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Review

Testing for drugs in hair
Critical review of chromatographic procedures since 1992

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Abstract

Up to now, more than 50 pharmaceuticals or drugs of abuse have been reported to be detectable in hair after oral or parenteral administration. The present paper reviews the literature devoted to drug testing in hair that has been published since 1992. Procedures for the detection of opiates, cocaine, amphetamines and cannabis in hair are described in detail. In particular, the papers on benzodiazepines show an increasing number of procedures using negative chemical ionisation with GC–MS and diode array detection with HPLC in hair analysis. For the most important benzodiazepines, diazepam and flunitrazepam, reliable methods now exist. On the other hand, the problem of the detecting tetrahydrocannabinol metabolites using different techniques is not yet solved. Some progress is observed in the detection of low dose drugs, like fentanyl and its derivatives or LSD. For most of the analyses using chromatographic techniques, the main data on sample preparation and analytical determinations are listed. Some new findings, based on the experience of the authors, are also added.

Keywords: Reviews; Hair analysis; Opiates; Cocaine; Cannabis; Amphetamines; Benzodiazepines; Hallucinogenics; LSD; Barbiturates; Antiepileptics; Antidepressants; Neuroleptics

Contents

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1. Introduction

At present, hair analysis is routinely used as a tool for detection of drug use in forensic science, traffic medicine, occupational medicine and clinical toxicology. The scientific community has expressed concerns about the role of hair drug testing in toxicological applications. The Society of Forensic Toxicologists (SOFT) and the Society of Hair Testing (SHT) have published consensus opinions, pointing out several deficiencies in the state of knowledge on drug incorporation and their detection in hair.

A previous review published by Moeller in 1992 [1] showed that most of the procedures for the detection of drugs in hair were based on gas chromatography–mass spectrometry (GC–MS). In the following years, GC–MS remained the most common analytical technique, but a great variety of extraction procedures have been published. However, comparisons have shown that these different procedures led to similar results at least for the common illegal drugs. In nearly all the studies GC–MS in the electron impact (EI) mode was used, but in special cases, in which sensitivity was crucial, chemical ionization (CI) and negative chemical ionization (NCI) or even GC–MS–MS where adopted.

In recent years, few articles have been published using radioimmunoassays (RIA), e.g. the study of Magura et al. [2] concerning cocaine abuse, and the examination of fentanyl in hair by Wang et al. [3]. For the determination of benzodiazepines, high-performance liquid chromatography (HPLC) combined with a diode array detector (DAD) is becoming more and more important. Supercritical fluid extraction has been introduced as a new extraction method and capillary electrophoresis (CE) is becoming more and more popular for detecting illegal drugs in hair.

Research dealing with contamination, and the influence of cosmetic treatments on the drug concentration in hair has also been carried out in recent years.

2. Opioids

The development of the methods used for opiates in the past 5 years is combined with the development of screening methods for opiates, cocaine, cannabinoids, and amphetamine (including its derivatives) simultaneously. Three methods dominate the literature, as is briefly described in Table 1: liquid–liquid extraction (LLE) after HCl-hydrolysis introduced by Kintz and Mangin [4] and solid-phase extraction (SPE) [5] after enzymatic hydrolysis with β-glucuronidase/sulfatase led to similar results, both with the disadvantage, that heroin and 6-O-acetylmorphine (MAM) might be hydrolyzed to morphine. Methanol extraction and direct detection with GC–MS was known since the early 1990s, but was not published in detail until 1996 by Kauert and Röhrich [6]. It is undoubtedly the simplest method with high sensitivity for heroin, cocaine, and tetrahydrocannabinol (THC), but poor sensitivity for their metabolites morphine, benzoylecgonine and THC-COOH. In 1995, Rothe and Pragst confirmed by systematical extraction studies that methanol and water had the best extraction capability for opiates, while with hydrophobic solvents like dioxane and acetonitrile, a low extraction rate was found [7].

The range of MAM concentrations in hair determined using the described procedures is listed in Table 2.

