually cross react with parent compounds and metabolites and, moreover, their results should be expressed as equivalents (since discrimination of parent substances and metabolites is not possible). In general, immunoassays have been used at (and beyond) their limits of detection (LOD).

Poor agreement has been reported between GC/MS and RIA (Sachs and Arnold 1989) and GC/MS and FPIA (Kintz, Ludes, and Mangin 1992b) analysis of hair for morphine at concentration below of 1 ng/mg, making the establishment of cut-offs levels, for immunoassays, unsafe. Immunoassays are considered only preliminary analytical tests. A second analytical method based on a different property of the analyte must be always performed, like HPLC, or GC/MS (Cassani and Spiehler 1993).

HPLC and Capillary Electrophoresis (CE)

Analysis of drugs in hair by HPLC has not found a wide application. It has been reported the detection in human hair, of haloperidol with HPLC-UV (Uematsu et al. 1992), of phenytoin and carbamazepine (Mei and Williams 1997), thiopental and ketamine (Gaillard and Pepin 1998a). By HPLC-UV analysis have been also detected in hair clonazepam, flunitrazepam, midazolam, diazepam and oxazepam (El Mahjoub and Staub 2001; McClean et al. 1999).

Liquid chromatography with fluorimetic detection has been reported for the determination in hair samples of: cocaine and morphine (Tagliaro et al. 2000); enantiomeric composition of amphetamine and methamphetamine (Phinney and Sander 2004); fenfluramine and non-fenfluramine, as biomarkers of N-nitrosofenfluramine ingestion (Kaddoumi et al. 2004); LSD (Rohrich, Zorntlein, and Becker 2000), MDM and MDA (Tagliaro et al. 1999) and also ofloxacin, norfloxacin, ciprofloxacin (Mizuno, Uematsu, and Nakashima 1994).

HPLC with chemical ionization mass spectrometric detection has been performed for the determination in hair of derivatized amphetamines (Stanaszek and Piekoszewski 2004), benzodiazepines and metabolites (Toyo'Oka et al. 2003; McClean et al. 1999), cocaine and metabolites (Clauwaert et al. 1998), corticosteroids and anabolic steroids (Gaillard, Vayssette and Pepin 2000). HPLC with coulometric detection (HPLC-Ch) has been applied for the detection of buprenorphine (Kintz et al. 1994) and illicit drugs (Achilli et al. 1996). Finally, HPLC has been used as a means for separation of testosterone (Wheeler et al. 1998) and thyroxin (Tagliaro et al. 1998) from other analytes present

in the hair digest while their confirmation in the fractions was performed with immunoassay.

Capillary electrophoresis has been applied for the determination in hair sample of heroin, cocaine and ecstasy (Tagliaro 2000), 1,4-benzodiazepines and metabolites (McClean et al. 1999) and methaqualone (Plaut, Girod, and Staub 1998).

LC tandem mass spectrometry has been used for the hair analysis of furosine as a marker compound of proteins' glycation (Takemura et al. 1997).

GC-MS, GC-MS/MS

The most frequently used analytical method in hair analysis is the GC-MS, which is superior to other methods in selectivity, sensitivity and specificity. Furthermore, it is obviously a carry over from urine analysis programs. The quantitation of drugs in hair is performed by selected ion monitoring (SIM) due to the low amounts of drug present while the deuterated target drugs are the usually used internal standards.

GC-MS has been used for the hair analysis of opiates, cocaine and related drugs, amphetamines, cannabinoids, other abused substances, benzodiazepines, therapeutical drugs, pesticides and other environmental pollutants, as well as doping substances.

In the next paragraph it will be presented the outcome of the hair analysis (mainly by various GC techniques) for every class of these substances as well as the limits and considerations that needed to be faced in each case.

In Tables 2–7 are listed the substances detected in hair, the analytical methods used and relevant references.

DETECTION OF SPECIFIC CLASSES OF SUBSTANCES BY HAIR ANALYSIS

Hair Analysis for Opiates

Hair analysis started in 1979 when Baumgartner and colleagues (Baumgartner et al. 1979) succeeded to detect opiates in the hair of heroin abusers by RIA and to estimate their opiate abuse histories by sectional analysis. In 1980, Klug detected morphine in hair in the range of 0.1–10 ng/mg and he was the first to fulfill the forensic toxicology requirements for hair analysis, because he confirmed RIA results by a chromatographic method (TLC with fluorescence detection) (Klug 1980). In 1986, Marigo et al. detected morphine in the alkaline digested hair of heroin addicts using HPLC with fluorimetric detection (Marigo et al. 1986). The use of GC-MS for the detection of opiates in hair started in 1991 with the identification of heroin and 6-MACM (Goldberger et al. 1991).

The treatment of hair with 10% HCl for one hour at 100°C has been considered to give quantitative extraction of morphine from hair (Nakahara et al. 1992). It was also found that the major components in hair of heroin addicts are 6-MACM together with morphine (Nakahara et al. 1992; Cone, Darwin, and Wang 1993). In another report, however, it was shown that the extraction with methanol/trifluoroacetic acid was the best for extracting 6-MACM and morphine with the minimum hydrolysis and the maximum recovery of 6-MACM (Nakahara, Kikura, and Takahashi 1994). The incorporation of codeine and morphine metabolites into hair samples and the recovery of opiates (6-MACM, acetylcodeine, morphine) during extraction has been also evaluated (Polletini et al. 1997). Moreover, combined extractions of cocaine, opiates and their metabolites from human head hair have been achieved in cases of polydrug poisonings (Hold et al. 1998).

Hair analysis for opiates is performed, in general, by using validated GC-MS methods, which are also used for the analysis of other specimen (Cone, Darwin, and Wang 1993; Hold et al. 1998).

Hair Analysis for Cocaine and Metabolites

The detection of cocaine metabolites (benzoylecgonine) in hair was firstly performed by RIA (Baumgartner, Black, and Jones 1982). In the 1990s was confirmed, by the use of GC-MS, that cocaine was the major component over its metabolites in hair of cocaine abusers (Cone et al. 1991; Nakahara, Ochiai, and Kikura 1992). In rat experiments was demonstrated by using both, normal and deuterated, cocaine, BE and ecgonine methylester (EME) that cocaine predominates in the incorporation into hair over its metabolites, BE and EME. It has been also shown that BE, probably, is produced after incorporation into the hair shaft since no BE was incorporated in hair from the circulation (Cone et al. 1991). The most likely binding site for cocaine in hair has been considered melanin (Joseph, Su, and Cone 1996) and the incorporation tendency has been black > brown > blond hair.

