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Review

Testing hair for pharmaceuticals

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Abstract

More than hundred pharmaceuticals, drugs of abuse or doping agents have been reported to be detectable in human hair. This article reviews the analysis of 90 drugs and drug metabolites by chromatographic procedures, including the pretreatment steps, the extraction methods, the reported limits of detection and the measured concentrations in real human hair samples. Some progress is observed in the detection of low dose drugs, like fentanyl or flunitrazepam. The general tendency in the last years, to highly sophisticated techniques (GC–MS–NCI, HPLC–MS, GC–MS–MS) illustrates well this constant fight for sensitivity. Some new findings, based on the recent experience of the authors, are also added.

Keywords: Hair; Reviews; Drug screening; Pharmaceuticals

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1. Introduction

The very first case of the determination of poison in human scalp hair was published by Casper [1] in 1857, in his very famous handbook 'Praktisches Handbuch der Gerichtlichen Medizin'. He had determined arsenic in the hair of a body exhumed after eleven years. About one hundred years later, in 1954, the first organic drug was detected in guinea-pig hair [2]. The paper that has really change the situation, however, was that of Baumgartner et al. [3] in 1979. The authors published a radioimmunological method for the detection of opiates in the hair of drug addicts and for the first time introduced the idea of tracking drug use. From this date, great interest has been shown in this matrix in forensic cases and the number of papers relating to drug detection in hair has increased exponentially during the last two decades.

The toxicological analysis taken from hair samples makes it possible to obtain long term information concerning the consumption of xenobiotics by the exposed person. Hair growth rate could vary among individuals and in different body locations in the same person, but in the head it is usually in the range of 0.7–1.5 cm/month [4]. By this aspect, the method is different from the urine analysis whose detection period generally lasts a few days. Indeed, it could be longer for some pharmaceuticals such as diazepam, phenobarbital or other molecules with long half-lives, or other drugs with multiple kinetic elimination profiles such as clenbuterol, nandrolone and other anabolic steroids. In practice, the two biological materials are often complementary. Sweat and saliva have also been proposed as an alternative matrix for the detection of drugs of abuse in the first h after their administration [5,6]. P. Kintz [7] has described a comparison of drug testing in addicts between urine, sweat and hair. Indeed, whole blood must be considered as the medium of choice for the correlation between analytical results and clinical observations.

The concept of hair analysis is very simple. The solid hair is transferred into a liquid phase (the hair itself or the molecules of interest) and then examined like a urine or blood sample. Initially nobody could imagine that one could successfully identify other compounds in hair other than those classically found in urine. This way of thinking ended during the 80s when cocaine was always quantified in hair at a higher concentration than that of benzoylecgonine.

Most of the procedures devoted to drug detection in hair are based on gas chromatography mass spectrometry (GC–MS) in the electron impact mode [8], but sensitivity is a constant fight and in recent years, positive and negative chemical ionization or even GC–MS–MS or high resolution MS (HRMS) techniques were adopted. For the determination of benzodiazepines or opiates, high-performance liquid chromatography (HPLC), combined with photodiode array detector (PDA) or MS (buprenorphine, theophylline), has proved to be more and more important. Capillary electrophoresis introduced by Tagliaro et al. [9,10] for analysis of illicit drugs should become a popular method for detecting pharmaceuticals.

2. Drug incorporation

Drug substances, which are present in the systemic circulation due to deliberate drug consumption and are taken up during histogenesis, represent the endogenous pathway. Molecules absorbed or transferred to the keratinized hair from perspiration (sweat, sebum and transdermal excretion) represent the endogenous-exogenous pathway. At last, drugs deposited from the external environment (pollution, cosmetic hair treatments) can enter the keratinized hair by absorption and represent the exogenous pathway.

The mechanism generally proposed for the endogenous pathway of molecules into growing hair is passive diffusion from the blood supply at the base of the hair follicle. When a drug wants to permeate a cell, it must indeed traverse the plasma membrane. Thus, we must consider the mechanisms by which drugs cross membranes and the physico-chemical properties that influence this transfer. Most drugs are weak bases or acids that are present in biological fluids both in their ionized and non-ionized forms. Plasma membrane exhibit low permeability to polar molecules, thus ionized compounds are rarely able to penetrate the lipid cell membrane. Only the free, non-ionized molecules that are sufficiently lipid soluble can diffuse across the cell membrane. Once inside the cell, the uncharged drug will dissociate

depending upon its pK_a value. The pH-dependence of dissociation and lipid solubility of a substance may result in the unequal distribution of the drug in various compartments with different pH values. This favors the accumulation of undissociated basic drugs in the compartment with the lower pH. The isoelectric pH of hair being close to 6 clearly indicates the acidic nature of the hair [11], and thus the good incorporation of basic drugs.

Another very important point is represented by the drug interaction with melanin. In contrast to matrix cells, the melanocytes are a highly specialized cell population. The intracellular pH for melanocytes has been estimated in the range 3–5 [12]. Accumulation of basic substances is favored in the cytosol. Many investigators have demonstrated the affinity of natural and synthetic melanins to various drugs [13]. It is generally accepted that the ability of melanin-containing tissue to accumulate and retain drugs is remarkable [14]. Being entrapped during melanin granule formation, a permanent concentration gradient will occur for drugs with a high melanin affinity and the influx into a melanocyte will be higher than into a matrix cell. C. Howells et al. [15] have investigated the binding of several drugs (including clenbuterol, chlorpromazine, diethylstilbestrol, nandrolone, salbutamol, salicylic acid and trenbolone) to melanin from *Sepia officinalis*. Basic and hydrophobic drugs were the most strongly bound. Desorption by ethanol was complete for neutral drugs but partial for the basic drugs. This suggests that the binding of these latter molecules also involves an ionic component. This also suggests that the methanolic sonication of a powdered hair sample should be an effective pretreatment of the matrix when testing for neutral drugs (like anabolic steroids) but rather ineffective for basic ones. The same authors have measured the melanin capacity to be higher for clenbuterol (5.9 nmol/mg) than for the neutral hydrophobic drug nandrolone (0.56 nmol/mg). According to the melanin and acidic protein concentrations contained in white or black hair, it is natural to consider that black hair can concentrate basic drugs more efficiently while white hair could incorporate acidic drugs slightly more effectively. In all cases the basic drugs have a better incorporating ratio than the acidic ones. Nevertheless, the low incorporating ratio of acidic drugs is well balanced by a high blood concentration which can be thousand times higher than the one of a basic molecule.

