



11 Detection of Doping Agents in Human Hair

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11.1 INTRODUCTION

The use of stimulants (amphetamine, ephedrine, strychnine) in sport to improve performances was reported in the early 1900s. The Medical Commission of the International Olympic Committee (IOC) established in 1967 the first list of prohibited substances and methods and adopted a medical code to protect the health of athletes and to ensure respect for the ethical concepts implicit in fair play, the Olympic spirit, and medical practice. More recently, and after the Tour de France in 1998, the concerns about doping resulted in the formation of the World Anti-Doping Agency (WADA).

The current rules governing doping in sport have as their core that a doping violation is deemed to occur upon finding in a body fluid a prohibited substance, a metabolite of a prohibited substance, or a compound chemically or pharmacologically related to a prohibited substance. In most cases, urine is the specimen of choice, but recombinant human erythropoietin and related compounds or hormones can be detected in blood (1). To date, hair is not accepted in doping control, although France passed in 2001 a law allowing biologists to use this matrix to document doping (décret n° 2001-35 from 11 January 2001).

The major practical advantage of hair testing compared with urine or blood testing for drugs is that it has a larger surveillance window (weeks to months, depending on the length of the hair shaft, against 2 to 4 days for most xenobiotics).

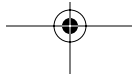
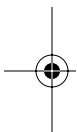


TABLE 11.1
Comparison between Urine and Hair for Testing Doping Agents in Sport

Parameters	Urine	Hair
Drugs	all, except some peptidic hormones	all, except hormones
Major compound	metabolites	parent drug
Detection period	2–5 days, except anabolic steroids	weeks, months
Type of measure	incremental	cumulative
Screening	yes	no
Invasiveness	high	low
Storage	–20°C	ambient temperature
Risk of false negative	high	low
Risk of false positive	low	undetermined
Risk of adulteration	high	low
Control material	yes	needed

For practical purposes, the two tests complement each other. Urinalysis and blood analysis provide short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis. While analysis of urine and blood specimens cannot distinguish between chronic use or single exposure, hair analysis can offer the distinction. Table 11.1 summarizes major characteristics of each specimen in regard to its place in doping control.

At this time, a possible inequity in doping control, linked to racial bias, has been pointed out by those who are against hair in such a situation. Drugs appear to be incorporated into the hair during at least three stages: from the blood during hair formation, from sweat and sebum, and from external environment. From various studies, it has been demonstrated that after the same dosage, black hair incorporates much more drugs than blond hair (2, 3). This has resulted in discussions about a possible racial bias of hair analysis and is still under evaluation.

The possibility of racial bias due to differences in melanin concentrations or in hair porosity is still in discussion. Melanins are responsible for the color of hair. Two types of melanin are present, eumelanin (with low sulfur content) and pheomelanin (with high sulfur content). Black and brown hair contain more eumelanin than red and blond hair. It appears that it is not simply the concentration of drugs in blood that determines the concentration in hair. Numerous factors may influence the incorporation of drugs into hair, such as the nature of the compounds (pKa, lipid solubility, metabolism pattern) and variation in hair growth cycles. Until these mechanisms are elucidated, the quantitative results and extrapolation to the amount of drug intake of such a hair analysis should be considered with extreme caution (4).

11.2 PROCEDURES

11.2.1 SPECIMEN COLLECTION

Collection procedures for hair analysis for drugs have not been standardized. Hair is best collected from the area at the back of the head, called the *vertex posterior*.

Au: Meaning is unclear.

Compared with other areas of the head, this area has less variability in the hair growth rate, the number of hairs in the growing phase is more constant, and the hair is less subject to age- and sex-related influences. Hair strands are cut as close as possible from the scalp, and the location **root-tip** must be mentioned. Samples can be stored at ambient temperature in aluminum foil, an envelope, or a plastic tube. The sample size varies considerably among laboratories and depends on the drug to be analyzed and the test methodology. For example, when nandrolone or betamethasone are investigated, a 100-mg sample is recommended. However, cocaine or amphetamine can be investigated on a 10-mg sample. Sample sizes reported in the literature range from a single hair to 200 mg, cut as close to the scalp as possible. 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.

11.2.2 DECONTAMINATION PROCEDURES

Contaminants of hair would be a problem if they were drugs of abuse or their metabolites or if they interfered with the analysis and interpretation of the test results. It is unlikely that anyone would intentionally or accidentally apply anything to their hair that would contain testosterone or any anabolic. The most crucial issue facing hair analysis is the avoidance of 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. Various approaches for preventing evidentiary false positives due to external contamination of the hair specimens have been described.

