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Review

Hair analysis, a novel tool in forensic and biomedical sciences: new chromatographic and electrophoretic/electrokinetic analytical strategies

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Abstract

Hair analysis for abused drugs is recognized as a powerful tool to investigate exposure of subjects to these substances. In fact, drugs permeate the hair matrix at the root level and above. Evidence of their presence remains incorporated into the hair stalk for the entire life of this structure. Most abusive drugs (e.g. opiates, cocaine, amphetamines, cannabinoids etc.) and several therapeutic drugs (e.g. antibiotics, theophylline, β_2-agonists, etc.) have been demonstrated to be detectable in the hair of chronic users. Hence, hair analysis has been proposed to investigate drug abuses for epidemiological, clinical, administrative and forensic purposes, such as in questions of drug-related fatalities and revocation of driving licences, alleged drug addiction or drug abstinence in criminal or civil cases and for the follow-up of detoxication treatments. However, analytical and interpretative problems still remain and these limit the acceptance of this methodology, especially when the results from hair analysis represent a single piece of evidence and can not be supported by concurrent data. The present paper presents an updated review (with 102 references) of the modern techniques for hair analysis, including screening methods (e.g. immunoassays) and more sophisticated methodologies adopted for result confirmation and/or for research purposes, with special emphasis on gas chromatography–mass spectrometry, liquid chromatography and capillary electrophoresis.

Keywords: Reviews; Hair analysis; Opiates; Cocaine; Amphetamines; Cannabinoids; Antibiotics; Theophylline; β_2-Agonists

Contents

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1. Introduction

In the scientific community, until recently, hair analysis was considered a quite esoteric method typical of forensic toxicology for investigating suspected poisoning in human remains. Famous was the finding of arsenic in Napoleon's hair [1] and the consequent debate about the supposed poisoning of the Emperor.

Another field of application concerned trace metals (e.g. zinc, lead, cadmium, iodine, copper, calcium, magnesium), with the aim of diagnosing nutritional deficiencies, learning problems or mental illnesses (schizophrenia, Down's syndrome) and general diseases (cystic fibrosis, coeliac disease). Although based on some intriguing evidence, hair analysis for trace metals, except in some instances, has never gained a general acceptance, because of unresolved problems posed by environmental contamination of samples and variability of results in different populations.

Even though the deposition of drugs in skin and skin annexes (hair, nails) had since long been demonstrated by dermatologists [2], only much later great attention was focused onto hair as a biological sample for drug analysis.

In reality, the international concern about the worldwide spread of drug abuses, with the related socio-economic problems, has prompted extensive research on methods to monitor this phenomenon, particularly in groups of population at risk (e.g. students, prisoners etc.) and in workers with a special degree of responsibility toward the collective whole (e.g. pilots, drivers, etc.).

As is well known, urine testing is the standard tool to investigate use of illicit drugs by individuals, but this method has shown several pitfalls, among which we mention the short time window (2–3 days for many drugs) during which the drug is detectable in this fluid (with consequent low epidemiological sensitivity), the embarrassing sample collection (with some potential for cheating), the impossibility of repeating the sampling later to control a previous disputed sample, the poor correlation between the "degree of addiction" and the actual drug concentration.

On the other hand, hair has been proposed as a record of past chronic drug intake, free from enzymatic metabolism, difficult to cheat, and proportional to drug ingestion.

After the pioneering paper by Baumgartner et al. [3], in a few years different researchers reported that the hair of chronic users contains a variety of drugs, including opiates, barbiturates, amphetamines, antidepressants, cocaine, phencyclidine, nicotine etc. [4]. Evidence of compounds embedded in this matrix was reported long after drug exposure, as witnessed by the finding of cocaine and metabolites in the hair of Peruvian mummies dating back to AD 1000 [5].

Hence, hair was proposed as an "alternative" or a "complement" to urinalysis offering several advantages, such as easy sample collection, sample stability at room temperature for a long time and, above all, a much wider time window of detection (months), with the goal of correlating chronologically the addiction behaviour with the distribution and concentration of the drugs in the hair stalk.

In recent years, extensive research has been dedicated to investigate the different ways of drug sequestration into hair, to develop analytical methods suitable for this unconventional matrix, and to verify the potential sources of errors.

Even if, at present, the pathways of drug entry are still not fully understood, it appears that drugs from blood and skin secretions (but also from the external environment) can enter the hair. The hair constituents in which drugs are bound or incorporated are also unclear: cheratin, melanin granules and the cell membrane complex have been hypothesized [100].