3. Sampling

3.1. Sample collection

Hair is best collected from the area at the back of the head, called the *vertex posterior*. This area has less variability in hair growth rate than other areas. Moreover, the hair is less subject to age and sex-related influences [4]. There is, however, a relatively large interindividual variability [16]. The sample size collected should be at least 200–250 mg. For aesthetic reasons, however, it could be dramatically less (10–50 mg). When head hair is not available or too short, pubic or axillae hairs could be collected. On the contrary to drugs of abuse (violation of laws on narcotics, probation, drug abstinence in detoxification treatments, child custody, divorce), testing hair for pharmaceuticals in forensic science is more frequently performed on deceased persons. Thus, the quantity of collecting hair could be significantly higher than for living peoples, except indeed for newborns, very young children, and on the opposite of age, for very old persons.

3.2. Decontamination and cosmetic treatments

The problem of external contamination is very important for drugs of abuse as people can be exposed to a smokey or dusty environment. Papers dealing with that problem which also concerns the establishment of cut-off values are numerous, and various studies were published by Mieczkowski [17], Blank [18], Kintz [19] or Kidwell [20]. The problem, though largely debated, is still not fully resolved and is the subject of fierce scientific discussion. The approach is, of course, very different for pharmaceuticals as the drugs are supposed to be normally taken by mouth or by another therapeutic route under normal pharmaceutical presentation. Another important serious concern is the change in the drug concentration induced by cosmetic treatments of hair. The strong bases used for such treatments

(ammonia for example) may cause hair damage resulting in lost from the hair matrix or alternatively under favorable environmental contamination conditions, a higher incorporating ratio [21].

4. Sample pretreatment

In fact, the history of hair analysis is the history of extraction procedures, because after the drug has been extracted once it can be handled as if it had been extracted from urine or blood samples. More than 15 procedures have been proposed between 1979 and 1992, including chronologically [22]: methanolic sonication, 0.1 *M* NaOH, 0.1 *M* HCl, water, buffers, 1 *M* NaOH, 0.1% Na docadecylsulfate, acetone, pronase, performic acid–pronase, methanol–5 *M* HCl, proteinase K dithiothreitol, phosphate buffer pH 7.4–β-glucuronidase-arylsulfatase and lastly introduced by Staub et al. [23], the supercritical fluid extraction (SFE). The subject has been previously reviewed by Chiarotti et al. [24] and later by other workers [25,26]. With the exception of SFE and methanolic sonication, the resulting mixture from the pretreatment step must be further purified prior to analytical examination. It could be as simple as a simple liquid–liquid extraction with common solvents or as complex as a multi-step trace enrichment procedure devoted to the analysis of 17-keto steroids and their esters [27].

5. Compounds tested

5.1. Carbamates

Kintz et al. [28] have reported a GC–MS method for the quantification of meprobamate in hair and have shown a correlation between drug levels in hair and in serum. In another study [29], Kintz has monitored the appearance of meprobamate in beard hair after single oral administration (placebo, 400, 800 or 1200 mg) in 16 healthy volunteers. Drug levels appeared to be dose-related. Aldicarb (Temik®) has been quantified by the same author in the hair of a 39-year-old man whose immediate family was suspected of poisoning him. Drug concentration in hair was found to be 0.09 ng/mg while post-mortem whole blood concentration was measured at 4.81 μg/ml [30]. Table 1 summarizes the pretreatment, extraction and analytical methods used and the measured concentrations for meprobamate.

5.2. Licit morphinomimetics

Morphine and codeine and, though a little bit later, 6-AM [31,32], have been analyzed since the beginning of the methodology, others for substitute treatments like methadone, buprenorphine, or levo-alpha acetylmorphine were only investigated recently [33]. The drugs used for their analgesic, anesthetic or antitussive properties were also analyzed. In most cases, these pharmaceuticals were detected in an

Table 1
Methodology and results published for meprobamate

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD[a]	Ref.
Meprobamate	1 *M* HCl, overnight at +60°C	L/L[b] using phosphate buffer pH=5, and chloroform	GC–MS after derivatization with a methylating agent (trimethylphenylammonium hydroxide)	3.32 and 4.21 ng/mg (n=2)	0.2 ng/mg	[28]
Meprobomate	Idem	Idem	Idem	Range was 0.2–17.64 ng/mg (n=16 during 21 days) in beard hair. Oral dose of 400 mg gives a peak concentration of 4.27–6.08 ng/mg. 800 mg: peak 8.54–13.01 ng/mg 1200 mg: peak11.89–17.64 ng/mg	Idem	[29]

[a] LOD: Limit of detection.
[b] L/L: Liquid–liquid extraction.

abusive context. Table 2 lists the main studied molecules and the different proposed analytical techniques.

5.3. Anticonvulsants

During trade drug monitoring, the level of the drug in blood is essential in adjusting individual dosages. Human hair analysis has been applied in monitoring dosage history over several months. Goullé et al. [52] have demonstrated that a group correlation exists between phenobarbital in hair and phenobarbital in serum on the one hand, and between phenobarbital in hair and some typical clinic observations on the other hand. Kintz et al. [53] have proved that the concentration of carbamazepine was significantly correlated with the daily dose. The authors also pointed out that the deviations observed in their study led to the conclusion that hair analysis of a sample cannot definitely evaluate the quantity of drug consumed. However, hair testing should be successfully applied for documenting clinical disorders by analyzing sequential segments corresponding to one-month periods. Williams et al. [54] confirmed that the technology can offer the identification of erratic or total non-compliance in patients retrospectively. Phenytoin has also been quantified in the hair of patients under anti-epileptic therapy [55]. Table 3 shows the different analytical procedures for this therapeutic class.

5.4. Benzodiazepines and related compounds

Benzodiazepines are one of the most used class of drugs throughout the world, and surprisingly were not assayed by chromatographic procedures until the late 90s. Strong acidic or alkaline conditions cannot be used for the pretreatment step since the drugs are unstable and can be readily converted into decomposed compounds including benzophenones. Several results were obtained using incubation in Söerensen buffer or a mixture of β-glucuronidase-arylsulfatase, mild acidic incubation or even direct methanolic sonication. Though this latter procedure produces very dirty samples that would dramatically decrease the signal-to-noise ratio of the chromatographic detection, GC–MS–MS has been proposed to reduce the interference [57]. Hair concentrations of individual benzodiazepines are generally low, so due to the high electrophilic character of the analytes, GC–MS–NCI represents the state-of-the-art method for testing these molecules. Sexual abuse of men and women, while under the influence of flunitrazepam (‘date-rape’ drug), has focused many investigators to work on this molecule of main interest in forensic science. As already mentioned, zolpidem and zopiclone are also widely used for this aim [46]. Table 4 gives the main procedures devoted to the analysis of benzodiazepines and other anxiolytic-hypnotic drugs in hair.

