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Human Hair: An Important Biological Matrix of Interest

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Review Article

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Abstract

Human hair can often be useful biological matrix, alternative to the conventional biological matrices such as blood, serum, urine, saliva etc for clinical and toxicological analyses. The objective of this article was to provide comprehensive details regarding the utilization of human hair as an analytical sample of interest. Such details involve presenting information on hair structure, mechanisms of active substances (drugs) incorporation into human hair, its merits as an analytical sample, collection and preparation for analysis, and various analytical techniques that have been used to determine active substances in the hair samples. The methodology used entailed obtaining information from published works in scientific journals, official books as well the internet websites.

The results indicate that numerous active substances belonging to various pharmacological classes namely central nervous system analgesics, antidepressants, antipsychotics, anticonvulsants, anti-hypertensives, anti-inflammatory agents, anxiolytics, cannabinoides, sedatives and steroids etc. that have been incorporated into human hair by passive diffusion from the bloodstream into the growing hair cells or through sweat, sebum, and the external environment have been successfully determined. Literature has also revealed that the speed and extent of active substances penetrating black hair are related to the lipid solubility, molecular weight, and polarity of the active substances. In conclusion, the study has shown that human hair is an important biological matrix in clinical and toxicological analyses as well as in nutritional, archaeological and forensic investigations.

Keywords: Human Hair; Mechanisms of Drug Incorporation; Analytical Techniques of Drug Determination

Introduction

Human hair is a proteinaceous fibre consisting of subunits of α-keratin chains that are strongly hierarchical organized. Hair has two separate domains namely the external domain (hair shafts) and internal domain (follicles) [1]. The hair shaft is a cylindrical structure being made of tightly compacted cells growing from the follicle. Hair shaft is composed of dead, keratinized cells arranged in three layers namely (i) the cuticle (outer layer); (ii) the cortex (second layer), and (iii) the medulla (central layer). Cortical cells contain pigment granules that are mainly melanin (synthesized in the melanocytes, located in the hair bulb). The amount, density and type of melanin in melanocytes provide the exact color of hair and likewise the hair fiber's diameter, determines the size of medulla. The hair follicle is a

small sac-like organ embedded in the epidermal epithelium of the skin and the cell membrane is composed of protein(65–95%),water (15–35%) and lipids (1–9%) [2].

Hoppe in 1858, was the first scientist to demonstrate that hair can act as a good biological matrix by determining arsenic content in the hair of exhumed body.

The report engineered other studies on hair samples, for example the methanolic extraction of opiates from hair and their determination using radioimmunoassay analysis [3]. Currently, human hair can serve as an alternative to the conventional samples such as blood, serum, cerebrospinal fluid, saliva, sweat, urine etc, for clinical and toxicological drug analyses. It has also found applications in archaeological, nutritional and forensic investigations.

For instance, in (a) environmental exposure to toxicants, (b) doping controls, (c) death investigations, (d) fatal acute poisoning, (e) repeated deliberate poisoning, (f) chronic drug consumption, (g) drug-facilitated crime (sexual assaults, robbery), (h) workplace drug testing, (i) gestational drug exposure, (j) violation of probation or parole, (k) driving license regranting [4-6].

Simple passive transfer model has been proposed as the mechanisms of drug incorporation into hair [7,8]. The model assumes that active substances (drugs) move by passive diffusion from the bloodstream into the growing hair cells at the base of the follicle. This incorporation depends on the active substance concentration in blood, which is a function of its ingested dose [9]. The implication of the passive diffusion model is that the position(s) the active substances are found along the hair shaft have correlation with the time these active substances were present in the bloodstream and hence forms the basis for hair segmental analysis.

The second model called multi-compartment model has also been proposed to be a better model than passive transfer model in explaining how active substances get into human hair. The model assumes that active substances are incorporated into human hair through: (i) the blood circulation during formation; (ii) sweat and sebum after formation; and (iii) the external environment after formation and after the hair has emerged from the skin. Active substances may also be transferred from multiple body compartments that surround the hair follicle [10].

Besides the above two models, active substances can be incorporated into human hair through external contamination. External contamination of hair can be summarized as: (a) deposition of active substances on the hair surface from air, water and cosmetic hair treatments etc. and (b) passive inhalation of active substances that are smoked [11,12].