Most but not all laboratories use a wash step; however, there is no consensus or uniformity in the washing procedures. Among the agents used in washing are detergents such as Prell shampoo, surgical scrubbing solutions, surfactants such as 0.1% sodium dodecylsulfate, phosphate buffer, or organic solvents such as acetone, diethyl ether, methanol, ethanol, dichloromethane, hexane, or pentane of various volumes for various contact times. Generally, a single washing step is used; sometimes a second identical wash is performed. In contrast to testing for crack, cannabis, or smoked heroin, decontamination when testing for doping agents does not appear as a critical step.

11.3 DETECTION OF DOPING AGENTS

11.3.1 DETECTION OF ANABOLIC STEROIDS

Au: Define DHEA at first occurrence?

Athletes use both endogenous (testosterone, **DHEA**) or exogenous (nandrolone, stanozolol, mesterolone) anabolic steroids because it has been claimed that they increase lean body mass, increase strength, increase aggressiveness, and lead to a shorter recovery time between workouts.

The first data available for endogenous steroids in hair were given late in 1995 by the German group of Scherer and Reinhardt (5), who used GC-MS (gas chromatography-mass spectrometry) to detect androstenediol (9 to 19 pg/mg), testosterone (13 to 24 pg/mg), androstenedione (5 to 15 pg/mg), DHEA (21 to 56 pg/mg),

dihydrotestosterone (2 to 8 pg/mg), and 17 α -hydroxy-progesterone (1 to 7 pg/mg). Some years later, Kintz et al. (6, 7) established the physiological concentrations of both testosterone and DHEA with a distinction between hair of male and female subjects. After decontamination with dichloromethane, 100 mg of hair was incubated in 1M NaOH in the presence of testosterone- d_3 . After neutralization, the extract was purified using solid-phase extraction with Isolute C18 columns followed by liquid-liquid extraction with pentane. After silylation, the drugs were analyzed by GC-MS. Concentrations for DHEA were in the range of 1 to 7 pg/mg (mean 4 pg/mg) and 0.5 to 11 pg/mg (mean 5 pg/mg) for the males ($n = 15$) and females ($n = 12$), respectively. Concentrations for testosterone were in the range 0.5 to 12 pg/mg (mean 4 pg/mg) and not detected to 2 pg/mg for the males ($n = 41$) and females ($n = 12$), respectively.

Unlike testosterone in urine, the interpretation of concentration findings in hair can be difficult and critical. The range between physiological concentrations of testosterone and those found in abusers seems to be rather small. Therefore, in complement of testosterone determination, the identification of unique testosterone esters in hair enables an unambiguous charge for doping because the esters are certainly exogenous substances. This approach was largely developed by Thieme et al. (8) and Gaillard et al. (9), and Rivier (10) recently claimed that although hair analysis alone cannot be useful for screening purposes, it could become a useful technique for obtaining additional information on long-term testosterone abuse.

Thieme et al. (8) published in 2000 a complete analytical strategy for detecting anabolics in hair. The preparation of the sample was carried out by a methanol extraction step with sonication for all the anabolics, except for stanozolol, which was incubated in NaOH. Extensive cleanup procedures were employed, such as HPLC (high-performance liquid chromatography) and solid-phase extraction, followed by derivatization to form the enol-TMS derivatives. Drugs were identified either by GLC-MS/MS or GC-HRMS (GC-high-resolution mass spectrometry). Metandienone and its metabolite 6 β -hydroxymetandienone, stanozolol and its metabolite 3'-hydroxy-stanozolol, mesterolone, metenolone **enantate**, nandrolone decanoate, and several testosterone esters, such as propionate, isocaproate, decanoate, and phenylpropionate, were identified in hair of several bodybuilders.

Au: meth-enolone?

Au: enantate?

Gaillard et al. (9) developed a method for testing both the anabolic steroids and their esters. A 100-mg amount of powdered hair was first treated with methanol for extraction of esters, then alkaline digested with 1M NaOH for the recovery of the other drugs. These preparations were extracted with ethyl acetate, pooled, then finally highly purified using a twin solid-phase extraction on amino and silica cartridges. After silylation, drugs were detected by GC-MS/MS. Nandrolone and testosterone undecanoate were identified in hair of two athletes at 5.1 and 15.2 pg/mg.