5.5. Antidepressants and psychostimulants

Various antidepressants or psychostimulants (amphetamine derivatives excepted) could be determined in hair including some of their nor-metabolites as shown in Table 5. As firstly noticed by Ishiyama [66] and lately confirmed by Tracqui [67] on amitriptyline, enormous interindividual variations could be observed and a rather tenuous relationship could be produced ($r=0.563$) between drug concentration in hair and oral dosage.

5.6. Antipsychotics

Sato et al. [72] have clearly demonstrated that the concentration of chlorpromazine in black hair collected from 23 Japanese patients who had been taking the drug orally was correlated with the daily dose and with the trough plasma concentration at steady state. Matsuno et al. [73] reported the same observation in 59 inpatients of a psychiatric hospital who were controlled on maintenance doses of haloperidol (HL) given three times per day. The correlation coefficient was better however, between the daily dose and the concentrations in hair for reduced haloperidol (RHL) ($r=813$) rather than for HL ($r=0.682$). Moreover it was slightly higher for the sum of the concentrations of the two substances in hair ($r=0.829$).

Sachs [74] reported the detection of clozapine in the hair of a 1-year-old boy exhumed several months after death. The mother was charged for murdering him with the drug prescribed to her depressive sister. The neuroleptics are detected in hair using the procedures listed in Table 6.

Table 2
Methodologies and results published for morphinomimetics

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD	Ref.
Buprenorphine (Bup)	0.1 *M* HCl overnight at +56°C	L/L with ammonium phosphate buffer pH=8.4, and chloroform–2-propanol–heptane	HPLC on a CN analytical column using phosphate buffer pH 4–acetonitrile–heptane sulfonic acid as the mobile phase with a coulometric detection	Bup: 20–590 pg/mg NorBup: ND-150 pg/mg (*n*=14 in both cases)	Bup: 20 pg/mg NorBup: 10 pg/mg	[34]
Buprenorphine	Idem	Idem	HPLC–MS on a C_{18} analytical column and acetonitrile–2 m*M* formiate buffer pH=3 as mobile phase	Bup: 4–140 pg/mg NorBup: ND-67 pg/mg (*n*=6 in both cases)	Bup: 4 pg/mg NorBup: 2 pg/mg	[35]
Dextrometorphan	Methanol (1% HCl) overnight at +37°C	SPE	GC–MS	2.3–466.7 ng/mg (*n*=7)	NR[a]	[36]
Dextromoramide	0.1 *M* HCl overnight at +56°C	L/L with ammonium phosphate buffer pH=8.4, and chloroform–2-propanol–heptane	GC–MS	Three segments of the same case: 1.09, 1.48 and 1.93 ng/mg	0.1 ng/mg	[37]
Dextromoramide	Idem	SPE on C_{18} cartridges at pH=8.6	GC–MS	1.55 ng/mg (*n*=1)	0.1 ng/mg	[38]
Dextropropoxyphene (PPX) Norpropoxyphene (NPPX)	Acetate buffer pH=4, arylsulfatase, β-glucuronidase	L/L at pH=4, and hexane–3-methylbutanol	HPLC–PDA (λ=220 nm) on a C_{18} analytical column and acetonitrile–phosphate buffer pH =2.3 as the mobile phase	PPX: 1.2–26.4 ng/mg (*n*=24 but 12 subjects) NPPX: 2.6–71 ng/mg (*n*=24 but 12 subjects)	PPX: below 1 ng/mg NPPX: 1.5 ng/mg	[39]
Dextropropoxyphene Norpropoxyphene	0.1 *M* HCl overnight at +56°C	SPE on C_{18} cartridges at pH=11	GC–MS in SIM[b] mode after conversion of NPPX to NPPX amide at pH=11	PPX: 0.2–27.4 ng/mg NPPX: 0.3–68.9 ng/mg (*n*=13 for both analytes)	PPX: 0.05 ng/mg NPPX: 0.04 ng/mg	[40]
Dihydrocodeine	Methanolic sonication, 4 h at +40°C	No further purification	GC–MS after derivatization with pentafluoropropionic anhydride	Three segments of the same case: 1.9, 3.0 and 3.2 ng/mg	0.1 ng/mg	[41]
Dihydrocodeine	NaOH (30 g/l)	Extrelut® and toluene–butanol at pH=8.5 followed by back extraction with H_2SO_4	GC–MS in SIM mode after derivatization with heptafluorobutyric anhydride	1.2–31.2 ng/mg (*n*=28 but 13 subjects)	0.03 ng/mg	[42]
Ethylmorphine	0.1 *M* HCl overnight at +56°C	L/L with ammonium phosphate buffer pH= 8.4, and chloroform–2-propanol–heptane	GC–MS after derivatization with N,O-bis-trimethylsilyltrifluoroacetamide	Three samples of the same case. head hair: 0.12 ng/mg, pubic hair: 0.18 ng/mg and axillae hair: 0.08 ng/mg	NR	[43]
Fentanyl	Dilute HCl overnight at +56°C	Triple L/L with acidic back extraction and re-extraction in methylene chloride	GC–MS for identification and GC–NPD for quantification	20 pg/mg (*n*=1)	8 pg/mg	[44]
Fentanyl and Sufentanil	Söerensen buffer pH=7.6, 2 h at +40°C	SPE on C_{18} end capped cartridges	GC–MS–MS on a triple quadrupole in the positive chemical ionization mode	Fentanyl: 100 pg/mg (*n*=1) Sufentanyl: 5–10 pg/mg (*n*=1)	2–5 pg/mg	[45]
Nalbuphine	0.1 *M* HCL overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS in SIM mode after derivatization with N,O-bis-trimethylsilyltrifluoroacetamide	Two segments of the same case: 0.30 and 0.38 ng/mg	NR	[46]
Pentazocine	Söerensen buffer at pH=7.6, 2 h at +40°C	NR	GC–MS after derivatization with pentafluoropropionic anhydride	200 ng/mg (*n*=1)	NR	[47]
Pethidine	0.1 *M* HCl overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	1.9 ng/mg (*n*=1)	0.1 ng/mg	[48]

(Cont.)

Table 2. Continued.