The detection of cocaethylene in the hair of cocaine abusers is produced from the combination of cocaine and alcohol and is considered to be a certain indicator of cocaine active use (Gaillard and Pepin 1997; Hold et al. 1998; Wilkins et al. 1995).

Hair Analysis for Amphetamines

It has been reported the detection in human hair by GC-MS of amphetamine (AP), MA, MDA, MDMA, MDEA, and MBDB (Ishiyama, Nagai, and Toshida 1983; Suzuki, Hattori, and Asano 1984).

It has been also performed the detection of MA and AP in a single hair by HPLC-chemiluminescence (HPLC-ChemLu) (Takayama, Tanaka, and Hayakawa 1997). It has been also stated that sectional hair analysis for MA corresponds to the reported drug history. Moreover, it has been reported that five minutes after administration of MDMA in rats, can be detected MDMA and MDA in hair root samples (Nakahara 1999). The detection limit of MDMA and MDA in hair has been reported to be approximately 0.125 ng/mg of hair (Han et al. 2005).

Hair Analysis for Cannabinoids

Hair analysis for cannabinoids is focused on the identification of different analytes (Staub 1999): Δ9-tetrahydrocannabinol

(Δ9-THC), cannabinol (CBN), cannabidiol (CBD) and the main metabolite of THC, 11-nor-Δ9-THC acid (THCA). The detection of THCA is the unequivocal and more elegant proof of cannabis consumption. However, the concentration of THCA in hair is extremely low due to the weak incorporation rate of acidic substances into the hair matrix. Furthermore, certain washing procedures (dichloromethane) could result in significant decrease of cannabinoid levels in hair (Uhl and Sachs 2004). The former together with the possibility of external contamination makes the detection and interpretation of results for cannabinoids in hair one of the most difficult problems to be dealt with.

In head and pubic hair have been detected, with GC-MS, derivatized THC and THCA in the range 0.26–2.17 ng/mg and 0.07–0.33ng/mL respectively. By GC-NCI-MS the range of derivatized THCA has been demonstrated to be 0.02–0.39 ng/mg (Kintz, Cirimelle, and Mangin 1995).

The cutoff for the hair total cannabinoid equivalents by immunodetection was reported to be 0.06 ng/mg, while the LOD for THC and CBN by GC-MS was 0.04 ng/mg. In the same study was also reported the determination of THCA by GC-MS/MS at a LOQ of 0.1pg/mg hair (Uhl and Sachs 2004).

The determination of THCA in hair demands a more laborious examination of hair like GC-tandem MS (Uhl and Sachs 2004), GC-MS-NCI (Moore, Guzaldo, and Donahue 2001) or additional cleaning steps prior to analysis (Uhl and Sachs 2004). Therefore, the exclusive detection of THC, CBN, and CBD, which is usually performed, will never raise the uncertainty for some cases.

In Table 2 are presented the parent-abused substances and their metabolites that have been detected in human hair so far and the analytical methods used for their detection.

Hair Analysis for Nicotine and Cotinine

Hair nicotine determination has been considered a useful biological marker for exposure assessment to tobacco smoke (Jaakkola and Jaakkola 1997). There are many reports on hair analysis for nicotine and cotinine in correlation with individual smoking habits and exposure (Kintz, Ludes, and Mangin 1992b Uematsu et al. 1995; Zahlsen, Nilsen, and Nilsen 1996; Jaakkola and Jaakkola 1997).

The nicotine concentration was determined in hair of neonates born from smoking or non-smoking mothers (Kintz and Mangin 1993; Eliopoulos et al. 1994) and of infants (Pichini et al. 1997). The mean concentrations were 2.4 ng/mg for nicotine and 2.8 ng/mg for cotinine in hair of neonates of smoking mothers, while hair of neonates of non-smokers had mean concentrations of 0.4 ng/mg and 0.26 ng/mg for nicotine and cotinine respectively (Eliopoulos et al. 1994). Nicotine levels in hair of non-exposed to tobacco smoke infants was reported to be (1.3 ± 1.7) ng/mg hair while in hair of infants passively exposed to smoke it was (15.4 ± 6.7) ng/mg hair (Pichini et al. 1997). These results indicate that environmental passive nicotine exposure is the dominate contributor to the overall nicotine found in hair both from smokers and non-smokers as it has been also suggested elsewhere (Zahlsen, Nilsen, and Nilsen 1996).

Hair Analysis and Chronic Alcohol Consumption

Hair samples have been analyzed for the determination of ethyl glucoronide (Janda et al. 2002; Jurado et al. 2004) and fatty acid ethyl esters (FAEE) (Hartwing, Auwarter, and Pragst 2003) which have been considered markers of chronic alcohol consumption. The analytical methods used for these determinations were GC-MS (Jurado et al. 2004) and LC-MS/MS (Janda et al. 2002). The presence of ethyl glucoronide in hair has been correlated with frequent alcohol misuse and not with social drinking (Janda et al. 2002).

Hair Analysis for Benzodiazepines

Human hair was first screened for the presence of benzodiazepines in 1992 (Kintz et al. 1992b). In 1992, was also reported the detection of diazepam with RIA (Sramek et al. 1992). In 1993 was detected in hair of neonates' diazepam and oxazepam as a result of gestational exposure to these substances (Kintz and Mangin 1993). The following years lots of other benzodiazepines and metabolites were detected in human hair: the detection of nordiazepam and oxazepam by GC-MS-NCI (Kintz et al. 1996), flunitrazepan and 7-aminofluritrazepan (Cirimelle et al. 1997), and alprazolam (Hold et al. 1997). Various benzodiazepines and metabolites have been also detected in hair with HPLC-UV or EI-MS detection (McClean et al. 1999), ELISA (Negrusz et al. 1999), capillary electrophoresis (McClean et al. 1999) or LC-MS-MS (Cheze et al. 2005; Kintz et al. 2005a; Kronstrad et al. 2004). The techniques now available (LC-ESI-MS/MS) allow the detection of benzodiazepines and benzodiazepine-like hypnotics (zopiclone and zolpiden) in hair even if they have been administered at a single therapeutic dose (Cheze et al. 2005). For most of them the LODs have been lower than 2 pg/mg. In Table 3 are listed all the benzodiazepines and metabolites that have been detected in hair so far, and the relevant methods used.

Hair Analysis for Antidepressants and Antipsychotic Drugs

Antidepressants and antipsychotic drugs have been detected in human hair as a means of long term exposure to these drugs and in order to test the compliance of patients to doctor's prescription. The substances of this category detected in human hair so far and the analytical methods used for their determination are listed in Table 4.