A sensitive, specific, and reproducible method for the quantitative determination of stanozolol in human hair was developed by Cirimele et al. (11). The sample preparation involved a decontamination step of the hair with methylene chloride and the sonication in methanol of 100 mg of powdered hair for 2 h. After elimination of the solvent, the hair sample was solubilized in 1 ml 1N NaOH, 15 min at 95°C, in the presence of 10 ng stanozolol- d_3 used as internal standard. The homogenate was neutralized and extracted using consecutively a solid phase (Isolute C18) and a liquid-liquid (pentane) extraction. After evaporation of the final organic phase, the

Au: Define MBHFA/TMSI? Au: Define MBHFBA?

dry extract was derivatized using 40 μl MBHFA /TMSI (1000:20, v/v), incubated for 5 min at 80°C, followed by 10 μl of MBHFBA, incubated for 30 min at 80°C. The derivatized extract was analyzed by a Hewlett-Packard GC-MS system with a 5989 B Engine operating in negative chemical ionization mode of detection. The assay was capable of detecting 2 pg of stanozolol per milligram of hair when approximately 100 mg hair material was processed. The analysis of a 3-cm-long hair strand, obtained from a young bodybuilder (27 years old) declaring to be a regular user of Winstrol® (stanozolol, 2 mg), revealed the presence of stanozolol at the concentration of 15 pg/mg.

Au: Define MSTFA?

More recently, Kintz et al. (12) published a method for the quantitative determination of methenolone in human hair. The sample preparation involved a decontamination step of the hair with methylene chloride. The hair sample (about 100 mg) was solubilized in 1 ml 1N NaOH, 15 min at 95°C, in the presence of 1 ng testosterone-d₃ used as internal standard. The homogenate was neutralized and extracted using consecutively a solid-phase (Isolute C18 eluted with methanol) and a liquid-liquid (pentane) extraction. The residue was derivatized by adding 50 μl MSTFA /NH₄I/2-mercaptoethanol (1000:2:5, v/v/v), then incubated for 20 min at 60°C. A 1.5- μl aliquot of the derivatized extract was injected into the column (HP5-MS capillary column, 5% phenyl-95 % methylsiloxane, 30 m \times 0.25 mm i.d., 0.25 mm film thickness) of a Hewlett-Packard (Palo Alto, CA) gas chromatograph (6890 Series). Methenolone was detected by its parent ion at m/z 446 and daughter ions at m/z 208 and 195 through a Finnigan TSQ 700 MS/MS system. The assay was capable of detecting 1 pg/mg of methenolone when approximately 100 mg hair material was used. The analysis of a strand of hair obtained from two bodybuilders revealed the presence of methenolone at concentrations of 7.3 and 8.8 pg/mg.

Au: We changed (11) to (13). OK?

Using quite the same method (except for the internal standard, where nandrolone-d₃ was used), the same group developed a procedure to test for nandrolone, the most abused anabolic agent (13). The limit of detection of the assay was 0.5 pg/mg. Nandrolone tested positive in the hair of three athletes at the concentration of 1.0, 3.5, and 7.5 pg/mg.

Nandrolone is metabolized to norandrosterone and noretiocholanolone. Other 19-norsteroids, such as norandrostenedione or norandrostenediol, classified as anabolic androgenic steroids by the IOC, are available over the counter or through the Internet and have the same metabolites as nandrolone. Although norandrostenediol and norandrostenedione are banned by the IOC, there is a great need in forensic science and for the survey of athletes, to discriminate nandrolone from other 19-norsteroids. This is obviously not possible in urine, as the metabolites are common. Hair can identify the exact nature of the parent compound (e.g., nandrolone, norandrostenediol, or norandrostenedione, in the case of positive urine for norandrosterone), as it has been accepted by the scientific community that the parent compound is the major analyte that is incorporated in hair. Thus, hair analysis would discriminate nandrolone abuse from over-the-counter preparations containing 19-norsteroids. Recently, our laboratory was requested by an attorney to evaluate potential doping practices of an athlete. The 30-year-old subject tested positive for norandrosterone in urine at 230 ng/ml. The analysis was done in an accredited laboratory, but the athlete denied the result. The analysis of a strand of hair obtained from the athlete