A novel extraction procedure for the determination of opiates in hair was developed by Edder et al. [9] using supercritical CO_2. The addition of polar modifiers like H_2O, methanol and triethylamine led to a subcritical fluid with high extraction efficiency. Up to now the method has been evaluated for the simultaneous extraction of heroin, MAM, morphine and methadone. The fast speed of extraction (30 min) is an advantage, but unfortunately the instrument costs are high compared with SPE or LLE. Conversely, only small amounts of nonhalogenated organic solvents are needed, causing little environmental pollution.

Unusual methods used for the determination of opiates in hair are the investigation of cross-sectional, laterally microtomed hair by Fourier transform infrared spectroscopy (FT-IR) and CE. On the basis of the results of Kalasinsky et al. [10] FT-IR seems to allow more detailed investigation of drug incorporation into hair. These results could not be confirmed, because it is very difficult to achieve the needed sensitivity with this instrumentation.

A completely different approach based on CE was used for the determination of morphine and cocaine

Table 1
Screening procedures for the detection of illegal drugs in hair

Reference	Kauert and Röhrich [6]	Moeller et al. [5]	Kintz and Mangin [4]
Analytes	Heroin, 6-MAM, dihydrocodeine, codeine, methadone, THC, cocaine, amphetamine, MDMA, MDEA, MDA	Heroin, 6-MAM, dihydrocodeine, codeine, methadone, THC, cocaine, amphetamine, MDMA, MDEA, MDA	Heroin, 6-MAM, dihydrocodeine, codeine, methadone, THC, cocaine, amphetamine, MDMA, MDEA, MDA
Decontamination step	Ultrasonic bath 5 min each 5 ml H_2O, 5 ml acetone, 5 ml petrolether	20 ml H_2O (2×) 20 ml acetone	5 ml Cl_2CH_2 (2×5 min)
Homogenization	100 mg hair cut into small sections in a 30-ml vial	Ball mill	Ball mill
Extraction	4 ml methanol ultrasonic bath 5 h, 50°C	20–30 mg powdered hair, 2 ml acetate buffer+β-glucuronidase/aryl-sulfatase, 90 min/40°C	50 mg powdered hair, 1 ml 0.1 *M* HCl, 16 h/56°C
Clean-up	None	$NaHCO_3$; SPE (C_{18}), elution with 2 ml acetone–CH_2Cl_2 (3:1, v/v)	$(NH_4)_2HPO_4$; extraction 10 ml $CHCl_3$–2-propanol–*n*-heptane (50:17:33, v/v); organic phase purified with 0.2 *M* HCl; HCl phase to pH 8.4; re-extraction with $CHCl_3$
Derivatization	Propionic acid anhydride	1000 μl PFPA–75 μl PF-*n*-propanol; 30 min/60°C; N_2/60°C; 50 μl ethyl acetate	40 μl BSTFA–1% TMS; 20 min, 70°C
GC conditions	Column: 20 m×0.25 mm×0.25 μm methyl silicone; inj. temp.: 280°C; temp. prog. 140°C, 20°C/min to 300°C, 8 min	Column: 12 m×0.2 mm×0.33 μm phenyl methyl silicone; inj. temp.: 260°C; temp. prog. 70°C, 30°C/min to 155°C, 10°C/min to 240, 1 min	
MS conditions	EI 70 eV; SIM at *m/z* 297, 313, 370 (THC) 82, 182, 303 (cocaine) 268, 310, 327, 341, 369, 383, 397 (opiates) 235, 250 (methaqualone)	EI 70 eV; SIM at *m/z* 118, 190 (amphetamines) 421, 300, 182, 303 (BZE and cocaine) 282, 284, 390, 414, 444, 447, 473, 577 (opiates)	

in hair, but the method introduced by Tagliaro et al. [11] is not yet suitable for general screening. The sensitivity for THC is very low, not to speak of carboxy-THC.

Pötsch et al. [12] found a decrease of opiates — even down to zero — after cosmetic treatment and UV irradiation in in vitro experiments. Jurado et al. [13] also found a big decrease of drug concentrations in some cases examining parallel strands — colored and noncolored, bleached and nonbleached — of drug user's hair, but a decrease to zero was only observed in a case with heavily damaged hair.