It has been reported the detection in hair of: phenobarbital (Fujii et al. 1996); phenytoin (Fujii et al. 1996; Mei and Williams 1997); carbamazepine and metabolites (Mei and Williams 1997; Shen et al. 2002); amitriptyline, climipramine, doxepine, imipramine, maprotiline, trihexylphenidyl, chlorpromazine, chlorprothixene, trifluoroperazine, clozapine, haloperidol and

TABLE 2
Drugs of abuse detected in human hair, methods of detection and relevant references

Substances	Methods	References
1. Opiates		
Codein, Acetyl-	GC-MS	Polletini et al. 1997
Codeine	GC-MS; HPLC-ECh; LC-MS/MS	Wilkins et al. 1995; Achilli et al. 1996; Kronstrand et al. 2004
Codeine, Dihydro-	HPLC-ECh	Achilli et al. 1996
Heroin	GC-MS; HPLC-ECh; HPLC, CE	Goldberger et al. 1991; Achilli et al. 1996; Tagliaro et al. 2000;
Hydrocodone	HPLC-ECh	Achilli et al. 1996
Morphine	TLC-Flu; HPLC-Flu; RIA; GC-MS; HPLC-ECh; LC-MS/MS	Klug 1980; Marigo et al. 1986; Sachs and Arnold 1989; Cone, Darwin, and Wang 1993; Tagliaro et al. 2000; Achilli et al. 1996; Kronstrand et al. 2004;
Morphine, 6-Monoacetyl-	GC-MS; HPLC-ECh; LC-MS/MS	Nakahara et al. 1992; Achilli et al. 1996; Kronstrand et al. 2004
Morphine, Ethyl-	HPLC-ECh; LC-MS/MS	Achilli et al. 1996; Kronstrand et al. 2004
Procaine	HPLC-ECh	Achilli et al. 1996
Thebaine	HPLC-ECh	Achilli et al. 1996
2. Cocaine		
Cocaine	RIA; GC-MS; HPLC-Flu,CE; HPLC-Ech; LC-MS/MS; HPLC-MS	Baumgartner et al. 1982; Cone, Darwin, and Wang 1993; Tagliaro et al. 2000; Achilli et al. 1996; Kronstrand et al. 2004; Paulsen et al. 2001;
Benzoylecgonine	GC-MS; HPLC-Flu-MS; LC-MS/MS; GC-MS	Cone, Darwin, and Wang 1993; Clauwaert et al. 1998; Kronstrand et al. 2004;
Ecgonine Methyl Ester		Henderson et al. 1992
Cocaethylene	GC-MS; HPLC-Flu-MS	Gaillard and Pepin 1997; Clauwaert et al. 1998
3. Amphetamines		
Amphetamine	HPLC-ChemLu; GC-MS; LC-MS/MS; HPLC- MS; HPLC-Flu	Takayama, Tanaka, and Hayakawa 1997; Rothe et al. 1997; Kronstrand et al. 2004; Stanaszek and Piekoszewski 2004; Phinney and Sander 2004
Ephedrine	HPLC-MS	Stanaszek and Piekoszewski 2004
Methamphetamine	HPLC-ChemLu; GC-MS; LC-MS/MS;HPLC-MS; HPLC-Flu;	Takayama, Tanaka, and Hayakawa 1997; Rothe et al. 1997; Kronstrand et al. 2004; Stanaszek and Piekoszewski 2004; Phinney and Sander 2004;
3,4-Methylenedioxyampheta-mine (MDA)	GC-MS; LC-MS/MS, HPLC-Flu; HPLC-MS	Rothe et al. 1997; Kronstrand et al. 2004; Tagliaro et al. 2000; Stanaszek and Piekoszewski 2004;
3,4-Methylenedioxymethamphe tamine (MDMA)	HPLC-Flu;HPLC-ECh; CE; LC-MS/MS; HPLC-MS	Tagliaro et al. 1999;Achilli et al. 1996; Tagliaro et al. 2000; Kronstrand et al. 2004; Stanaszek and Piekoszewski 2004;
3,4-Methylenedioxyethylamphe tamine (MDEA)	GC-MS; HPLC-MS; HPLC-Flu;	Rothe et al. 1997; Stanaszek and Piekoszewski 2004; Tagliaro et al. 1999;
MBDB*	GC-MS	Rothe et al. 1997
Paramethoxyamphetamine	HPLC-MS	Stanaszek and Piekoszewski 2004
Fenfluramine	HPLC-Flu	Kaddoumi et al. 2004
Norfenfluramine	HPLC-Flu	Kaddoumi et al. 2004
N-Nitrosofenfluramine	HPLC-Flu	Kaddoumi et al. 2004
Methcathinone	HPLC-MS	Stanaszek and Piekoszewski 2004
4. Cannabinoids		
Δ9-THC	GC-MS; GC-MS/MS	Cirimele et al. 1996; Uhl and Sachs 2004
Cannabinol	GC-MS	Cirimele et al. 1996
Cannabidiol	GC-MS	Cirimele et al. 1996
11-Nor- Δ9-THCA	GC-MS; GC-MS/MS	Moore, Guzaldo, and Donahue 2001; Uhl and Sachs 2004;

*MBDB: N-methy-1-1-(1,3-benzodioxol-5-yl)-2-butylamine.

TABLE 3
Benzodiazepines detected in human hair, methods of detection and relevant references

Substances	Methods	References
Alprazolam	GC-MS; HPLC-MS	Hold et al. 1997; Toyo'oka et al. 2003
Alprazolam, 1-Hydroxy-	HPLC-MS	Toyo'oka et al. 2003
Alprazolam, 4-Hydroxy-	HPLC-MS	Toyo'oka et al. 2003
Bromazepam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Clobazam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Clonazepam	GC-MS; HPLC-UV; LC-MS/MS	Negrusz et al. 2000; El Mahjoub and Staub 2001; Chèze, Villain, and Pepin 2005
Clonazepam, 7-Amino-	GC-MS	Negrusz et al. 2000
Clotiazepam	GC-MS, HPLC-UV	Gaillard and Pepin 1997
Diazepam	RIA; GC-MS; HPLC-UV; LC-MS/MS	Sramek et al. 1992;Kintz and Mangin 1993; El Mahjoub and Staub 2001; Kronstrand et al. 2004
Diazepam, Nor-	GC-MS; HPLC-MS/MS	Kintz et al. 1996; McClean et al. 1999
Estazolam	HPLC-MS	Toyo'oka et al. 2003
Estazolam-metabolite*	HPLC-MS	Toyo'oka et al. 2003
Flunitrazepam	GC-MS; ELISA; HPLC-UV	Cirimele et al. 1997; Negrusz et al. 1999; El Mahjoub and Staub 2001
Flunitrazepam, 7-Amino-	GC-MS; ELISA; LC-MS/MS	Cirimele et al. 1997; Negrusz et al. 1999; Kronstrand et al. 2004
Loprazolam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Lorazepam	LC-MS/MS; GC-MS	Kintz et al. 2004; Cirimele, Kintz, and Mangin 1996
Lormetazepam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Midazolam	HPLC-UV; GC-MS; HPLC-MS	El Mahjoub and Staub 2001; Cirimele et al. 2002; Toyo'oka et al. 2003
Midazolam, 1-Hydroxy-	HPLC-MS	Toyo'oka et al. 2003
Midazolam, 4-Hydroxy-	HPLC-MS	Toyo'oka et al. 2003
Nitrazepam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Nordazepam	GC-MS, HPLC-UV	Gaillard and Pepin 1997
Oxazepam	GC-MS; HPLC-UV	Kintz et al. 1996; El Mahjoub and Staub 2001
Prazepam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Temazepam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Tertazepam	LC-MS/MS	Chèze, Villain, and Pepin 2005
Triazolam	HPLC-MS	Toyo'oka et al. 2001
Zolpidem	LC-MS/MS	Kintz et al. 2005a
Zopiclone	LC-MS/MS	Chèze, Villain, and Pepin 2005