It has also been observed that some lipophilic compounds are found in higher concentrations in hair than hydrophilic metabolites, even if the latter have a longer half-life in circulating fluids, e.g. 6-monoacetylmorphine versus morphine [6] and cocaine versus benzoylecgonine [7].

Hair growth averages between 0.9 and 1.2 cm/month, with some differences depending on anatomical location, sex, race, age and with a relatively large interindividual variability [8]. Since one theory of drug incorporation in the hair matrix is that it takes place at the root level, it has been hypothesized that information on time and severity of addiction could be obtained from segmental analysis of hair

[8]. Some reports support this hypothesis, but on this point serious questions have been raised [9] and there is not complete agreement in the literature.

Some other important issues are still open in hair analysis. First of all, it has been demonstrated that hair can be contaminated from the environment, if exposed to solutions or airborne particles of drugs. Several attempts have been made to distinguish external contamination from the presence of drugs in hair due to active use [10], but this point is still highly controversial (just some examples can be found in Refs. [11–15]). Other controversies regard possible bias of the results due to color, age, gender, differences in the hair structure, the correlation between dosage and drug concentration in hair, the optimal sample treatment, the cut-off levels suitable for different purposes etc. The stability of drugs in hair, although generally considered excellent, has also been found less satisfactory after cosmetic treatments (e.g. bleaching and permanent waving) [101,102].

Despite some unresolved problems in both analytical aspects and interpretation of results, there is much interest in hair testing applications in clinical, epidemiological and legal environments [16].

Medical professionals and legal authorities in many jurisdictions use hair analysis to prove or exclude chronic (or repeated) use of illicit drugs in cases of:

- revocation of the driving licence for drug abuse and/or licence re-issuing after the claimed end of addiction;
- assessment of compliance to detoxication treatments;
- probation and parole monitoring;
- violation of laws on narcotics;
- drug-related deaths;
- child custody, adoption, divorce etc.;
- specific civil and criminal cases with allegation of drug abuse.

Employment-related hair testing has been proposed as a complement or an alternative to urinalyses, but serious questions remain about the appropriateness of basing decisions solely on hair test results; thus, hair testing is generally not accepted in this context.

The increasing attention paid to hair analysis is paralleled by a great production of scientific literature, particularly in recent years. This means that no review can be comprehensive of all the analytical, physiological and applicative aspects. Hence, the present work is intended just to give a glimpse of the main current analytical methodologies applied to hair analysis, with a special focus on the most recent chromatographic and capillary electrophoretic techniques.

2. Target drugs in hair

A great variety of drugs has been reported as detectable in the hair of chronically treated subjects, but, for the forensic environment in which hair analysis has been developed, by far the main attention of researchers has up to now been paid to drugs of abuse.

Opiates and cocaine, because of their major social relevance in Europe and in the USA, respectively, have been studied with particular interest, as witnessed by three recent reviews (on opiates: [17], on cocaine: [18,19]).

Amphetamines were among the first drugs whose incorporation in hair was studied [20]. Japanese groups have traditionally concentrated their research on amphetamine and methamphetamine, as the traditionally abused drugs in the far East, but the recent spread of illicit stimulants, including amphetamine analogues and hallucinogenic amphetamines (e.g. methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA) etc.) into the West has made this class of compounds the third most prevalent in hair analysis. This subject has been recently reviewed by Nakahara [21]; after this review a relevant paper on MDA and MDMA analysis has been published by Kintz et al. [22]. Lysergic acid diethylamide (LSD) poses particular problems to analysts because of its rapid metabolism and low dosage; only recently has the incorporation of LSD in hair been demonstrated by Nakahara et al. [23], together with other hallucinogenic compounds such as MDA, MDMA and phencyclidine (PCP).

The use of marijuana and hashish is widespread worldwide, however, relatively few reports have been published on the detection of cannabis-related compounds in hair. This is probably due to the very low levels at which Δ9-tetrahydrocannabinol (THC) and, particularly, its metabolite 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) are detectable in this matrix. Moreover, because of the prevalent use of cannabis products by smoking, external contamination remains a major issue.

A recent research paper contains also a short review of the previous works in this field [24].