Table 2
Published concentration ranges of 6-O-acetylmorphine in the hair of heroin users

Reference	Concentration range 6-MAM (ng/mg)
Kauert and Röhrich [6]	0.03–79.8
Kintz and Mangin [4]	0–84.3
Moeller et al. [5]	2.0–74
Pepin and Gaillard [8]	0.3–131.2

Pubic and axillary hair showed higher drug levels than scalp hair [14,15], which can be due to the lower rate of growth of pubic hair. Pubic and scalp hair have very different telogen–anagen ratios and consequently drug concentrations cannot be directly compared. Because of individual differences in rate of growth [16] and telogen–anagen ratios [17], dose–concentration correlation studies should only be performed on hair samples grown from the shaved skin before drug administration and under control of the hair growth rate.

Understandably, there were no studies on dose–

Table 3
Extraction of opioids from human hair

Substance	Extraction	Reference
Buprenorphine	0.1 *M* HCl (12 h, 56°C); toluene	[20]
Dextromoramide	0.1 *M* HCl (12 h, 56°C); CPH	[21]
Dihydrocodeine	NaOH (30 g/l) neutralized with HCl; SPE	[22]
Ethylmorphine	0.1 *M* HCl (12 h, 56°C); CPH	[23]
Fentanyl	MeOH (12 h, 40°C)	[24]
Sufentanil	MeOH (ultrasonic bath 5 h/50°C)	[24]
Pentazocine	Söerensen buffer, pH 7.4	[25]
Zipeprol	0.1 *M* HCl (12 h, 56°C); CPH	[26]

concentration correlation of heroin, MAM and morphine in the hair of humans. In a study in patients with painful syndromes using high amounts (30–4000 mg/day) only an intra-individual correlation could be found [18].

The problem of false positive urine analyses for opiates (morphine) due to ingestion of poppy seeds could by solved by examining hair for morphine. Goldberger [19] did not find any morphine after normal poppy seed consumption and Sachs [18] found only traces (<0.2 ng/mg) of morphine after a consumption of as much as 250 g of poppy seed in 3 days.

While morphine and codeine have been analyzed in hair since the start of this methodology, others like methadone were only investigated later (see Table 3). Recent studies on methadone [27] and meprobamate [28] showed a dose–concentration correlation, independently of individual hair growth and telogen–anagen ratio.

A controlled study with 450 mg codeine given to seven subjects showed substantially greater concentrations in proximal than in distal segments; morphine could not be detected [29].

3. Cocaine

The literature concerning cocaine up to 1994 was reviewed by Selavka and Rieders [30]. The fact that consumption of the drug leads to higher concentrations of the parent drug than of the metabolite benzoylecgonine is well known since 1991. In Table 1, some routine analytical methods for cocaine are included, but one should be additionally mentioned [31]. The hair sample is cut into approximately 1-mm segments. After a washing procedure with methanol the specimens are incubated overnight at 37°C in 0.05 *M* sulfuric acid. The acid extracts are neutralized with 1.0 *M* NaOH and then pH is adjusted to 4.0 with 1 ml sodium acetate. SPE extraction with methylene chloride–2-propanol (8:2, v/v) containing 2% ammonium hydroxide is followed by evaporation and derivatization with BSTFA (with 1% TMCS). Cocaine, benzyolecgonine, ecgonine methyl ester, norcocaine, cocaethylene and norcocaethylene are quantified in the same run. Typical concentration ranges are listed in Table 4.

Unlike heroin, cocaine consumption can be detected by measurable metabolites which cannot be caused by cocaine contamination. Table 5 shows the

Table 4
Concentration ranges of cocaine in the hair of cocaine users

Reference	Concentration range cocaine (ng/mg)
Kauert and Röhrich [6]	0.04–129.7
Kintz and Mangin [4]	0.4–78.4
Moeller et al. [5]	0.3–127.0
Pepin and Gaillard [7]	0.89–242.0

Table 5
Concentrations ranges of cocaine and metabolites [31]

Analyte	Concentration range (ng/mg)
Cocaine	6.4–19.2
Benzyolecgonine	0.3–2.5
Ecgonine methylester	0–1.9
Norcocaine	Traces to 0.7
Cocaethylene	0–2.6
Norcocaethylene	0 or traces

concentration of cocaine and cocaine metabolites in hair reported by Cone et al. [31].