*8-chloro-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine-4-one.

their nor-metabolites (Pragst et al. 1997; Shen et al. 2002); and thiopental (Gaillard and Pepin 1998a).

The relevant studies performed have shown that the incorporation of such drugs and their metabolites in hair is facilitated by the lipophilicity, basicity and molecular weight of each particular compound (Shen et al. 2002) as it has been reported for other substances (including cocaine and heroin) as well (Nakahara 1999; Wenning 2000).

Sectional hair analysis for antidepressant and antipsychotic drugs may provide their detection at least 16 months after intake (Shen et al. 2002).

Hair Analysis for Pesticides and Persistent Organic Pollutants (POPs)

In the recent years the analysis of hair has been expanded in the field of risk assessment of exposure to organic chemicals such as pesticides and POPs (Table 5).

In hair have been detected various organochlorine pollutants, such as polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo-dioxins, polychlorinated dibenzodifurans, coplanar polychlorinated, biphenils and total biphenils (Schramm 1997; Tsatsakis and Tutudaki 2004). Moreover, the detection in hair of pesticides belonging to the families of organophosphates,

TABLE 4
Antidepressants and antipsychotics detected in human hair, methods of detection and relevant references

Substances	Methods	References
Amisulpride	HPLC-UV	Gaillard and Pepin 1997
Amitriptyline	GC-MS	Shen et al. 2002
Amitriptyline-M*1	GC-MS	Shen et al. 2002
Amitriptyline-M2	GC-MS	Shen et al. 2002
Carbamazepine	HPLC-UV; GC-MS	Mei and Williams 1997; Shen et al. 2002
Carbamazepine-M	GC-MS	Shen et al. 2002
Chlorpromazine	GC-MS	Shen et al. 2002
Chlorpromazine-M1	GC-MS	Shen et al. 2002
Chlorpromazine-M2	GC-MS	Shen et al. 2002
Chlorprothixene	GC-MS	Shen et al. 2002
Chlorprothixene-M	GC-MS	Shen et al. 2002
Clomipramine	GC-MS	Pragst et al. 1997
Clomipramine, Nor-	GC-MS	Pragst et al. 1997
Clozapine	GC-MS	Shen et al. 2002
Clozapine-M	GC-MS	Shen et al. 2002
Cyamemazine	HPLC-UV; GC-MS	Gaillard and Pepin 1997; Alunni-Perret et al. 2003
Desipramine	GC-MS	Ishiyama, Nagai, and Toshida 1983
Dosulepine	HPLC-UV	Gaillard and Pepin 1997
Doxepine	GC-MS	Shen et al. 2002
Doxepine, Nor-	GC-MS	Pragst et al. 1997
Doxepine-M1	GC-MS	Shen et al. 2002
Doxepine-M2	GC-MS	Shen et al. 2002
Haloperidol	GC-MS; HPLC-UV	Shen et al. 2002; Uematsu et al. 1992
Imipramine	GC-MS	Pragst et al. 1997
Loxapine	HPLC-UV	Gaillard and Pepin 1997
Maprotiline	GC-MS	Pragst et al. 1997
Maprotiline, Nor-	GC-MS	Pragst et al. 1997
Moclobemide	GC-MS	Gaillard and Pepin 1997
Phenobarbital	GC-MS	Goulle et al. 1995
Phenytoin	HPLC-UV	Mei and Williams 1997
Pipotiazine	HPLC-UV	Gaillard and Pepin 1997
Thionizadine	HPLC-UV	Gaillard and Pepin 1997
Toloxatone	GC-MS	Gaillard and Pepin 1997
Trifluoperazine	GC-MS	Shen et al. 2002
Trihexyphenidyl	GC-MS	Shen et al. 2002
Trihexyphenidyl-M	GC-MS	Shen et al. 2002
Trimeprazine	GC-MS	Alunni-Perret et al. 2003
Triptyline, Nor-	GC-MS	Ishiyama, Nagai, and Toshida 1983
Valproic acid	GC-MS	Gaillard and Pepin 1997

*M: metabolite.

carbamates and pyrethroides, like lindane (Neuber, Merkel, and Randow 1999), DDTs, permethrin, chlorpyriphos, malathion (Liu and Pleil 2002) and methomyl (Tsatsakis et al. 2001) has been also reported.

The most critical step in the hair analysis of these compounds is their quantitative extraction from the hair matrix. Many extraction methods have been used with recoveries ranging between 35 and 120%. The extraction procedures include acidic

TABLE 5
Pesticides and POPs detected in human hair, methods of detection and relevant references