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD	Ref.
Pholcodine	Acidic hydrolysis	L/L at pH=0–9	GC–MS after acetylation	NR	NR	[49]
Zipeprol	0.1 *M* HCL overnight at +56°C	Triple L/L with ammonium phosphate buffer pH=8.4, and chloroform–2-propanol–heptane followed by a back extraction process	GC–MS in SIM mode after derivatization with N,O-bis-trimethylsilyltrifluoroacetamide	7.34 and 33.1 ng/mg (n=2)	NR	[50, 51]

[a] NR: Not reported.
[b] SIM: Selected ion monitoring.

5.7. Cardiovascular drugs

Cardiovascular drugs like β-blocking agents were extracted from the hair with diethyl ether–dichloromethane after alkaline digestion with 1 *M* NaOH for 10 min at +100°C. The organic phase was subsequently purified with 0.1 *M* H_2SO_4 and analyzed by HPLC–UV onto a CN analytical column using water–acetonitrile–phosphate buffer pH=3 as the mobile phase. The detector was operated at λ=215 nm [76]. Measured concentrations in individuals were atenolol=0.9 ng/mg (1 case), betaxolol=1.2, 2.4 and 2.7 ng/mg (3 cases), propranolol=1.6 and 2.4 ng/mg (2 cases) and sotalol=4.4 and 5.3 ng/mg (2 cases).

Metoprolol was assayed by Kintz et al. [77] in the hair of a 34-year-old specialist in shooting. The reported concentration in hair was 8.41 ng/mg while the man alleged a chronic ingestion of 50 mg of the drug daily for a few months. The hair was incubated

Table 3
Methodologies and results published for anticonvulsants

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD	Ref.
Carbamazepine	Phosphate buffer pH=5.5, arylsulfatase, β-glucuronidase	L/L at pH=5.5 with hexane–diethylether–propanol	GC–MS	1.2–57.4 ng/mg (n=30)	0.1 ng/mg	[53]
Carbamazepine	0.1 *M* NaOH, overnight at +40°C	L/L with phosphate buffer at pH=7.6, and methyl-*tert*-butyl ether	HPLC–UV (λ=214 nm) on C_{18} analytical column and acetonitrile–methanol– water as the mobile phase	20.4–200.1 ng/mg (n=135 in 23 patients)	NR	[54]
Carbamazepine	2 m*M* NaOH, 15 min at +80°C, then 37% HCl for 20 min at +80°C	SPE on C_{18} cartridges at pH=9	Fluoroescence polarization immunoassay on the Abbott TDx® or GC	15.4–69.2 ng/mg by FPIA 13.9–66.3 ng/mg by GC (n=17 in both cases)	0.5 ng/mg (FPIA) NR or GC	[56]
Phenobarbital	Acidic buffer pH =2 during 10 min	Extrelut® and chloroform–isopropanol–heptane at pH=2	GC–MS	1.5–194.0 ng/mg (n=40)	0.2 ng/mg	[52]
Phenobarbital	Water, overnight at +56°C	SPE on C_{18} cartridges at pH=2	GC–MS	1.2 and 1.5 ng/mg (n=2)	0.1 ng/mg	[48]
Phenytoin (Carbamazepine)	1.5 *M* NaOH, overnight at +37°C	L/L with phosphate buffer at pH=7.6, and methyl-*tert*-butyl ether	HPLC–UV (λ=214 nm) on C_{18} analytical column and acetonitrile–methanol–water as the mobile phase	6.0–157.8 ng/mg for phenytoin (0.6–63.7 ng/mg for carbamazepine) (n=14 in both cases)	2 ng/mg phenytoin 1.3 ng/mg carbamazepine)	[55]

Table 4
Methodologies and results published for benzodiazepines and related compounds

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD	Ref.
Alprazolam	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	HPLC–PDA (λ=230 nm) on C_{18} analytical column and acetonitrile–phosphate buffer pH=3.8 as mobile phase	0.30 and 0.59 ng/mg (n=2)	0.1 ng/mg	[46, 48]
Alprazolam	1 *M* NaOH, overnight at +40°C	L/L with toluene–methylene chloride at pH=9	GC–MS–NCI after derivatization with *N,O*-bis-trimethylsilyltrifluoroacetamide	NR in human hair 60–100 pg/mg in rat hair	Limit of quantification: 25 pg/mg	[58]
Alprazolam Flunitrazepam Lorazepam Nordiazepam Oxazepam	Söerensen buffer pH=7.6, 2 h at +40°C	L/L at pH=7.6 with diethyl ether–chloroform	GC–MS–NCI after derivatization with *N,O*-bis-trimethylsilyltrifluoroacetamide	Alprazolam: 0.30–1.24 ng/mg (n=2) Flunitrazepam: 19–148 pg/mg (n=31) Lorazepam: 31–49 pg/mg (n=4) Nordiazepam: 0.20–18.87 ng/mg (n=42) Oxazepam: 0.10–0.50 ng/mg (n=14)	1 pg/mg 15 pg/mg 1 pg/mg 4 pg/mg 1 pg/mg	[59]
Diazepam 7-amino-flunitrazepam Lorazepam Lormetazepam Nordiazepam Oxazepam	Acetate buffer pH=4, arylsulfatase, β-glucuronidase	SPE on C_{18} end capped cartridges	GC–MS in SIM mode	Diazepam: 0.01–2.21 ng/mg (n=15) 7-amino-flunitrazempam: 0.02–9.50 ng/mg (n=8) Lorazepam: 4.91 ng/mg (n=1) Lormetazepam: 4.07–29.05 ng/mg (n=3) Nordiazepam: 0.13–1.83 ng/mg (n=20) Oxazepam: 0.20–3.44 ng/mg (n=15)	0.01 ng/mg 0.02 ng/mg 1 ng/mg 2 ng/mg 0.1 ng/mg 0.2 ng/mg	[60]
Flunitrazepam 7-amino-flunitrazepam	Söerensen buffer pH=7.6, 2 h at +40°C	L/L at pH=7.6 with diethyl ether–chloroform	GC–MS–NCI after derivatization with heptafluorobutyric anhydride	Flunitrazepam: 31–129 pg/mg (n=14) 7-amino-flunitrazepam: 3–161 pg/mg (n=26)	15 pg/mg 3 pg/mg	[61]
Flunitrazepam	Methanolic incubation, overnight	No further purification	GC–MS–MS–NCI without derivatization	NR	NR	[57]
Flunitrazepam	Söerensen buffer pH=7.6, 2 h at +40°C	SPE using a mixed-mode cartridge	Micro-plate enzyme immunoassay	0.1–1.0 ng/mg (n=4)	0.1 ng/mg	[62]
Flunitrazepam 7-amino-flunitrazepam	Methanolic sonication for 1 h followed by 0.1 m*M* HCl incubation, overnight at +50°C	SPE using a mixed-mode cartridge	GC–MS–NCI after derivatization with heptafluorobutyric anhydride	Flunitrazepam: ND and 23 pg/mg 7-amino-flunitrazepam: 26 and 48 pg/mg (n=2 in both cases)	NR	[63]
Lorazepam	Söerensen buffer pH=7.6, 2 h at +40°C	L/L at pH=7.6 with diethyl ether–chloroform	GC–MS–NCI after derivatization with *N,O*-bis-trimethylsilyltrifluoroacetamide	Three segments: 31, 40 and 49 pg/mg of the same case	1 pg/mg	[64]
Nordiazepam Oxazepam	Söerensen buffer pH=7.6, 2 h at +40°C	L/L at pH=7.6 with diethyl ether–chloroform	GC–MS–NCI after derivatization with *N,O*-bis-trimethylsilyltrifluoroacetamide	Nordiazepam: 0.25–18.87 ng/mg (n=13) Oxazepam: 0.11–0.50 ng/mg (n=5)	0.01 ng/mg 0.005 ng/mg	[65]
Zolpidem	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	2.2, 2.9 and 9.2 ng/mg (n=3)	0.1 ng/mg	[46]
Zopiclone	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	4.2 ng/mg (n=1)	NR	[46]