Discussion

Collection of Hair Sample

The vertex of the head has been chosen by most analytical chemists as the site of interest for hair collection. The choice is probably because its analysis can (i) reveal drug use during time intervals (ranging from a week to years) before sampling, (ii) show that the site has less variability in the hair growth rate and more constant number of hairs in the growing phase (iii) show the hair to be less subjected to age and sex related influences (iv) present vertex hair as the most uniform in growth and the most consistent in the growth phase.

However, its (vertex) limitation is the exposure to sweat secretions, air, dust and water contaminants and modification by cosmetic treatments [2].

Beside the vertex, other hair samples are the beard hair (produced from male sexual hair follicles) and pubic hair respectively. Collected hair samples should be stored in a dry aluminum foil; an envelope or a plastic bag at ambient temperature.

Preparation of Hair for Analysis

Prior to human hair analysis, decontamination is required in order to avoid false positive results. Such false positive results might be due to chemical substances introduced into the body by inhalation, passive environmental exposure [13]. It is assumed that decontamination process by appropriate washing is capable of removing loosely bound chemical substances or drugs on the surface of the human hair.

Some Procedures for Hair Samples Decontamination Include

(a) washing with polar organic solvents namely acetone, ethanol, methanol etc [14,15], or non-polar organic solvents such as dichloromethane, isopropanol, diethyl ether, pentane, and hexane [16,17] (b) washing with sodium dodecyl sulfate solution or other detergents [18], and (c) using organic solvents and repetitive washings with phosphate buffers [13,19]. The non-polar solvent is preferred over polar solvent because it does not expand the hair or promote the dissolution of the binding drugs.Washing procedure is aimed at removing only (i) the dirt, grease and external contaminants from the hair surface (ii) drugs that have penetrated the epidermal layers and not drugs inside the

hair shaft [20-22]. The wash ratio method which compares the drug concentration found in the washing solution (all washings) with that found in hair after washing has been proposed as a criterion to distinguish passive exposure from active use of the chemical substances [19,23].

Extraction Procedures

The extraction procedures are used to remove any drugs remaining in the hair matrix after the extending washing of the hair samples and after the drug concentration of the washing solvents has produced a plateau (the drug fraction in the inaccessible domain of hair). They represent drugs that were incorporated from the interior.

The Extraction Procedures for Drugs are Categorized Into

(a) acidic extraction- using dilute hydrochloric acid or sulphuric acid (0.1–0.6 M HCl or 0.005 H2SO4) or acidified methanol (methanol-trifluoroacetic acid-acetic anhydride) at room temperature or 37°C overnight followed by neutralization and solid phase extraction Nakahara Y, et al. [24]; (b) alkaline digestion- using dilute sodium hydroxide (0.1 ~ 2.5 M NaOH) at 37°C overnight. The pH been adjusted to pH = 9 followed by solid phase extraction [25]; and (c) enzymatic digestion- aimed at the destruction of the hair structure followed by the release of the incorporated drugs to the digestion buffer [26,27]. Typical examples of enzymes employed are biopurase [28], β-glucoronidase/arylsulfatase (*glusulase*) [26], protease VIII [27], proteinase K [29] and protease E [28].

Of the three extraction procedures, the enzymatic extraction (digestion) at neutral pH, has been proposed as a universal extraction procedure for all chemical substances incorporated in hair, because the hair matrix is completely dissolved leading to the best recoveries [23].

Analytical Techniques for Active Substances Determination

Analysis of active substances in human hair requires minimum analytical parameters namely: specificity, sensitivity, and absence of matrix effects.

The analytical techniques are not quite different from those used for other biological matrices such as blood, serum, urine, saliva etc. Such analytical techniques may include but not limited to immunological assay, gas chromatography, liquid chromatography, capillary electrophoresis, infrared microscopy. Examples of such determinations are:

1. **Radioimmunoassays (RIA)**

(i) morphine [30], (ii) buprenorphine [31], (iii) cocaine/ benzoylecgonine [32], (iv) methadone [33], (v) fentanyl [34], (vi) benzodiazepines [35], (vii) thyroxin [36].

2. **High performance liquid chromatography**

(i) morphine [37], (ii) anticonvulsants [38], (iii) fluoroquinolone antibacterials [39], (iv) amphetamine and methamphetamine [40], (v) ecstasy [41].

3. **High performance liquid chromatography-mass spectrometry**

(i) prednisone [42], (ii) corticosteroids [43].