Substance	Methods	References
Aldrin	GC-MS	Liu and Pleil 2002
Biphenyls, Coplanar Polychlorinated	GC-MS	Schramm 1997; Tsatsakis and Tutudaki 2004
Biphenyls, Total	GC-MS	Schramm 1997; Tsatsakis and Tutudaki 2004
Chlorpyriphos	GC-MS	Liu and Pleil 2002
Cyalothrin	GC-MS	Liu and Pleil 2002
Cypermethrin	GC-MS	Liu and Pleil 2002
Diazinon	GC-MS	Liu and Pleil 2002
Dieldrin	GC-MS	Liu and Pleil 2002
Dioxins, Polychlorinated Dibenzo-p-	GC-MS	Schramm 1997; Tsatsakis and Tutudaki 2004
DDTs	GC-MS	Liu and Pleil 2002
Endrin	GC-MS	Gaillard and Pepin 1997
Furans, Polychlorinated Dibenzo-	GC-MS	Schramm 1997; Tsatsakis and Tutudaki 2004
Lindane	GC-MS	Neuber, Merkel, and Randow 1999
Malathion	GC-MS	Liu and Pleil 2002
Methomyl	HPLC, ELISA	Tsatsakis et al. 2001
Permethrin	GC-MS	Liu and Pleil 2002

hydrolysis (Neuber, Merkel, and Randow 1999), soxhlet extraction (Schramm 1997), extraction under reflux (Nakao et al. 2002), methanolic extraction (Tsatsakis and Tutudaki 2004) and liquid extraction with various solvents (Liu and Pleil 2002; Tsatsakis and Tutudaki 2004). The analysis of these compounds is usually performed by gas chromatography coupled usually to mass spectrometry (Liu and Pleil 2002). For the detection of organophosphates has been also used nitrogen phosphorus detector (NPD) (Tsatsakis, and Tutuduki 2004). Hair analysis for pesticides and POPs has been proved a useful tool in risk assessment, in monitoring indoor air pollution by organic chemicals, or occupational and environmental exposure through inhalation, or even through the food chain (Tsatsakis et al. 2001).

Hair Analysis in Doping Controls

Hair is not yet approved to be a valid specimen in the field of doping control by the International Olympic Committee (IOC), although it is accepted in most courts of justice that have dealt with such cases (Kintz 1998; Gaillard, Vayssette, and Pepin 2000).

The first report concerning the detection by GC-MS of endogenous steroids in hair was published in 1995 and included the detection of androstendiol, testosterone, androstenedions, dehydroepiandrosterone (DHEA), dehydrotestosterone and 17-α-hydroxy-progesterone (Scherer and Reinhardt 1995). In 1997, were reported the physiological concentrations of testosterone and DHEA in hair for males and females subjects and was found that the differences in range between testosterone abusers and not abusers were small (Kintz, Cirimele, and Ludes 1999). For this reason the identification of testosterone esters in hair has been established as an unambiguous prove of doping since the esters are exclusively exogenous substances (Thieme et al. 2000; Gaillard, Vayssette, and Pepin 2000; Dumestre-Toulet et al. 2002). In hair of several bodybuilders were detected the testosterone esters propionate, isocaproate, decanoate and phenylpropionate (Thieme et al. 2000), together with the following exogenous anabolics stanozolol and its metabolite 3-hydroxystarozolol, mestenolone, metanolone enantate, nontrolone decanoate and nandrolone (Gaillard, Vayssette, and Pepin 1999). It has been also reported the detection of stanozolol in human hair by GC-NICI-MS (Cirimelle et al. 2000) and methanolone by GC-MS/MS (Kintz et al. 2002).

Sample preparation for the detection of anabolic steroids in hair has been rather laborious with several cleaning steps, including HPLC and solid phase extraction, followed by derivatization to form various derivatives (Thieme et al. 2000; Gaillard, Vayssette, and Pepin 1999).

Corticosteroids have been detected in hair since 1999, when the detection of prednisone by LC-MS, in the hair of chronic users was reported (Cirimele et al. 1999). The same group reported later the detection, with the same procedure, of 10 corticosteroids with detection limits ranging from 30 to 170 pg/mg (Cirimele et al. 2000). By applying modified cleaning and extraction procedures was attained the detection of the most common corticosteroids used as doping agents with a limit of sensitivity about 100 pg/mg (Gaillard, Vayssette, and Pepin 2000).

The first detection of clenbutanol in the hair from two bodybuilders was reported in 1996 and was attained after alkaline hair digestion, liquid extraction and immunoassay confirmation by HPLC-immunoassay (Gleixner, Sauerwein, and Meyer 1996). It has been suggested in the case of salbutanol, whose use is permitted for specific therapeutic purposes (after a medical prescription), that sectional hair analysis can provide the possibility of discriminating acute administration from chronic use, which is necessary to obtain the anabolic effect (Polletini et al. 1996). For the detection of β-adrenergic stimulants (β2-agonists and β-blockers) has been developed a procedure that involves overnight acidification, SPE, derivatization and GC-MS detection. The limits of detection have been 2 pg/mg for salbutanol and clenbutanol (Kintz, Cirimele, and Ludes 2000a).

A well-recognized advantage of hair over urine in doping control has been considered the ability to discriminate by performing hair analysis, which was the parent substance received by the subject. It has been reported the discrimination

of nandrolone (prohibited) from over the counter preparations containing 19-nonsteroids (non-prohibited) which have the same metabolites as nandrolone and result all in positive urine test (Kintz, Cirimele, and Ludes 2000b). In the same direction, have been used the ratios of parent compounds to their metabolites (stanozolol to its major long-term metabolite 3-hydroxy-stanozolol in hair, metandienone to epi- and 6β-hydroxymetandienone) (Thieme et al. 2000).

So far peptide hormones and glycoproteins have not been detected in hair, and we believe this will not be possible even in the future, since the destruction of hair matrix destroys these molecules.

In our opinion hair analysis is difficult, even in the near future, to become accepted for official doping analysis. Firstly, there are different restrictions for different classes of doping agents, and consequently hair analysis could not offer any advantage over urine analysis, e.g. for substances that are prohibited during competition but not always during training. As a result, a substance that was administered shortly before competition could not be detected by hair analysis. Secondly, the relationship of urine and hair results, rational bias due to cosmetic treatment and ethnic differences etc. should be established before considering hair as a valid specimen by the IOC and the International Sport Federations. Despite these, hair analysis could provide useful information in the confirmation of repetitive intake or abuse and the identification of the exact nature of the molecule administered.

The substances of this category detected in human hair and the relevant analytical methods are presented in Table 6.

Hair Analysis for Other Drugs

Hair analysis has been applied successfully for the detection in human hair of several other drugs, abused and prescribed ones. Phencyclidine and its metabolites 1-(1-phenylcyclohexyl)-4-hydroxypiperidine and trans-phencyclidine have been detected in human hair by RIA and by tandem MS (Baumgartner et al.1981; Nakahara et al. 1997). The detection in hair of lysergic acid diethylamide (LSD) and metabolites with HPLC-fluorescent detection (Rochrich, Zornlein, and Becker 2000) and GC-MS (Nakahara 1999) has been also reported. Fentanyl has been detected in hair by GC-MS/MS (Kintz et al. 2005), as well as lidocaine (Gaillard and Pepin 1998b). The enantioselective determination of methadone and its metabolite in hair has been also achieved with capillary electrophoresis (Frost et al. 1997).

The substances detected in human hair that are not categorized in one of the aforementioned classes and the relevant analytical methods are presented in Table 7.