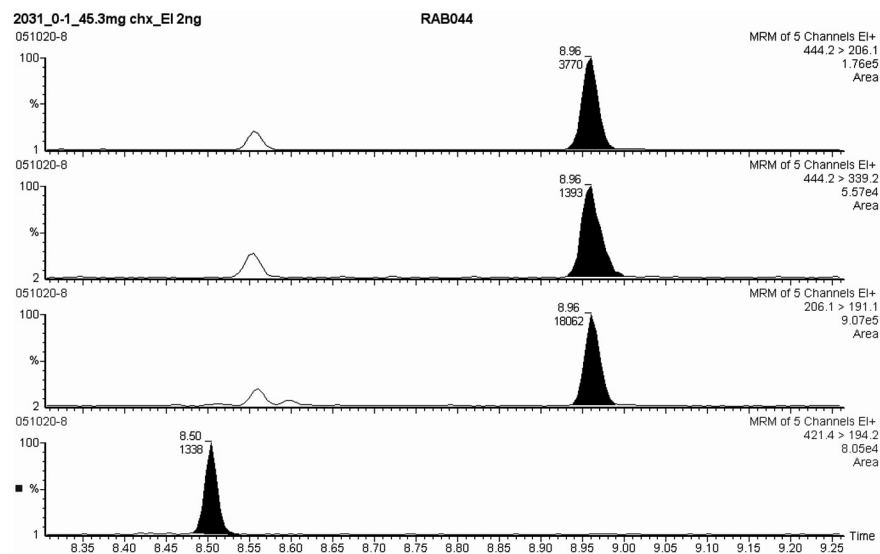


FIGURE 11.1 Chromatogram obtained after extraction by the established procedure of a 45.3-mg hair of an athlete. Metandienone was quantified at the concentration of 79.1 pg/mg. Top: quantification transition for metandienone. Middle: two qualifying transitions of metandienone. Bottom: nandrolone- d_3 with its daughter ion at m/z 194.2.

revealed the presence of 19-norandrostenedione at the concentration of 7 pg/mg (14), making a unique distinction from nandrolone doping.

In 1999 (15), two male bodybuilders were arrested by the French customs in possession of 2050 tablets and 251 ampules of various anabolic steroids. It was claimed that the steroids were for personal use and not for trafficking, as suggested by the police. Hair from both males were positive for nandrolone (196 and 260 pg/mg), testosterone (46 and 71 pg/mg), and stanozolol (135 and 156 pg/mg), clearly indicating steroid abuse.

In a series of seven steroid abusers, Deng et al. (16) identified nandrolone (20 pg/mg) and methyltestosterone (170 pg/mg). More recently, Dumestre-Toulet et al. (17), in a case of trafficking of doping agents, used GC-MS to identify nandrolone (1 to 7.5 pg/mg, $n = 3$), stanozolol (2 to 84 ng/mg, $n = 4$), methenolone (17 and 34 pg/mg), testosterone enanthate (0.6 to 18.8 ng/mg, $n = 5$), and testosterone cypionate (3.3 and 4.8 ng/mg) in the hair of bodybuilders.

Au: enantate?

This laboratory identified metandienone in the hair of an athlete who denied the use of the drug that had been identified by a WADA-accredited laboratory during a control. The analysis of a 5-cm strand of hair obtained from the athlete revealed the presence of metandienone at the concentrations of 78 pg/mg for the segment of 0 to 1 cm, 7 pg/mg for the segment of 1 to 2 cm, 10 pg/mg, for the segment of 2 to 3 cm, and 108 pg/mg for the last segment (3 cm to the end of the strand). The transition m/z 444 to 206 has been used to determine the concentrations of metandienone in the different segments. Figure 11.1 is the chromatogram obtained for the first segment (0 to 1 cm) of the athlete's hair.

TABLE 11.2
Compendium of Results for Endogenous Anabolics in Hair

Compounds	Mean (pg/mg)	Mini (pg/mg)	Maxi (pg/mg)
Testosterone	8.4	1.5	64.2
Epitestosterone	2.4	0.5	17.6
Au: Define DHT at first occurrence?	DHEA 16.9	0.8	94.2
	DHT 1.8	0.5	4.2

As a full example, our procedure to test for anabolics is described in detail. The hair was decontaminated twice using 5 ml of methylene chloride, for 2 min at room temperature, and then pulverized in a ball mill. Then 100 mg of decontaminated hair was incubated in 1 ml 1*N* NaOH, 15 min at 95°C, in the presence of 1 ng of testosterone-d₃ used as internal standard (IS). After cooling, the homogenate was neutralized with 1 ml 1*M* HCl, and 2 ml of 0.2*M* phosphate buffer (pH 7.0) were added.