4. **High performance liquid chromatography-¬tandem mass spectrometry**

(i) anabolic androgenic steroids and their esters namely androsterone, boldenone, dehydroepiandrosterone, methandienone, methyltestosterone, nandrolone, phenylcholinone, stanozolol, testosterone, trenbolone [44,45], (ii) zolpidem [46], (iii) furosine [47], (iv) antidepressant and antipsychotic drugs [48].

5. **Gas chromatography-mass spectrometry**

(i) Opiates, cocaine and its derivatives [49]; (ii) benzodiazepines [50]; (iii) beta-2-agonists [51], (iv) amphetamine and methamphetamine [52], (v) codeine [53].

6. **Gas chromatography-tandem mass spectrometry**

(i) cocaine and its derivatives [54], (ii) anabolic steroids and their esters [55], (iii) fentanyl and sufentanil [56].

7. **Capillary electrophoresis**

(i) methadone [57], (ii) benzodiazepines [58], (iii) heroin [59].

Generally, by using any of the analytical techniques above, scientists do in certain cases, prefer to utilize human hair as a biological matrix due to some of its merits namely (i) sample collection is non-invasive (ii) easy to be performed under conditions that prevent adulteration and substitution, (iii) exogenous active substances are stable (iv) diagnostic tool for the determination of chronic alcohol consumption (v)extended detection windows (from 3 days to years), depending on the length of the hair shaft, (vi) capable of distinguishing chronic (prolonged exposure) or acute (single exposure) poisoning in conventional biologic samples, (vii) capable of reflecting long term drug abuse history. Despite these merits of human hair few demerits make analysts turn to conventional biological matrices and they include absence of (a) its propensity to incorporate different substances from the circulation (b) standardized techniques between laboratories, (c) laboratory certification program (d) consistent proficiency-testing materials, and (e) consistent results within and between laboratories.

Active substance	Method of analysis	Experimental conditions
Buprenorphine	GC-MS	Column: fused-silica capillary column mobile phase: helium, flow rate: 1.0 ml/min
Clonazepam	LC/MS/MS	column C18, mobile phase: methanol/ 0.1 % formic acid/2mM ammonium acetate flow rate: 0.20 ml/min
Cocaine	HPLC	Column: C18, flow rate: 1.0 ml/min mobile phase: phosphate buffer (pH 3.0)/ acetonitrille/methanol
Cocaine	GC-MS	Column: fused-silica OV-1 capillary column mobile phase: helium, flow rate: 1.0 ml/min
Carbamazapine and phenytoin	HPLC	Column: C18, mobile phase: acetonitrille/methanol/water, flow rate: 1.0 ml/min
Fentanyls	GC-MS/MS	Column: DB-5MS, mobile phase: helium, flow rate: 1.0 ml/min
Fentanyls	LC-MS/MS	Column: UPLC HSS T3, mobile phase: 0.1 % formic acid/ ammonium acetate/acetonitrile flow rate: 0.20 ml/min
Heroin	GC-MS/MS	Column: fused-silica capillary column mobile phase: helium, flow rate: 1.0 ml/min
Methadone	GC-MS	Column: fused-silica DB-1 capillary column mobile phase: helium, flow rate: 1.0 ml/min
Morphine	HPLC	Column: C18, mobile phase:
Morphine	GC-MS	Column: fused-silica capillary column, mobile phase: helium, flow rate: 1.0 ml/min
Testosterone, nandrolone, amphetamine, codeine, benzoylecgonine, ephedrine, terbutaline, phencyclidine,propranolol, salbutamol and stanozolol.		Column: Zorbaz C18, mobile phase: Solvent A;10 mM ammonium formate/0.02 M formic acid in water, B: 0.02 M formic acid in acetonitrile, flow rate:0.4 ml/min
Zolpidem	HPLC	Column: C18, mobile phase: acetonitrile/ammonium buffer, 0.02 M, (pH 8), acetate flow rate: 1.0 ml/min

Table 1: Illustration of few active substances (drugs) in human hair analyzed by chromatographic methods.

The (Table 1) above, provides few active substances in human hair that have been determined by chromatographic analytical methods.

Conclusion

The unique nature of the human hair (melanin content enhancing the incorporation of active substances into the hair), its merits as biological matrix and the availability of specific, sensitivity, accurate and precise analytical techniques, make human hair the biological matrix of interest.

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