INTERPRETATION OF HAIR ANALYSIS RESULTS

Despite the fact that hair is considered an important—if not the most important—non-conventional biological sample and despite the bulk literature that exists on hair analysis the interpretation of hair analysis results, in many cases, still remains a question under debate for the scientific community.

Through the years the main issue in hair analysis has been the efficacy of different decontamination procedures in removing the external deposited substances from hair since it is a body specimen that can be easily contaminated from exterior due to its high surface to volume ratio. Substances deposited in hair from the environment are loosely bound to either the surface of the hair or to the hair matrix, and thus can be removed by appropriate decontamination procedures. It is fundamental to be able to distinguish between passive exposure (environmental contamination) and active consumption; consequently decontamination procedures for hair are compulsory (Welch et al. 1993; Blank and Kidwell 1995; Wenning 2000).

To date, an unequivocal decontamination procedure, widely accepted for hair analysis of all substances, does not exist, as it was mentioned in the relevant section in this review. Relevant studies have reported that the application of an effective washing procedure could result in greater than 90% removal of the contaminated substance. However, still remains the most critical point: the successful identification of those samples that possibly exceed the cutoff levels as false positives, due to the remaining portion of contaminating drug (<10%) that has not been removed by the washing procedure.

Therefore, in order to avoid relying entirely on the removal or not of the contamination certain criteria have been proposed which allow the evaluation of the passive exposure. The first of these criteria, which has been suggested by several authors, is the wash criterion. The value of the wash criterion was disputed, in other studies, since its application failed to identify the contaminated samples, in many cases, even after various decontamination steps (Koren et al. 1992; Blank and Kidwell 1995; Romano, Barbera, and Lombardo 2001). More recent studies, however, concerning large number of samples from demonstrated drug (cocaine and amphetamines) users and workplace subjects, have shown that the application of the wash criterion, after an effective decontamination procedure (for 3.75 hours), had resulted in the identification of all hair positive samples as compared to urine-positive results. Moreover, non-drug users did not produce any false positive results even if the hair had not been washed (Cairns et al. 2004a; Cairns et al. 2004b; Cairns et al. 2004c).

In our opinion the appearing disagreement between previous studies on decontamination procedures and more recent ones is due to the different methodology used. All relevant studies relied on soaking experiments where hair samples had been either soaked in concentrated drug solutions or exposed to drug vapors for long periods of time in such way as it is not possible to happen in real world circumstances. Moreover, the currently in use washing procedures have succeeded in removing and identifying external contamination even in these extreme samples (Cairns 2004a).

The second criterion that has been suggested for the discrimination of passive exposure from active use is the “metabolite

TABLE 6
Doping agents detected in human hair, methods of detection and relevant references

Substances	Methods	References
Androstendiol	GC-MS	Scherer and Reinhardt 1995
Androstenedion	GC-MS	Scherer and Reinhardt 1995
Androsterone, 19-Nor-	GC-MS	Thieme et al. 2000
Androsterone, Dehydroepi-	GC-MS	Thieme et al. 2000
Beclomethasone	LC-MS	Cirimele et al. 2000
Clenbutanol	HPLC-Immun; GC-MS	Glexner et al. 1996; Kintz et al. 2000
Cortisol	LC-MS	Cirimele et al. 2000
Cortisone	LC-MS	Cirimele et al. 2000
Flumethasone	LC-MS	Cirimele et al. 2000
Hydrocortisone Acetate	HPLC-MS	Gaillard, Vayssette, and Pepin 2000
Mestenolone	GC-MS	Gaillard et al. 1999
Metandienone	GC-MS	Thieme et al. 2000
Metandienone, 6β-Hydroxy	GC-MS	Thieme et al. 2000
Metandienone, Epi-	GC-MS	Thieme et al. 2000
Metanolone Enantate	GC-MS	Gaillard et al. 1999
Methasone, Beta-	LC-MS	Cirimele et al. 2000
Methasone, Dexa-	LC-MS	Cirimele et al. 2000
Nandrolone	GC-MS	Gaillard et al. 1999
Nandrolone Decanoate	GC-MS	Gaillard et al. 1999
Prednisolone	LC-MS	Cirimele et al. 2000
Prednisolone, Methyl-	HPLC-MS	Gaillard, Vayssette, and Pepin 2000
Prednisone	LC-MS	Cirimele et al. 1999
Progesterone,17-α-Hydroxy-	GC-MS	Scherer and Reinhardt 1995
Salbutanol	GC-MS	Kintz et al. 2000
Stanozolol	GC-MS	Gaillard et al. 1999
Stanozolol, 3-Hydroxy-	GC-MS	Gaillard et al. 1999
Testosterone	GC-MS; HPLC-RIA	Scherer and Reinhardt 1995; Wheeler et al. 1998
Testosterone Decanoate	GC-MS	Thieme et al. 2000
Testosterone Isocaproate	GC-MS	Thieme et al. 2000
Testosterone Phenylpropionate	GC-MS	Thieme et al. 2000
Testosterone Propionate	GC-MS	Thieme et al. 2000
Testosterone Undecanoate	GC-MS	Gaillard, Vayssette, and Pepin 2000
Testosterone, Dehydro-	GC-MS	Scherer and Reinhardt 1995
Triamcinolone	LC-MS	Cirimele et al. 2000
Triamcinolone Acetonide	HPLC-MS	Gaillard, Vayssette, and Pepin 2000

criterion". The application of this criterion consists of both the determination of the metabolites present in hair, after washing and digestion of the hair matrix and the determination of the metabolite to parent drug ratio.

It is critically important to demonstrate whether or not the metabolite detected in hair could be produced under in vitro conditions (normal hair care or environmental degradation) or in the presence of hair bound enzymes (esterases present in sweat). Moreover, it has been shown that labile molecules, like heroin and cocaine, could be hydrolyzed in hair fibers and cocaine could be hydrolyzed to benzoylecgonine during normal hygiene procedures with basic detergents (Nakahara and Kikura 1994). In cases where drugs do not have metabolites available, the metabolite criterion could not be applied at all. Therefore, only in the cases where a hair sample is above the cutoff for incorporated parent drugs, the ratios of metabolite to parent drug should be applied, as suggested for every substance, e.g. for cocaine abuse BE/cocaine >0.05 and for heroin abuse 6-MACM/morphine

TABLE 7
Other drugs and substances detected in human hair, methods of detection and relevant references