in mild acidic medium and extracted using SPE on C_{18} cartridges at pH=8.6 before a derivatization with trimethylboroxime and an analysis by GC–MS.

The determination of digoxin in hair was realized using a commercially available kit of a microparticulate enzyme immunoassay [78]. Hair was digested overnight at +40°C using protease–sodium dodecyl sulfate–dithiothreitol at pH=7.2. Digoxin concen-

Table 5
Methodologies and results published for antidepressants and stimulants

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD	Ref.
Amitriptyline Nortriptyline Imipramine Desipramine	1.5 *M* NaOH, overnight at +25°C	L/L with heptane and back extracted using 0.1 *M* HCl	GC–MS	Amitriptyline: 12.8 and 23.9 ng/mg (n=2) Nortriptyline: 11.2, 68.4 and 118.5 ng/mg (n=3) Imipramine: 16.5, 46.9 and 69.2 ng/mg (n=3) Desipramine: 16.5, 20.4 and 22.6 ng/mg (n=3)	NR	[66]
Amitriptyline	1 *M* NaOH for 30 min at +100°C	L/L with heptane–isoamylalcohol at pH =8.5	GC–MS	ND-17.21 ng/mg (n=30)	NR	[67]
Amitriptyline Clomipramine Doxepine Imipramine Maprotiline (and their nor-metabolites)	1 *M* NaOH for 30 min at +80°C	Extrelut® with ethyl acetate–ether	GC–MS after derivatization with pentafluoropropionic anhydride	Amitriptyline: 0.6–11.0 ng/mg Nortriptyline: 0.5–7.9 ng/mg (n=25 for both analytes) Clomipramine: 0.4–3.9 ng/mg Norclomipramine: ND-1.5 ng/mg (n=7 for both analytes) Doxepine: 1.0–3.0 ng/mg Nordoxepine: 0.5–2.1 ng/mg (n=6 for both analytes) Imipramine: 0.9–9.5 ng/mg Desipramine: 0.6–5.3 ng/mg (n=5 for both analytes) Maprotiline: 1.4–40.0 ng/mg (n=13)	Between 0.17 and 1.7 ng/mg	[68]
Amitriptyline Nortriptyline Imipramine Desipramine Dothiepin Northiaden	1 *M* NaOH for 30 min +70°C	L/L at pH=9.6 with hexane–butanol followed by a back extraction in 0.2% orthophosphoric acid	HPLC–UV (λ=214 nm) on a C_{18} analytical column with acetonitrile–phosphate buffer pH=7.0–diethylamine as the mobile phase	NR	NR	[69]
Clomipramine	1 *M* NaOH for 10 min at +100°C	L/L with ammonium phosphate buffer pH=8.4, and chloroform–2-propanol–heptane	GC–MS	0.37 and 0.79 ng/mg (n=2)	0.03–0.1 ng/mg	[70]
Fenfluramine	NaOH for 60 min at +100°C	L/L with ammonium phosphate buffer pH=8.4, and chloroform–2-propanol–heptane	GC–MS	14.1 ng/mg (n=1)	NR	[71]
Fluoxetine	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	4.3 ng/mg (n=1)	0.1 ng/mg	[48]
Moclobemide	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	4.0 ng/mg (n=1)	0.1 ng/mg	[48]

trations in the hair of 35 elderly patients who had been taking the drug for 1–5 years (60–250 μg/day) ranged from 3.6–11.4 pg/mg.

5.8. Anti-infection drugs

Using HPLC and fluorescence detection, oflox-

Table 6
Methodologies and results published for antipsychotics

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD	Ref.
Alimemazine	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	2.0 ng/mg (n=1)	NR	[48]
Chlorpromazine	2 *M* NaOH for 30 min at +80°C	L/L with hexane–isoamylalcohol	HPLC equipped with a coulometric detector Mobile phase was methanol–accetonitrile–phosphate buffer pH=6.8 on a C_{18} column	1.6–27.5 ng/mg (n=23) for oral dosage of 30–300 mg/day	0.5 ng/mg	[72]
Clozapine	NR	SPE	GC–MS in SIM mode	3.2 ng/mg (n=1)	NR	[73]
Cyamemazine	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	11.2 ng/mg	NR	[48]
Haloperidol (HL) (reduced haloperidol= RHL)	2 *M* NaOH for 30 min at +80°C	L/L with hexane–isoamylalcohol	HPLC equipped with a coulometric detector. Mobile phase was methanol–acetonitrile–phosphate buffer pH=6.8 on a C_{18} column	HL: 3.44–208.11 ng/mg RHL: 4.11–106.52 ng/mg (n=59 for both analytes)	Limit of quantification: 0.5 ng/mg	[74]
Haloperidol	1 *M* NaOH for 30 min at +70°C	L/L at pH=9.6 with hexane–butanol followed by a back extraction in 0.2% orthophosphoric acid	HPLC–UV (λ=214 nm) on a C_{18} analytical column with acetonitrile–phosphate buffer pH=7.0–diethylamine as the mobile phase	NR	NR	[69]
Haloperidol	0.1 *M* HCl, overnight	L/L	GC–MS in SIM mode	Fifty segments of the same case, concentrations were in the range 0.12–0.68 nmg/mg	NR	[75]
Hydroxyzine	0.1 *M* HCl, +56°C	SPE on C_{18} cartridges	GC–MS	8.34 ng/mg	NR	[46]
Promethazine	0.1 *M* HCl, overnight at at pH=8.6 +56°C	SPE on C_{18} cartridges	GC–MS	5.7 ng/mg	NR	[46]
Thioridazine Chlorpromazine	1 *M* NaOH for 30 min +70°C	L/L at pH=9.5 with butylchloride followed by a back extraction in 0.2% orthophosphoric acid	HPLC–UV (λ=255 and 265 nm) on a Phenyl analytical column column with acetonitrile–phosphate buffer pH=3.0 as the mobile phase	NR	NR	[69]
Tiapride	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	8.9 ng/mg (n=1)	NR	[48]