The Isolute C18 columns were conditioned with 3 ml of methanol, followed by 3 ml of deionized water. After sample addition, the columns were washed twice with 1 ml of deionized water. After column drying, analyte elution occurred with the addition of 3 aliquots of 0.5 ml of methanol. The eluant was evaporated to dryness under nitrogen flow, and the residue reconstituted in 1 ml of 0.2*M* phosphate buffer (pH 7.0). A further purification step was achieved by addition of 100 mg of Na₂CO₃/NaHCO₃ (1:10, w/w) and 2 ml of pentane. After agitation and centrifugation, the organic phase was removed and evaporated to dryness. The residue was derivatized by adding 5 μl MSTFA-NH₄I-2-mercaptoethanol (250 μl, 5 mg, 15 μl, respectively) and 45 μl MSTFA, then incubated for 20 min at 60°C.

A 1-μl aliquot of derivatized extract was injected into the column of a Hewlett-Packard (Palo Alto, CA) gas chromatograph (6890 Series). The flow of carrier gas (helium, purity grade 99.9996%) through the column (HP5-MS capillary column, 5% phenyl-95% methylsiloxane, 30 m × 0.32 mm i.d., 0.25 μm film thickness) was 1.5 ml/min.

The injector temperature was 270°C, and splitless injection was employed with a split-valve off time of 1.0 min. The column oven temperature was programmed to rise from an initial temperature of 60°C to 295°C at 30°C/min and maintained at 295°C for the final 10 min.

The detector was a Waters Quattro Micro operated in the electron-impact and in selected-reaction monitoring mode. The parent ions are selected in the first quadrupole. The corresponding daughter ions are selected in the third quadrupole after collision with argon at a cell pressure of 1.00 × 10⁻⁴ Pa. The electron multiplier was operated at 650 V.

Results from about 100 hair samples are presented in Table 11.2.

11.3.2 DETECTION OF CORTICOSTEROIDS

Cortisone and hydrocortisone, naturally occurring hormones, influence metabolism, inflammation, and electrolyte and water balance. Their synthetic derivatives are used

Au: Is NH4I correct?

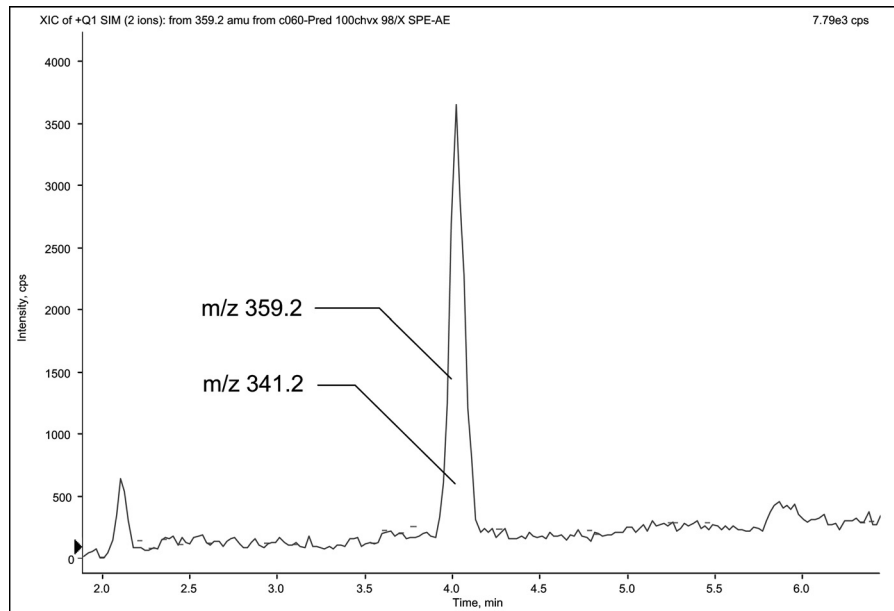


FIGURE 11.2 Typical LC-MS chromatogram of a subject under prednisone treatment. Concentration was 130 pg/mg (quantitation ion: m/z 359.2, corresponding to the M+1).

in therapeutic programs for their anti-inflammatory and immunosuppressive actions. They are also used in certain sports to improve the performances of the athletes (euphoria, motor activity).

Cirimele et al. (18) published in 1999 the first identification of such a drug, in this case prednisone, in the hair of a subject treated for years. A 50-mg hair specimen was incubated overnight in Sorensen buffer, then extracted by solid-phase extraction using an Isolute C18 column. Prednisone was detected by LC-MS at 1280 pg/mg. Using the same preparation technique, and cortisol- d_3 as an internal standard, the same group published (19) several months later a screening procedure for ten corticosteroids, with detection limits in the range 30 to 170 pg/mg. Two applications were documented for prednisone and beclomethasone, identified in hair at 140 and 230 pg/mg, respectively.