Substances	Methods	References
1. Other drugs and pharmaceuticals		
Acenocoumarol	HPLC-UV	Gaillard and Pepin 1997
Acetazolamide	HPLC-UV	Gaillard and Pepin 1997
Albendazole	HPLC-UV	Gaillard and Pepin 1997
Amobarbital	GC-MS	Gaillard and Pepin 1997
Atrazine	HPLC-UV	Gaillard and Pepin 1997
Buclizine	GC-MS	Gaillard and Pepin 1997
Buprenorphine	RIA, LC/ECD; HPLC-ECh; ELISA	Kintz et al. 1994;Achilli et al. 1996; Cirimele et al. 2004
Buprenorphine, Nor-	RIA, LC/ECD	Kintz et al. 1994
Chloroquine	GC-MS	Gaillard and Pepin 1997
Ciprofloxacin	HPLC-Flu	Mizuno, Uematsu and Nakashima 1994
Cyclophosphamide	HPLC-UV	Gaillard and Pepin 1997
Diclofenac	HPLC-UV	Gaillard and Pepin 1997
Embutramide	HPLC-UV	Gaillard and Pepin 1997
Etodolac	HPLC-UV	Gaillard and Pepin 1997
Fenfluramine	GC-MS	Gaillard and Pepin 1997
Fentanyl	RIA; GC-MS/MS	Wang, Cone, and Zacny 1993; Kintz et al. 2005b
Floctafenine	GC-MS	Gaillard and Pepin 1997
Fluconazole	HPLC-UV	Gaillard and Pepin 1997
Furosemide	HPLC-UV	Gaillard and Pepin 1997
Halofantrine	GC-MS	Gaillard and Pepin 1997
Ibuprofen	HPLC-UV	Gaillard and Pepin 1997
Ketamine	HPLC-Flu	Gaillard and Pepin 1998a
Ketoprofen	GC-MS	Gaillard and Pepin 1997
Levamisole	HPLC-UV	Gaillard and Pepin 1997
Lidocaine	HPLC-ECh	Achilli et al. 1996
Lysergic Diethylamide	HPLC-Flu	Rohrich, Zorntlein, and Becker 2000
Lysergic Diethylamide, Nor-	HPLC-Flu	Rohrich, Zorntlein, and Becker 2000
L-α-Acetyl Methadol	GC-MS	Wilkins et al. 1997
L-α-Acetyl Methadol, dinor-	GC-MS	Wilkins et al. 1997
L-α-Acetyl Methadol, nor-	GC-MS	Wilkins et al. 1997
Meprobamate	GC-MS	Gaillard and Pepin 1997
Methadone	RIA;HPLC-ECh;LC-MS	Marsh and Evans 1994; Achilli et al. 1996; Wilkins et al.1996
Methaqualone	GC-MS, CE	Plaut , Girod , and Staub 1998
Metoclopramide	HPLC-UV	Gaillard and Pepin 1997
Moramide, dextro-	GC-MS	Gaillard and Pepin 1997
Naloxone	HPLC-ECh	Achilli et al. 1996
Niflumic acid	HPLC-UV	Gaillard and Pepin 1997
Norfloxacin	HPLC-Flu	Mizuno, Uematsu, and Nakashima 1994
Ofloxacin	HPLC-Flu	Mizuno, Uematsu, and Nakashima 1994
Paracetamol	GC-MS	Gaillard and Pepin 1997
Pentazocine	GC-MS	Gaillard and Pepin 1997
Pentobarbital	GC-MS	Gaillard and Pepin 1997
Pethidine	GC-MS	Gaillard and Pepin 1997
Phencyclidine	RIA;GC-MS/MS	Baumgartner et al. 1981; Nakahara 1999

(*continued on next page*)

TABLE 7
Other drugs and substances detected in human hair, methods of detection and relevant references (*Continued*)

Substances	Methods	References
Phencyclidine, Trans-	GC-MS	Nakahara 1999
Phenobarbital	GC-MS	Goulle et. al. 1995
Piperidine, 1-(1-Phenyl-cyclohexyl)-4-Hydroxy-	GC-MS	Nakahara 1999
Propoxyphene, Dextro-	HPLC-UV	Mersch, Yegles, and Wennig 1997
Propoxyphene, Nor-	HPLC-UV	Mersch, Yegles, and Wennig 1997
Proquanil	HPLC-UV	Gaillard and Pepin 1997
Quinine	HPLC-UV	Gaillard and Pepin 1997
Thiopental	HPLC-Flu	Gaillard and Pepin 1998a
Warfarine	HPLC-UV	Gaillard and Pepin 1997
2. Other substances		
Caffeine	GC-MS	Gaillard and Pepin 1997
Cotinine	LC-MS/MS	Kronstrand et al. 2004
Eserine	GC-MS	Gaillard and Pepin 1997
Ethyl Glucoronide	GC-MS; LC-MS/MS	Jurado et al. 2004; Janda et al. 2002
FAEE	GC-MS	Hartwing, Auwarten, and Pragst 2003
Furosine	LC-MS/MS	Takemura et al. 1997
Nicotine	GC-MS; LC-MS/MS	Kintz and Mangin 1993; Kronstrand et al. 2004
Sparteine	GC-MS	Gaillard and Pepin 1997
Thyroxin	HPLC-Immun	Tagliaro et al. 1998

>1.3 (Wenning 2000), in order to differentiate with certainty between external contamination and active use.

Other sources of possible bias in interpreting hair analysis results have been considered the hair color, the hair type and the various cosmetic hair treatments. However, by reviewing the relative literature we came to the conclusion that the actual concern should be the differences in the hair matrix and more specifically in the hair color and in the hair texture.

The consideration regarding hair color has been that darkly pigmented hair could bind larger quantity of drugs than lightly pigmented hair. Since the natural color component of hair is melanin several studies have dealt with the effect of the melanin content of hair on drug incorporation into hair. It is generally accepted that melanin binds, to some degree, a variety of drugs. However, it has been shown, by using non-pigmented hair, that drugs would bind in hair even in the absence of melanin (Uematsu 1992). On the other hand, melanin accounts for only 5% by weight of the hair matrix. Therefore, the question, which arises, is regarding the extent of contribution of the melanin drug binding to the overall sequestration of drugs in hair.

The studies that support the effect of melanin on drug incorporation into hair are based exclusively on the results of in vitro experiments with human hair soaked in cocaine or opiates (mostly codeine) solutions (Kronstrand et al. 1999) or amphetamines (Kelly et al. 2000). However, the in vitro soaking models are not valid models of in vivo incorporation into the growing fiber within the hair follicle. Previous clinical studies which indicated that drug incorporation might be affected by hair color were limited in a small number of subjects (5 to approximately 30) (Cone, Darwin, and Wang 1993; Henderson 1993). Moreover, the studies were performed with no effective and properly validated washing procedures, neither were used enzymatic digestions of hair (complete destruction of the hair matrix).