Although not illicit, nicotine can be considered pharmacologically close to abusive stimulants. The parent drug and its metabolite cotinine have been determined in the hair of smokers [25,26] and of subjects assuming nicotine [27], but also an important contribution from environmental exposure to nicotine vapours has been demonstrated [28]. Morphine, cocaine and nicotine/cotinine measurement in the hair of neonates has been examined as a means of quantifying fetal risk consequent to exposure to heroin, cocaine and nicotine during pregnancy [29–32].

Several therapeutic, but potentially toxic, drugs such as anti-depressants, antipsychotics and tranquillizers have been determined in hair; for tricyclic and tetracyclic anti-depressants readers can refer to the papers by Couper et al. [33,34] and for haloperidol to the papers by Uematsu et al. [35] and Matsuno et al. [36]. Benzodiazepine analysis in hair has also been published [37,38], as well as the determination of meprobamate [39]. The incorporation of phenobarbital [40,41] and other anti-epileptic drugs has been reported and their measurement in different sections of hair has been proposed to monitor the compliance to anti-epileptic therapy [42]. The anaesthetic drug fentanyl has been determined by Wang et al. [43], the antitussives dihydrocodeine and zipeprol by Sachs et al. [44] and Kintz et al. [45], respectively, and the analgesic dextromoramide by Kintz et al. [46].

Other compounds detected in hair include therapeutic drugs such as quinolone antibiotics ofloxacin and temafloxacin [47] and β_2-agonists clenbuterol, salmeterol and salbutamol [48], susceptible also to illicit use as anabolizing agents.

Recently, some researchers have focused their attention also onto low-molecular-mass endogenous substances which could theoretically be incorporated into the hair through mechanisms comparable to drugs. Although still preliminary, hydroxybutyric acid, a neurotransmitter, and biogenic polyamines (spermidine, spermine and putrescine) have been reported to be present in the hair of normal subjects [49], but the meaning of these findings is, at present, still obscure.

3. Analytical methods

Hair analysis requires a relatively complex and not yet standardized sample preparation. This includes washing steps to remove non-specific endogenous (sweat, sebum) and exogenous substances (environmental dusts, hair treatments) adsorbed onto the hair in order to expose a clean surface to the further extraction or digestion treatments. To this aim, the use of surfactants and shampoos, aqueous solutions and organic solvents (ethyl ether, 2-propanol, methanol, hexane, acetone, etc.) has been reported [8]. Particular effects have also been made to decontaminate hair from drugs possibly adsorbed from the environment in the form of powders, smokes or solutions, which could lead to misinterpretation of the results. This problem has recently been reviewed [50], but is still unresolved and, as above mentioned, is the subject of a fierce scientific debate [9–15], which is beyond the scope of the present paper. In any case, it has been demonstrated that heavily contaminated hair can not be entirely 'cleaned' by washing and that, on the other hand, extensive washings can also remove drugs present in hair due to personal use.

The washing step is generally followed by a further treatment to solubilize the drugs contained in the hair. This can be achieved in many different modes, including digestion of the hair matrix in alkali or with enzymes, incubation in acid media or in organic solvents to elute the analytes from the matrix without complete dissolution. The subject has been reviewed in detail by Chiarotti [51] and later discussed by Sachs and Moeller [52] and by Cirimele et al. [53].

In general, the mixture resulting from the incubation of hair in the digestion or extraction solutions is not suitable for direct analysis and must be further

purified and concentrated using liquid-liquid (LLE) or solid-phase extraction methods (SPE). To the best of our knowledge, no papers exist reviewing the sample purification techniques used for hair analysis which, however, are similar to those adopted for the pre-treatment of biological fluids such as urine or blood. Supercritical fluid extraction (SFE) methods have recently become available for many matrices and analytes. SFE has been applied in hair analysis for opiates by Edder et al. [54], who used subcritical conditions, and for opiates, cocaine and, tentatively, cannabinoids by Cirimele et al. [55].

Hair analysis poses peculiar problems which can summarized as follows:

- frequently, only small amounts of the sample (20–50 mg) can be collected because of aesthetic reasons, raising the issue of specimen preservation for re-test;
- the hair matrix is complex and its composition variable (genetic and cosmetic treatments);
- analyte concentrations are low, ranging from ng/mg (e.g. cocaine) to pg/mg (e.g. clenbuterol);
- often, medium-to-high sample throughput is needed;
- methods of analysis are not yet standardized, as well as quality control/quality assurance;
- the results should be defensible in court.