The determination of the pyrolysis product of cocaine, the androhydroecgonine methyl ester (AEME) was reported to be helpful in distinguishing between cocaine and crack users. Kintz et al. [32] found AEME in a range of 0.2–2.4 ng/mg in hair from seven crack users.

Literature and scientific debates on cocaine in hair are dominated by discussions on the effectiveness of decontamination procedures and a possible racial bias. These issues are important when hair analysis is used as stand-alone evidence, such as for workplace drug testing. Baumgartner and Hill [33] proposed a washing procedure which reportedly removes external contamination but maintains drugs unchanged in an 'inaccessible domain' which could only be reached by enzymatical dissolution of the matrix. He argues that the drug found in this area can have only been incorporated by consumption, when it exceeds a certain value, the used cut-off value.

According to the report of Kidwell and Blank [34], heavy contamination (with solutions of 1 μg/ml and more) cannot be eliminated even by intensive washing, as used in the procedures in Table 1. The authors state that after contamination of the hair, small amounts will penetrate into the hair matrix. Because of normal hygiene treatments the external contamination could then be washed away, but not the small amounts which have passed the cuticula. Thus, the analysis of those samples will lead to positive hair tests. Smith found cocaine, even after several washings, in the hair of young children living with cocaine using parents. He assumed that they could not be drug users [37].

Conversely, Koren et al. stated in 1992 [35] that after normal contamination by sitting in the same room with crack smoking persons cocaine is present in hair samples, but it can be washed out. Also, Mieczkowski [36] did not find cocaine in the hair of narcotic officers who reported relatively frequent handling of cocaine.

An important study on disposition of cocaine-d_5 was published by Henderson et al. in 1996 [38]. The deuterium labeled cocaine was administered intravenously and/or intranasally in doses of 0.6–4.2 mg/kg under controlled conditions. A single dose could be detected for 2–6 months, the minimum detectable dose appeared to be between 22 and 35 mg, but within the range of the doses used in the study, the hair test did not provide an accurate record of either the amount, time, or duration of drug use.

Cocaine, benzoylecgonine and ecgonine methylester were also found in the hair of mummies of ancient Peruvian coca leaf chewers. In contrast to today's cocaine users, the cocaine–benzoylecgonine ratio was less than 1 [39]. Whether cocaine was really known in the ancient Egypt, remains unclear. Balabanova et al. [40] found cocaine and/or benzoylecgonine in the hair of Egyptian mummies. This could not be confirmed by other researchers and was disputed by anthropologists.

Unlike previous studies, a time course experiment for cocaine in rabbit hair led to high concentrations even after the first day, which decreased to zero after 10 days [41].

4. Cannabis

Simultaneously, Cirimele et al. [42] and Jurado et al. [43] reported the first results by using GC–MS. Both determined in the same run Δ9-tetrahydrocannabinol (THC) and its major metabolite 11-nor-Δ9-THC carboxylic acid (THC-COOH). The first procedure was specifically devoted to cannabis, while the second was included in a general screening for opiates, cocaine and cannabis. As the measured concentrations were low, particularly in comparison with other drugs, some authors suggested the use of NCI to target the drugs [44,45] or the application of MS–MS [46,47]. More recently, Cirimele et al. [48] developed a simpler method, based on the simultaneous identification of cannabinol (CBN), cannabidiol (CBD) and THC. This procedure appears to be a screening method that is rapid, economic and does not require derivatization prior to analysis. Fig. 1 shows a typical chromatogram obtained with this method. As THC, CBD and CBN are present in smoke, to avoid potential external contamination, THC-COOH, the endogenous metabolite should be secondly tested to confirm drug use.