All the aforementioned pitfalls in the interpretation of hair analysis results have been disproved in the view of more recent studies which were performed with large number of subjects (more than 1,000 to several thousands) (Henderson et al. 1998; Kidwell 1999; Hoffmann 1999; Kelly 2000; Mieczkowski 2001; Mieczkowski 2003). For example, in hair samples positive for morphine could not be identified a melanin effect (Mieczkowski 2001). In a sample of approximately 1,800 individuals was not identified a "race" or color effect in hair analysis for cocaine and cannabinoids (Hoffmann 1999). Analysis of approximately 2,000 hair specimens for a number of drugs, including amphetamines did not support a color effect (Kelly 2000). In other studies, where side-by-side analyses of paired hair and urine specimens of the same individual were performed, was shown that the use of proper methodology provides no statistically significant hair color or hair type effects (Mieczkowski 2002; Cairns 2004b; Cairns 2004c). Consequently, although drugs could bind to melanin in hair, this does not really constitute a problem in

hair analysis since, so far, it provides a statistical insignificant effect.

Regarding hair texture is determined by genetic factors (thick or fine, porous or not) and can be affected by cosmetic hair treatments and hair habits. In the past, it was considered that thick hair incorporated larger quantities of drug and was more resistant to decontamination than fine hair. Cosmetic hair treatments can alter hair texture and all hair components due to coloring, bleaching, perming and UV-radiation and the ingredients of hair cosmetic products are capable of reacting with drug molecules (Skopp et al. 1997; Scopp, Potch, and Moeller 1997; Cirimele, Kintz and Mangin 1995; Andersen 2005). Bleaching involves oxidative alkaline treatment and lightens the natural hair shade. Permanent waving, on the other hand, is based on the cleavage of the existing hair disulfide bonds by mercaptens and their reconstruction in a new position. In experiments performed with hair exposed externally to fluorescent dyes has been shown that the cosmetic treatments could alter the helical portion of hair thus increasing its accessibility to contamination (DeLauder and Kidwell 2000).

In other studies has been suggested that cosmetic treatments, involving bleaching or dying, could result in loss of drugs from the hair matrix (Marques et al. 1993; Jurado et al. 1997). In order for the hair damage or the increased porosity (as a result of any treatment or influence) to be evaluated, it has been suggested the measurement of the hair uptake of methylene blue dye, followed by microscopic examination of the hair fibers (Baumgartner and Hill 1993). The decontamination procedure that would follow could vary depending on this evaluation. However, objective criteria to evaluate porosity have not been reported yet.

On the other hand, it has been shown an increased, although not severe, drug (cocaine, BEG, 6-MACM, morphine, codeine) uptake into bleached or permanent waved hair fibers from the sebum or the sweat (Skopp et al. 1997). It has been also reported that permed hair absorbs less cocaine, during exposure to crack smoke, compared to the untreated hair, irrespective of the hair type (Henderson et al. 1993) and that bleaching lowers significantly cocaine binding for all hair types (Joseph, Su, and Cone 1996). Moreover, both treatments, as well as, the environmental conditions and hair care habits could potentially affect the drug molecules incorporated in the hair shaft either by converting them to other chemical forms or by completely destroying them. It has been reported loss of up to 50% of methamphetamine incorporated in hair after five months (Nakahara, Shimanine, and Takahashi 1992) and of deuterated cocaine after six months (Henderson 1993). On the other hand, under certain circumstances, such as absence of moisture, drugs have been reported to remain in hair for long periods of time, even for centuries (Springfield et al. 1993; Baez et al. 2000).

All these data suggest that cosmetic treatments of hair may alter (enhance or reduce) drug binding to it and may affect the hair texture. However, unlike hair color effects there are not sufficient studies to weight the overall effect they might have on the results of hair analysis. Consequently, the physical condition of hair, the type and degree of cosmetic manipulation the hair specimen appears to have undergone, should be assessed and considered for proper interpretation of the results.

Last but not least issue for proper interpretation of hair analysis results is the purpose of the hair testing. Hair analysis has been applied in forensic investigations (past drug use/abuse, acute drug poisoning, rape cases etc), in legal cases (criminal responsibility, addiction assessment, adoption and protective cases etc.), in historical research, in doping control, in risk assessment of prenatal exposure or chronic exposure, as well as, in other scientific or legal cases (Baez et al. 2000; Tsatsakis et al. 2001; Clauwaert et al. 2000; Kintz et al. 2005b; Berti et al. 2003). Also, it is worth mentioning that it has become a frequently used method for testing sensitive employment positions (Cook 1997; Mieczkowski 2004).

It is generally accepted that sectional hair analysis could be used to prove the drug history of a drug addict. Hair analysis could provide valuable information on the type and extent (systematic, occasional or coincidental) of the specific drugs used. Furthermore, it could be also proven valuable in discriminating between legal and illegal drug use (as reported in the doping control section), since parent compounds are mainly found in hair over their metabolites. Besides hair testing is considered valuable for proving retrospective use from days to months, it has been also proposed that hair root might be a good specimen for proving acute drug poisoning (Nakahara et al. 1997). Recently hair analysis has been suggested to document addiction of anesthesiologists to certain drugs, such as fentanyl, sufentanyl, codeine, alfentanyl and midazolam (Kintz et al. 2005b).

Sectional hair analysis has been also used to test the compliance of patients to doctor's prescription. More specifically it has been applied to compliance studies of patients who received haloperidol (Uematzu et al. 1989), carbamazepine and phenytoin (Mei and Williams 1997) and various tricyclic antidepressants (Pragst et al. 1997).

In the field of prenatal exposure assessment, hair analysis has been performed in many cases in order to confirm the birth of "cocaine babies" (Martinez et al. 1993; Koren et al. 1992) and "methamphetamine babies" (Nakahara 1999). Hair of newborns has been considered as a biological indicator of intrauterine exposure to many substances, such as cocaine (Koren et al. 1992; Chiarotti et al. 1996; DiGregorio et al. 1994), methylephedrine, dihydrocodeine, caffeine, chloropheniramine; cocaine, opiates and cannabinoids (Nakahara 1999) and nicotine (Eliopoulos et al. 1994).

In conclusion, a positive result in hair analysis for drugs of abuse (the most studied field), after a sufficient decontamination process, should constitute specific identification of the metabolites and the parent compounds, by using the suggested cutoff levels (Table 1), and application of the metabolite and washing criteria, as well as, analysis of other body specimens for comparison.

There is no doubt that hair analysis can provide valuable and valid information on previous drug use in the fields of forensic

sciences and clinical toxicology. However, the confirmation of chronic environmental exposure to substances, by performing hair analysis, represents a challenge for the toxicologist and careful evaluation of the restrictions in the analysis of every class of substances is further required.

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