3.1. Immunological methods

Radioimmunoassay (RIA) was adopted since the introduction of hair analysis for drugs of abuse [3]. In fact, for screening purposes, this method meets almost all the needs of hair-analysis laboratories, particularly in terms of sensitivity, sample throughput and simplicity, and, consequently, it is still widely adopted as a first test. However, RIA methods are usually standardized for blood or urine testing and need to be thoroughly validated for the hair matrix [56]. Moreover, the need for radioactive reagents limits the application of RIA methods to the authorised laboratories.

The lack of sensitivity has hindered the application of most of the homogeneous phase non-radioisotopic methods (e.g. EMIT®); however, a commercial fluorescence polarization immunoassay was proposed by Franceschin et al. [57] and by Kintz et al. [58] (see [59] for comments). Heterogeneous-phase immunoassays, and, particularly, enzyme-linked immunosorbent assays (ELISA) are intrinsically more sensitive than immunoassays in the homogeneous phase, and recently have proved suitable for hair analysis [60,61].

The commercial availability of antisera specific for the analytes of interest and the cross-reactivity of antibodies towards structurally related compounds are well known limitations of immunometric methods, and they require confirmation with alternative techniques based on different analytical principles.

3.2. Chromatographic methods

The use of chromatographic techniques for the analysis of hair for drugs of abuse has already been reviewed by Moeller in 1992 [62]. Therefore, we can present here only a methodological update and a brief discussion on the different chromatographic choices. For references to specific methods, readers can refer also to the papers quoted in Section 2.

Thin-layer chromatography (TLC) with fluorescence detection was among the first methods to be applied to the determination of morphine in hair [63]. However, the limited mass sensitivity of the technique (2–3 μg) required that as much 1 g of hair was collected, which is clearly unacceptable for real applications. This technique was further improved by using high-performance TLC (HPTLC) and the more sensitive dansyl derivatization [64]. However, the moderate mass sensitivity of TLC techniques and, more importantly, the inherent limits in the accurate quantitation have hindered the diffusion of planar chromatography in hair analysis.

Gas chromatography (GC) with the traditional flame ionization (FID) or nitrogen–phosphorus flame ionization (NPD) detection has also found very limited applications in hair analysis, because of poor sensitivity and/or selectivity towards matrix components. However, especially in the early studies, GC was used to detect cloroquine, amphetamines and antidepressants (see Ref. [62]) and, more recently, cocaine and cocaethylene [65]. To the best of our knowledge, the more sensitive electron capture detection (ECD) has not found application in this field, conceivably for insufficient selectivity towards natu-

rally occurring interferent compounds in the hair matrix.

Gas chromatography–mass spectrometry (GC–MS) is by far the most important analytical technique presently used in analytical toxicology. It has become the 'gold standard' in terms of admissibility and defensibility in court, because this technique offers good sensitivity, excellent selectivity and mass specificity, and displays a high degree of standardization. On the other hand, GC–MS still shows moderate sample throughput and instrumental ruggedness. Moreover, to achieve optimal analytical performance, sample preparation, mostly by using solid-phase extraction (SPE), is crucial, and derivatization is almost mandatory in most cases.

Electron-impact ionization (EI) using selected ion monitoring (SIM) is generally adopted for routine purposes, and is considered a compromise between sensitivity and selectivity. In fact, often the sensitivity required for hair analysis is not always achievable by the more informative full-scan detection. GC–MS using EI–SIM (two ions) has also been proposed for the simultaneous analysis of multiple drugs of abuse, including amphetamines, opiates, cocaine and methadone [66].

Recent improvements in instrumentation allowed more routine use of chemical ionization (CI), which offers superior sensitivity in GC–MS [67,68].

In most instances, the sensitivity achieved by the widespread 'low-cost' GC–MS instrumentation is sufficient to determine routinely drugs present in hair down to concentrations of 0.5–1.0 ng/mg [62], which is what is required for most drugs of abuse and chronically administered therapeutic drugs.

When much lower concentrations are to be determined, as in the case of THC metabolites, LSD and clenbuterol, the sensitivity of most chromatographic techniques is challenged.

THC and THC-COOH were determined in the low pg/mg range of concentration by using GC–MS–MS [69]. Also, GC–MS with negative-ion chemical ionization (NCI), was reported by Kintz et al. [70] as an alternative method for testing human hair for cannabis, although it is less selective and, conceivably, slightly less sensitive. A method using GC–NCI-MS has also been reported by the same group for the analysis of lorazepam in human hair, showing a sensitivity of 2 pg/mg [38].