Using a 2.0-mm i.d. column, Cirimele et al. (20) demonstrated in ten patients treated with prednisone a low but not insignificant correlation ($r^2 = 0.578$, $p < 0.03$) between the total amount of ingested drug and the measured concentrations in hair. The procedure was sensitive enough to detect prednisone in the hair of patients treated with a low 5-mg/day dose. A typical chromatogram of one of the patient is given in Figure 11.2.

Repetitive abuse of corticosteroids by athletes can be demonstrated by segmental analysis along the hair shaft, in contrast to punctual **urinalysis**. A single treatment of about 1 week will show positive in a single 1-cm segment, while long-term abuse will lead to the identification of the corticoid(s) in several segments. For such an application, particularly in the case of a longitudinal survey of athletes, hair analysis

Au: We changed this to "punctual." OK?

appears as the solution of choice to document doping practices. Raul et al. (21) demonstrated that a single oral therapeutic treatment with 4-mg/day betamethasone for nine consecutive days is detectable through hair analysis. The drug tested positive at a concentration of 4.7 pg/mg in the corresponding hair segment, whereas no betamethasone could be identified in the distal hair strand. Extraction of the drug was classic from this group; however, to enhance sensitivity, a MIC 15 CP Nucleosil C18 column (150 × 1.0-mm i.d.) was used.

Bévalot et al. (22) published a confirmatory method for the quantitative determination in hair of the most common corticosteroids used as doping agents by athletes. They extracted drugs from 50 mg of powdered hair by methanolic extraction followed by a solid-phase extraction on a C18 column. Detection was performed with an electrospray ionization mass spectrometer in negative ion mode. The limits of sensitivity were about 100 pg/mg. Hair from athletes revealed the presence of hydrocortisone acetate, methylprednisolone, triamcinolone acetonide, and dexamethasone at 430, 1350, 280, and 1310 pg/mg, respectively. According to the authors, who tested in parallel the corresponding urine specimens, the comparison of the results demonstrated once again the dramatic complementarity of urinalysis and hair analysis (23).

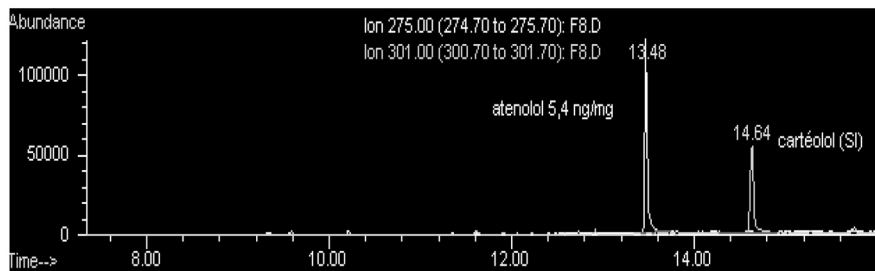
It has been advocated that high doses or repeated intake of synthetic corticoids leads to a lowering of endogenous cortisol synthesis. Raul et al. (24) postulated that a chronic use of corticoids could influence the physiological concentrations of cortisol and cortisone in hair. Using LC-MS, they established these concentrations at 5 to 91 pg/mg (mean 18 pg/mg) for cortisol and at 12 to 163 pg/mg (mean 70 pg/mg) for cortisone in a population of 17 males and 27 females, aged from 2 to 90 years. The authors did not observe influence of hair color or sex, but they did observe significantly higher cortisone concentrations before the age of 20.

11.3.3 DETECTION OF β -ADRENERGIC COMPOUNDS

β_2 -agonists are banned because of their sympathomimetic properties (stimulant effects) and their activity as anabolic agents at higher dosages. However, salbutamol is permitted by inhalers only and must be declared in writing prior to the competition.

To date, only three papers are available in the literature for these drugs in human hair, two for clenbuterol and one for salbutamol. In their paper, Gleixner et al. (25) identified clenbuterol after incubation in 1,4-dithiothreitol, NaOH, and tertiary butylmethyl ether by enzyme immunoassay (EIA) and confirmation by HPLC-EIA. Clenbuterol accumulated in hair after 10 μ g/day for 25 days at concentrations ranging from 23 to 161 pg/mg, with relatively high concentrations in dark hair. The drug was also found in the hair from two bodybuilders at 50 and 92 pg/mg.