acin, an antimicrobial quinolones derivative was assayed in hair samples of 12 volunteers who had received the drug orally for 1 to 3 days [79]. HPLC quantitation was achieved on a C_{18} column using a mixture of phosphate buffer (pH=2.6)–acetonitrile as the mobile phase. A strong significant positive correlation was observed between the total dose and the concentration of ofloxacin in the analyzed hair segments. Measured concentrations were in the range 5–45 ng/mg. Temofloxacin and fluoroquinoline were similarly extracted with chloroform after preparation with 1 *M* NaOH for 30 min at +80°C [80,81]. Ofloxacin, norfloxacin and ciprofloxacin have also been analyzed while the purification step involved a

Table 7
Methodologies and results published for miscellaneous drugs

Drugs	Pretreatment	Extraction	Analytical method	Measured concentrations	LOD	Ref.
Amobarbital	1 *M* NaOH for 10 min at +100°C	L/L with chloroform–2-propanol–heptane under acidic conditions	GC–MS	31.4 and 41.6 ng/mg (n=2)	0.01–0.1 ng/mg	[70]
Chlorpyriphos Diazinon Diuron Endosulfan Fenoxicarb Folpel Vinchlozolone	Methanol, overnight at +45°C	No further purification	GC–MS and GC–MS–NCI	Chlorpyriphos: 7.97 ng/mg (n=1) Diazinon: 4.46 ng/mg (n=1) Diuron: 3.46–4.61 ng/mg (n=5) Endosulfan: 2.01 ng/mg (n=1) Fenoxicarb: 1.65 ng/mg (n=1) Folpel: 60.96 ng/mg (n=1) Vinchlozoline: 0.17 ng/mg (n=1)	NR	[88]
Cyclophosphamide	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	0.05 ng/mg (n=1)	NR	[46]
5-Fluorouracil	0.5 *M* phosphate buffer pH=8	L/L with ethyl acetate and purification on silica gel column	HPLC with fluoroescence derivatization of the molecule with 18-crown-6-ether and 4-bromomethyl–7-methoxycoumarin	0.01–2.125 ng/mg (n=33)	0.01 ng/mg	[86]
Ketamine (Norketamine)	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	Three segments of the same case: Ketamine: 8.17, 8.56 and 17.26 ng/mg Norketamine: 0.33, 0.57 and 2.07 ng/mg	0.1 ng/mg	[87]
Ketoprofen	Water, overnight at +56°C	SPE on C_{18} cartridges at pH=2	HPLC–PDA (λ=260 nm) on C_{18} analytical column and acetonitrile–phosphate buffer pH=3.8 as mobile phase	3.2 ng/mg (n=1)	NR	[48]
Lidocaine (Desetyl-lidocaine)	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	Lidocaine: 115.89 ng/mg Desetyl-lidocaine: 1.57 ng/mg (n=1)	0.15 ng/mg	[38]
Metoclopramide	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	HPLC–PDA (λ=260 nm) on C_{18} analytical column and acetonitrile–phosphate buffer pH=3.8 as mobile phase	0.51 ng/mg (n=1)	NR	[46]
Nefopam	0.1 *M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	6.4 ng/mg (n=1)	NR	[48]
Niflumic acid	Water, overnight at +56°C	SPE on C_{18} cartridges at pH=2	HPLC–PDA (λ=290 nm) on C_{18} analytical column and acetonitrile–phosphate buffer pH=3.8 as mobile phase	9.4 ng/mg (n=1)	NR	[48]
Paracetamol	0.1 m*M* HCl, overnight at +56°C	SPE on C_{18} cartridges at pH=8.6	GC–MS	3.0–5.1 ng/mg (n=2)	NR	[48]
Secobarbital	1 *M* NaOH for 10 min at +100°C	L/L with chloroform–2-propanol–heptane under acidic conditions	GC–MS	21.6–58.9 ng/mg (n=3)	0.03–0.1 ng/mg	[70]
Thiopental (Pentobarbital)	Water, overnight at +56°C	SPE on C_{18} cartridges at pH=2	HPLC–PDA (λ=215 and 285 nm) on C_{18} analytical column and acetonitrile–phosphate buffer pH=3.8 as mobile phase	Three segments of the same case: Thiopental: 4.85, 6.65 and 7.74 ng/mg Pentobarbital: 5.68, 7.38 and 17.06 ng/mg	0.1 ng/mg (0.3 ng/mg)	[87]
Thyroxin	Methanol, overnight at +45°C	No further purification or HPLC separation on a reversed-phase column and collection of the fractions	Radioimmunoassay (direct or on the collected fractions)	Thyroxin: 16.6–48.7 pg/mg in 12 hypothyroid infants	NR	[85]

sample clean-up procedure on C_{18} cartridges instead the previously described chloroformic liquid–liquid extraction [82,83].

Chloroquine and monodesethylchloroquine were determined in the hair of patients under antimalarial therapy [84]. Dissolution of the hair matrix was achieved using 60% KOH solution for 10 min at +100°C. Liquid–liquid extraction was thus conducted by adding diethyl ether. The residue was then subjected to thin layer chromatography using ethyl acetate–isopropanol–20% ammonia as the eluent. The fluorescent spots (λ=350 nm) were removed and assayed by GC–MS–NCI. GC equipped with a nitrogen-phosphorus detector performed quantification. In two patients taking a daily oral dosage of 100 mg of chloroquine, the measured concentrations were 156 and 333 ng/mg for chloroquine, and 11 and 23 ng/mg for its monodesethyl metabolite, respectively.