Because of its inherent excellent selectivity, GC–MS–MS performed on triple quadrupole has been used for the determination of heroin and metabolites in hair, after direct silylation of the sample and avoiding any purification step, thus achieving a sensitivity of about 25 pg/mg [71].

On the other hand, derivatization with methyl boronic acid followed by GC–MS allowed the detection in the SIM mode of clenbuterol and salbutamol down to about 10 pg/mg of hair [72].

GC–MS analysis of LSD has been hampered by its extremely low levels in hair. To the best of our knowledge, the only GC–MS application so far reported was in combination, for quantification, with a sensitive liquid chromatographic assay using fluorescence detection (see later), and was mostly applied to experimentally treated animals, but also to some human cases [23,73].

High-performance liquid chromatography (HPLC) has also been used in hair analysis. In effect, HPLC is generally considered more suitable than GC for analysing biological extracts, particularly for polar and thermally labile compounds, and rarely requires derivatization. Sensitivity and selectivity can be excellent, especially when electrochemical and fluorescence detectors are used. Also, with UV absorbance detectors, sensitivity and specificity are acceptable; the latter can be improved by the use of a diode array or fast scanning detection systems, which record on-line the UV spectra of the eluted peaks. The coupling of HPLC to MS is possible through several interfaces, and today it is widely used to study drug metabolism. Finally, HPLC can stand high workloads and can easily be connected with automated sample-preparation devices, thus being extremely suitable for routine and quantitative work.

Notwithstanding these interesting features, HPLC still plays a minor role in hair analysis, although several methods have been published since the middle 1980s for the analysis of morphine, amphetamines and haloperidol [62].

In the more recent literature, HPLC–UV with GC–MS confirmation, has been reported for the determination of antidepressants (nortriptyline, amitriptyline, imipramine etc.), antipsychotics (haloperidol, chlorpromazine, thioridazine) and benzodiazepines in scalp hair with moderate sensitivity (about 1 ng/mg) [35,36].

The use of reversed-phase HPLC coupled with more selective detectors, such as electrochemical (EC) detectors for morphine [74,75] and fluorimetric (FL) for cocaine [76], has permitted the achievement of excellent sensitivities (about 0.02 ng/mg for both analytes in routine conditions). No sample derivatization was needed and sample clean-up was limited to a single-step liquid–liquid extraction, instead of the almost universally used, yet more complex and expensive, SPE. Low-level determinations using HPLC with EC detection have been reported for buprenorphine by Kintz et al. [77] and for clenbuterol in bovine hair by McGrath et al. [78].

HPLC–FL was also used for the determination of ofloxacin, norfloxacin and ciprofloxacin in human hair seriate sections [79,80].

HPLC–FL has also proved useful for the analysis of compounds present in hair at trace concentrations, such as clenbuterol and LSD. In particular, reversed-phase HPLC with fluorescence detection (ex. 310 nm, em. 470 nm) after on-line post-column photoderivatization by UV irradiation, has allowed the determination of clenbuterol in cattle hair, down to concentrations of 0.2 pg/mg [81]. LSD was also quantitatively determined in hair using ion-pair reversed-phase HPLC and direct fluorescence detection (ex. 315 nm, em. 420 nm) [23,73].

Only quite recently HPLC–MS using electrospray ionization (ESI) has been introduced in hair analysis, where the reported results on cocaine-related substances and opiates are extremely promising in terms of both sensitivity and selectivity [82]. Capillary HPLC with fast atom bombardment–mass spectrometry (FAB–MS) has been used for the determination of theophylline in the hair of patients undergoing chronic theophylline therapy for bronchial asthma [83].

3.3. Electrophoretic/electrokinetic methods

In the last decade, capillary electrophoresis (CE) has established itself as a sound, powerful and extremely versatile analytical tool in forensic toxicology, as witnessed by recent book chapters and review papers [84–86]. CE has been successfully applied to the analysis of seized preparations of illicit or controlled drugs and for the determination of drugs of abuse in biological fluids. However, CE applications in hair analysis are still limited and, to the best of our knowledge, all come from our group.

In our opinion, CE has several potential advantages which could be useful in hair analysis, among which we can mention: minimal need of sample (with consequent possibility of repeating the analysis many times on the same material), negligible consumption of reagents, peculiar separation mechanisms (which are highly 'orthogonal' to chromatography), high mass sensitivity (even with UV detectors), good-to-excellent concentration sensitivity (with EC or laser-fluorimetric detectors) and suitability for coupling with MS, particularly with ESI. As the main weak point of CE, we can mention a limited sensitivity in terms of concentration, particularly with absorbance detectors, due to the short pathlength (usually 20–100 μm) and to the minimal volume of sample which can be injected (in the order of tens of nl).