In 1999, Machnik et al. (26) tested clenbuterol in the hair of four females who had therapeutically taken the drug as a tocolytic. Hair was incubated in 1M KOH, and the drug was extracted with tertiary butylmethyl ether, followed in some cases by immunoaffinity chromatography, then derivatized with MSTFA-ammonium iodide-TMS ethanethiol. High-resolution mass spectrometry was used to identify clenbuterol. Limit of detection was about 0.8 pg/mg. The levels of clenbuterol determined in hair ranged from 2 to 236 pg/mg.



Au: Define SIM at first occurrence?

FIGURE 11.3 Typical GC-MS SIM chromatogram of a subject under atenolol treatment. Concentration was 5.4 ng/mg.

More recently (27), Kintz et al. developed a screening procedure for the simultaneous identification of β 2-agonists and β -blockers. The procedure involved overnight incubation in 0.1M HCl followed by neutralization, solid-phase extraction with an Isolute C18 column, derivatization with trimethylboroxine/ethyl acetate, and GC-MS detection. Limits of detection were 2 pg/mg for both salbutamol and clenbuterol. In nine asthmatic patients, the salbutamol concentrations in hair were in the range 27 to 192 pg/mg. In two asthma deaths, salbutamol concentrations in hair were 210 and 87 pg/mg. Finally, the laboratory identified salbutamol in the hair of a swimmer positive in urine at 71 pg/mg.

Most of the positive specimens are reported for salbutamol (46% of the total urines in 1999 for the IOC laboratory in France). However, as this drug is permitted for specific therapeutic purposes, together with a medical prescription, it appears very easy to evade the test, and almost all cases are considered as justified, even if it is known that the drug can be used to enhance performance. By comparison with concentrations measured in asthmatic patients, segmental analysis (repetitive positive segments) of hair would document unambiguously a doping attitude on the part of the athlete. Salbutamol (15 to 31 pg/mg, $n = 3$) and clenbuterol (15 to 122 pg/mg, $n = 6$) were identified in the hair of bodybuilders arrested for trafficking of a number of doping agents (17).

In addition to their medical use in the treatment of cardiac arrhythmias and hypertension, β -blockers have found a place in some athletic events, particularly disciplines in which good psychomotor coordination is required. In those sports, athletes can benefit from the peripheral blockade of several symptoms associated with anxiety. β -blockers are listed in the classes of drugs subject to certain restrictions, and tests will be conducted in some sports, at the discretion of the responsible authorities. A typical GC-MS chromatogram of a subject under atenolol treatment is given Figure 11.3.

11.4 DISCUSSION

There are essentially three types of problems with urinalysis drug testing: false positives when not confirmed with GC-MS, degradation of observed urine collection, and evasive maneuvers, including adulteration. These problems can be greatly mitigated

Au: This paragraph appeared verbatim later in this chapter. We deleted the second instance.

or eliminated through hair analysis. It is always possible to obtain a fresh, identical hair sample if there is any claim of a specimen mix-up or breach in the chain of custody. This makes hair analysis essentially fail-safe, in contrast to urinalysis, since an identical urine specimen cannot be obtained at a later date.

Another potential use of hair analysis is to verify accidental or unintentional ingestion of drinks or food that have been laced with drugs. In the case of a single use, the hair will not test positive, particularly for anabolics or corticosteroids, that are poorly incorporated in hair. Its greatest use, however, may be in identifying false negatives, since neither abstaining from a drug for a few days or nor trying to “beat the test” by diluting urine will alter the concentration in hair. Urine does not indicate the frequency of drug intake in subjects who might deliberately abstain for several days before biomedical screenings. While analysis of urine specimens cannot distinguish between chronic use or single exposure, hair analysis can make this distinction.

Although hair is not yet a valid specimen for the International Olympic Committee or the World Anti-Doping Agency, it is accepted in most courts of justice. A key issue is that some conflicting results are observed, all involving athletes that tested positive in urine in accredited WADA laboratories and negative in hair in certified forensic laboratories.

A lot of experience has been acquired in the detection of opiates, cocaine, and more recently benzodiazepines or hypnotics in hair. In contrast, there is a serious lack of suitable references to interpret the analytical findings for doping agents. In hair, doping agents concentrations, such as anabolic steroids, corticosteroids, or β 2-agonists, are in the range of picograms per milligram, whereas cocaine, amphetamines, or opiates are generally found in the range of several nanograms per milligram. Therefore, the Society of Hair Testing (SOHT) sought to obtain a consensus on hair testing for doping agents (28).