5.9. Miscellaneous

Many other drugs have been assayed for clinical application in forensic science, or even most recently, for occupational medicine or doping control.

As a tool to investigate a presumed maternal thyroxin supply to the congenital hypothyroid fetus, Tagliaro et al. [85] have evaluated the use of thyroxin measurement in the hair of newborns. Determination of 5-fluorouracil in scalp hair was investigated as a possible index of patient compliance when under chemotherapy [86].

As an example in forensic sciences, we reported on a polydrug use developed by the concomitant consumption of heroin, cocaine, cannabis, thiopental, ketamine and chloroform [87]. We have also described an unusual case where the victim ingested two morphinomimetics (methadone and dextromoramide) as well as cocaine, and surprisingly, lidocaine. The presence of lidocaine was explained since the victim, who could not tolerate pain in any form, had performed local infiltration of the drug before episodes of self-mutilation [38]. Many other cases are described in the literature.

Concerning occupational medicine, Cirimele has demonstrated the interest of hair analysis through the identification of several pesticides of exposed farmers or vine growers [88]. Dauberschmidt et al. [89], in a preliminary study, have focused their attention to DDT, PCBs and lindane concentration in hair of people exposed to environmental pollutants.

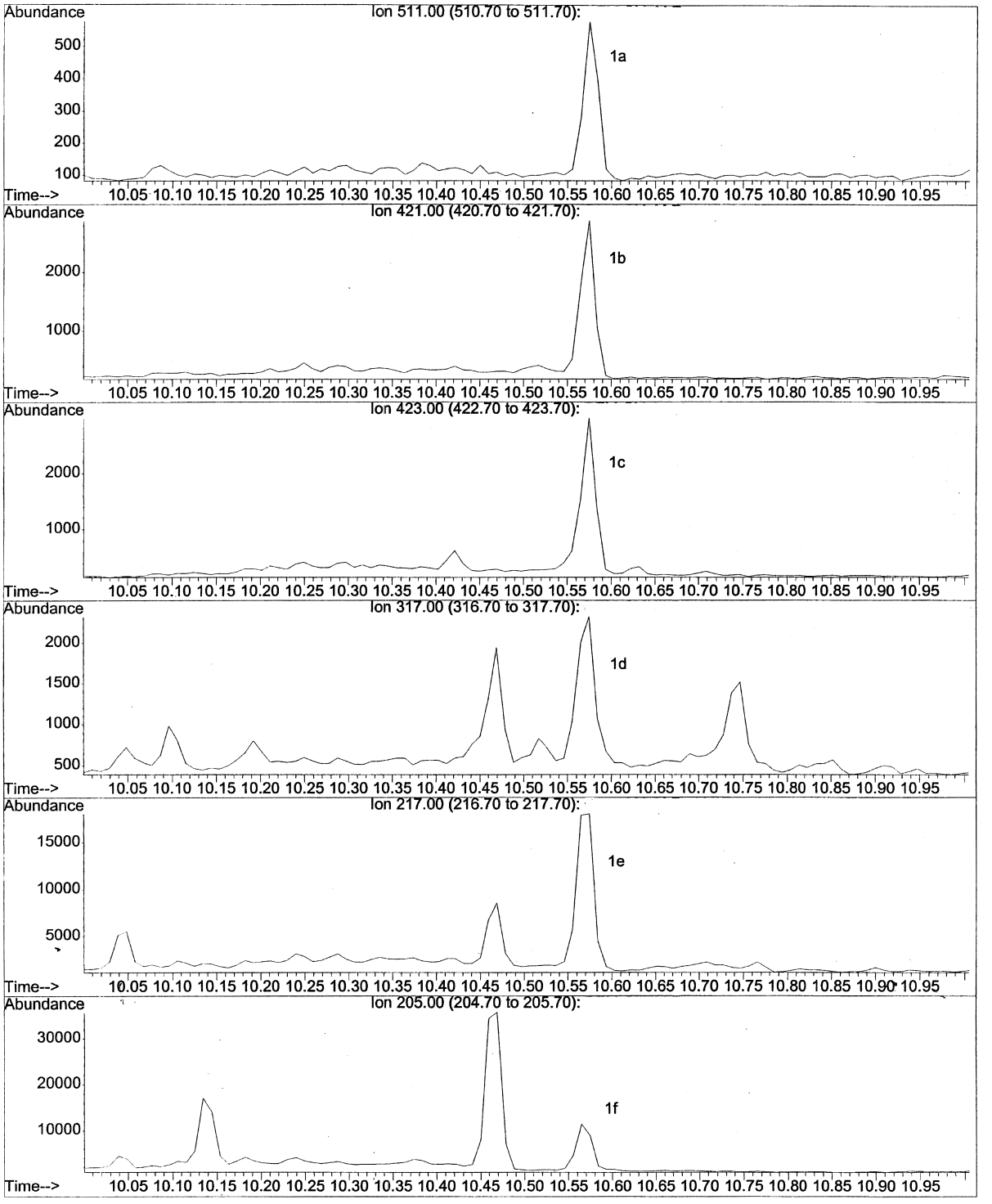
The different drugs found in hair using the procedures are listed in Table 7.

Lastly, hair analysis would be a promising tool for testing for banned substances in sports. International literature is very new about this subject but though young, the methodology has already proved to be extremely powerful. Kintz et al. [77,90,91], Gaillard et al. [27,92], and Thieme et al. [93] have demonstrated the interest of the method on various molecules including anabolic steroids, corticosteroids and β-agonists. The review of such compounds however is outside the scope of the present paper.