In our first experiences, we used a simple capillary zone electrophoresis (CZE) separation mode with UV detection at 238 nm for cocaine and 214 nm for morphine analysis [87]. The electropherograms showed excellent selectivity, with virtually no interference from the matrix, but, due to the limited amounts of sample (few nl) which could be injected into the capillary, the sensitivity was moderate (0.2 ng/mg). Later, stacking techniques were developed in order to increase sample loadability with a resulting increase in sensitivity (about 5 times), without sacrificing efficiency [88]. Micellar electrokinetic capillary chromatography (MECC) has also been tentatively applied to hair analysis, showing the potential of extending the analytical spectrum to basic, neutral and acidic drugs simultaneously [89].

Quite recently, CZE–ESI-MS has been successfully tested on a large panel of drugs of forensic interest, and, even if not yet applied to hair analysis because of some problems in sensitivity, it looks potentially suitable for application in this challenging field [90]. The recent commercial availability of 'nl electrospray', reportedly permits the coupling of CZE systems to MS, without the need for 'make up' liquid flow, so far necessary to match the μl flow required by the current interfaces. Avoiding the high dilution of the CZE effluent caused by the make up flow, such a development should allow high sensitivity and more efficient CZE–MS–MS operation.

3.4. Other methods

Several other methods have been used to analyze hair for drugs of abuse, mainly for qualitative purposes. Here, we simply mention MS–MS with direct probe injection, which allowed the determination of heroin and 6-monoacetylmorphine in the hair of heroin users, at a time when only morphine was believed to be present [91]. This finding was later valuable for the unambiguous identification of heroin abuse, because the simple presence of morphine in hair per se can derive from past morphine treatments or from other opiates metabolized to morphine.

Tandem mass spectrometry was also used to support studies on the deposition of drugs of abuse into human hair and on hair decontamination [92,93]; although expensive and instrumentally complex, MS–MS, because of its excellent selectivity and identification power, is currently used for investigating real forensic cases in some leading laboratories [94,95].

Recently, ion trap MS (daughter ion spectrometry) has been proposed as a cheaper alternative to MS–MS for cocaine and morphine analysis in hair [96]. Also, ion mobility mass spectrometry (IMS) has been reported as a perspective technique suitable for hair analysis, and applied to cocaine determination [97].

Infrared microscopy was reported to examine the interior of the hair to differentiate passive contamination from the environment and incorporation due to drug use. By microtoming the hair cross-sectionally, infrared spectra were obtained of the cortex and medulla of single hairs. Three-dimensional infrared imaging with Fourier-transform infrared (FTIR) microscopy suggested that hydromorphone was in the axial region of hairs of a chronic abuser of this drug [98,99].

4. Conclusion

Hair analysis is probably the most powerful method for investigating past "exposure" to drugs (illicit or therapeutic) by individuals. However, some basic physiological and analytical problems are not yet satisfactorily resolved (e.g. passive contamination of the sample, dose-to-concentration correlation, distribution of the drugs along the hair stalk, in vivo stability, in vitro stability) and, consequently, the results from hair analysis must be interpreted with great caution, especially if they have legal or administrative consequences. The combination of different methods is mandatory to achieve an acceptable degree of analytical reliability; in this respect, GC–MS plays a major role, but also HPLC with selective detectors can be useful, especially for routine applications. At present, CE is a promising complementary technique, already suitable for hair analysis with moderate sensitivity, but excellent selectivity.

However, interpretation problems still remain, which limit the acceptance of this methodology, especially when the results from hair analysis represent a single piece of evidence and can not be supported by concurrent data.

On the other hand, hair analysis could become more suitable for direct application for epidemiological purposes (e.g. to investigate the degree of prevalence of a certain drug in a population), in which "exposure" of a general population to illicit drugs yields useful population data. Moreover, an increasing use of hair analysis may result in reliable methods to investigate chronic treatments with drugs for which the environmental contamination of the sample can be excluded, for instance illicit use of doping agents in sport or of anabolizing drugs in food animal treatment (e.g. clenbuterol), and to control the patients' compliance to long-term therapies. Also, hair analysis for low-molecular-mass endogenous compounds could become a tool to study the average long-term behaviour of metabolites or hormones, without too frequent blood or urine analyses.

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