This consensus is as follows :

1. Hair analysis can essentially contribute to doping analysis in special cases, in addition to urine.
2. Hair specimens are not suitable for general routine control.
3. In the case of positive urine results, the negative hair result cannot exclude the administration of the detected drug and cannot overrule the positive urine result.
4. In the case of negative urine results, the positive hair result demonstrates drug exposure during the period prior to sample collection.
5. Before using hair analysis for doping control, sample collection and analytical methods have to be harmonized with respect to the sophisticated requirements already valid for urine.
6. The SOH feels responsible to support efforts that lead to this harmonization.

This statement was adopted on June 16, 1999, by the Society of Hair Testing.

It is clear that there is a great deal of research to be performed before the scientific questions and curiosity surrounding hair drug testing is satisfied. Some of this is due to a lack of consensus among the active investigators on how to

interpret the results of an analysis of hair. Among the unanswered questions, five are of critical importance:

1. What is the minimal amount of drug detectable in hair after administration?
2. What is the relationship between the amount of the drug used and the concentration of the drug or its metabolites in hair?
3. What is the influence of hair color?
4. Is there any racial bias in hair testing?
5. What is the influence of cosmetic treatments?

Several answers were recently addressed by Kintz et al. (29) on these specific topics.

Point 5 of this discussion can be resolved by using alternative sources of hair, and it has been recommended that pubic hair be collected in the case of bleached or colored head hair. However, care should be taken when sampling hair from other anatomic regions, as the concentrations can be highly variable according to the specimens. When comparing the physiological concentrations of DHEA and testosterone in hair collected from the head, pubis, and axillae, we were surprised to find an unusually high amount of DHEA sequestered in axillary hair (29).

Unfortunately, according to the WADA, basic scientific knowledge in hair biology is still lacking to make scalp hair analysis a valid tool in the field of doping control, and the following points will have to be resolved before applications:

1. Analytical methods are missing for several doping compounds, such as diuretics.
2. Peptide hormones are not incorporated in hair.
3. Hair washing, discoloring, tinting, and hair color (resulting in potential ethnic discrimination) appear to influence the concentration of drug measured in hair.
4. Drug incorporation within the hair longitudinal axis and over time is not proved to be regular in all occasions.

11.5 CONCLUSIONS

It appears that the value of hair analysis for the identification of drug users is steadily gaining recognition. This can be seen from its growing use in preemployment screening, in forensic sciences, and in clinical applications. Hair analysis may also be a useful adjunct to conventional drug testing in doping control. Specimens can be more easily obtained with less embarrassment, and hair can provide a more accurate history of drug use. However, there are still controversies on how to interpret the results, particularly concerning external contamination, cosmetic treatments, ethnic bias, or drug incorporation. Pure analytical work in hair analysis has reached a sort of plateau, with almost all of the analytical problems having been solved.

Although GC-MS is the method of choice in practice, GC-MS/MS (30) or LC-MS/MS are today used in several laboratories, even for routine cases, particularly to target low-dosage compounds such as anabolics or corticoids. In the case of doping control, drugs are screened in urine specimens according to validated

standard operating procedures in accredited laboratories. Because forensic laboratories can also be involved in testimony dealing with doping agents, the idea of using hair for doping control has emerged, as hair analysis has been accepted in court in other cases. Courts can request additional information on patterns of the use of doping substances, such as during the 1998 Tour de France cycling competition, when blood, urine, and hair were simultaneously collected. Hair can both confirm repetitive abuse and identify the exact nature of the parent compound (e.g., nandrolone, norandrostenediol, or norandrostenedione in the case of a positive result for norandrosterone in urine). Moreover, long-term use (over several months) of restricted compounds (only authorized under specific conditions and for a short period), such as salbutamol or corticoids, can be documented through hair analysis. The determination of testosterone esters in hair should allow a definitive, unambiguous confirmation of the administration of exogenous testosterone.

However, some issues remain to be resolved before hair can be considered as a valid specimen by the IOC and the International Sport Federations. The relationship between urine and hair results is not yet established, and a negative hair result does not mean “no doping.” The potential for ethnic discrimination must also be evaluated to avoid inequality during doping control. On the other hand, external contamination of hair samples does not constitute a major problem when testing for doping agents, in contrast to the problems associated with cosmetic treatments or the absence of specimen (bald or fully shaved subject).

Au: Is this the formal name of an organization?

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