5.10. Screening procedure

The sole screening procedure that has been described to date is that of Gaillard and Pépin [48], who published a method to screen for a wide range of pharmaceuticals in hair. Fifty to one hundred mg of powdered hair are incubated overnight at +56°C in water (acidic compounds) or 0.1 *M* HCl (neutral and basic compounds). A twin SPE on C_{18} cartridges is used for sample clean-up procedure. Acidic drugs are fixed at pH=2.0 and eluted with 1% ammoniacal methanol while neutral and basic drugs are retained on the column at pH=8.6 and eluted with 0.5% acetic acid in methanol [48]. Separation and identification of the drugs was performed using both GC–MS and HPLC–PDA techniques. The HPLC system

Fig. 1. Chromatogram of an extract of 50 mg a hair from a case of chloralose homicide. Peak: 1=chloralose–triTMS measured at 0.31 ng/mg. (a) m/z=511; (b) m/z=421; (c) m/z=423; (d) m/z=317; (e) m/z=217; (f) m/z=205. The gas chromatograph was a 6890 from Hewlett Packard (Les Ulis, France), a HP 6890 automatic injector and equipped with the HP 5973 mass selective detector. Analytical column was a CP SIL 8 CB, 30 m length, I.D. 0.25 mm (0.25 μm film thickness) from Chrompack (Les Ulis, France). Helium was used as the carrier gas at a flow-rate of 1.3 ml/min in the constant flow mode (i.e. 101.5 kPa at 50°C). Temperatures were: interface=300°C, ion source=230°C, quadrupole=106°C. Pulsed splitless injection was done at 290°C and 200 kPa during 1 min. The initial oven temperature was 50°C for 2 min and was increased to 310°C at 15°C/min and held for 3.67 min. The chromatographic run time was 23 min. Dwell time per ion was set at 75 ms. Derivatization was performed with Tri-Sil©/BSA in dimethylformamide at +80°C during 20 min.


Abundance
Ion 511.00 (510.70 to 511.70):
500
400
300
200
100
1a
Time-->
10.05 10.10 10.15 10.20 10.25 10.30 10.35 10.40 10.45 10.50 10.55 10.60 10.65 10.70 10.75 10.80 10.85 10.90 10.95
Abundance
Ion 421.00 (420.70 to 421.70):
2000
1000
1b
Time-->
10.05 10.10 10.15 10.20 10.25 10.30 10.35 10.40 10.45 10.50 10.55 10.60 10.65 10.70 10.75 10.80 10.85 10.90 10.95
Abundance
Ion 423.00 (422.70 to 423.70):
2000
1000
1c
Time-->
10.05 10.10 10.15 10.20 10.25 10.30 10.35 10.40 10.45 10.50 10.55 10.60 10.65 10.70 10.75 10.80 10.85 10.90 10.95
Abundance
Ion 317.00 (316.70 to 317.70):
2000
1500
1000
500
1d
Time-->
10.05 10.10 10.15 10.20 10.25 10.30 10.35 10.40 10.45 10.50 10.55 10.60 10.65 10.70 10.75 10.80 10.85 10.90 10.95
Abundance
Ion 217.00 (216.70 to 217.70):
15000
10000
5000
1e
Time-->
10.05 10.10 10.15 10.20 10.25 10.30 10.35 10.40 10.45 10.50 10.55 10.60 10.65 10.70 10.75 10.80 10.85 10.90 10.95
Abundance
Ion 205.00 (204.70 to 205.70):
30000
20000
10000
1f
Time-->
10.05 10.10 10.15 10.20 10.25 10.30 10.35 10.40 10.45 10.50 10.55 10.60 10.65 10.70 10.75 10.80 10.85 10.90 10.95

gives an elution of the drugs following a gradient elution mode from a C_8 analytical column with acetonitrile–phosphate buffer pH=3.8 [94,95]. The drugs investigated in this study, 84 molecules belonging to more than 15 pharmacological classes, were selected so as to represent various characteristics and classes, as well as to cover a wide range of GC and HPLC chromatographic behaviors. Extraction of the drugs can be divided into two groups considering the pK_a values of the compounds. Neutral drugs, however, could be successfully extracted by both systems (oxazepam, nordiazepam, lidocaine, meprobamate, diclofenac and etodolac for example). The same distinction could be made between the drugs that are exclusively analyzed by one of the two chromatographic system: meprobamate by GC because of the absence of useful UV absorption of the drug, and acenocoumarol by HPLC–PDA because the drug is a non-volatile anticoagulant.

6. Applications

6.1. Forensic case 1

Most of the time, the presence of psychotropic drugs in the blood is detrimental to the necessary vigilance that driving an automobile requires. In this case, conversely, the absence of medicine could explain part of the behavior and consequently, the accident.

A 33-year-old automobile driver took a highway the wrong way. He very rapidly caused an accident in which he lost his life, caused the death of another and gravely injured two other victims. At the site of the accident a sample of his blood and hair was taken. In his blood, no toxin (medicine, alcohol or drugs of abuse) was found. However, the analysis of a 4.5 cm portion of hair showed the presence of the following molecules: haloperidol=5.41 ng/mg, cyamemazine=0.59 ng/mg, cyamemazine sulfoxide=1.78 ng/mg, carbamazepine=6.36 ng/mg. The police investigation later showed that this man was under psychiatric treatment. According to his immediate family, he had stopped taking his medication a few days before the occurrence, which was well proven by the comparison with the analyses of his blood and hair. It was likely that this accident resulted from a decompensation during the time that the antipsychotic treatment was discontinued.

6.2. Forensic case 2

An 8-year-old boy was admitted to the emergency care unit in a deep coma. He rapidly recovered after adequate treatment. Toxicological analysis realized in our laboratory revealed the presence of alpha-chloralose in urine and blood of the young child, a rodenticide also used as a narcotic agent to control avian pests [96]. The mother will later confess that she has given him the drug under the form of capsules of paracetamol previously emptied of their contents. Her husband died 4 years ago of an unexplained manner but no autopsy was requested at that time. In the light of the present case, the attorney thus ordered an exhumation of the corpse. A detailed toxicological analysis showed the presence of chloralose in the liver at a concentration of 0.68 μg/g and in hair at 0.31 ng/mg. Fig. 1 displays coincident ions at m/z 511, 421, 423, 317, 217 and 205 of α-chloralose-tri-TMS of an extract of 50 mg of the deceased man's hair.

7. Conclusion

Hair analysis is certainly one of the most powerful methods for epidemiological purposes (drug prevalence in a population) and for investigating past exposure to drugs (licit or not) by individuals. Its application in therapeutic drug monitoring has proved to be not very applicable except as an all-or-nothing compliance test. In forensic science however, it is a matrix of major interest, since numerous published cases have been resolved by this mean, notably the establishment of homicide by poisoning in exhumed corpses. Indeed, some basic physiological and analytical problems still remain not satisfactorily revolved. The passive contamination of the sample, the effects of cosmetic treatments, the distribution of the drug along the hair stalk, the dose-to-concentration correlation, are as many problems that makes a result to be interpreted with great attention and caution. The methodology however, is perfectly valid. A very recent increase in the international literature of papers dealing with doping tests in

sports or with the surveillance of banned substances (anabolic steroids, β-agonists, antibiotics, PCBs, lindane) in meat producing animals shows well the interest of this matrix.

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