After decontamination with various mixtures (organic solvents, aqueous solvents, alone or in combination), the hair specimens are generally hydrolyzed in a strongly alkaline medium to obtain complete

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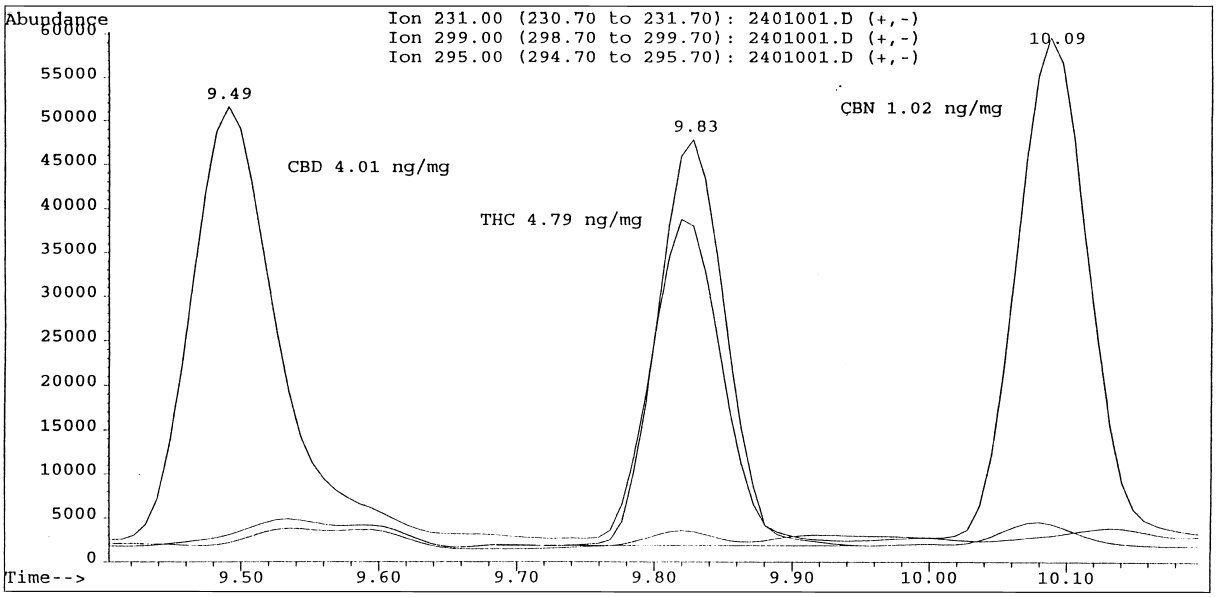

Fig. 1. Chromatogram obtained after extraction of a hair sample from a cannabis user. Concentrations measured were: 1.02, 4.01, and 4.79 ng/mg for cannabinol (CBN), cannabidiol (CBD), and THC, respectively.

dissolution of the matrix. However, extraction of THC was also proposed using methanol sonication [6] or supercritical fluid extraction [49].

The analytical procedures that appear in the literature are summarized in Table 6. As mentioned by Cirimele et al. [42], acid or enzymatic hydrolyses were inefficient in removing the target compounds. Silylated derivatives induced peaks interfering with THC.

As reported in Table 7, the concentrations of

Table 6
Analytical procedures for cannabis testing in hair

Ref.	Compound	Hydrolysis	Extraction	Derivatization	Analytical system	LOD (ng/mg)
[42]	THC/THC-COOH	1 *M* NaOH	L–L	PFPA/PFPOH	GC–MS-EI	0.1
[43]	THC/THC-COOH	11.8 *M* KOH	L–L	HFBA/HFPOH	GC–MS-EI	0.01
[44]	THC/11-OH-THC/THC-COOH	1 *M* NaOH	L–L	TFAA+MeOH–BF_3	GC–MS-NCI	0.01–0.25
[45]	THC-COOH	1 *M* NaOH	L–L	PFPA/PFPOH	GC–MS-NCI	0.005
[46]	THC-COOH	10 *M* KOH	SPE	PFPA/HFPOH	GC–MS–MS	<0.0002
[47]	THC-COOH	10 *M* NaOH	L–L	HFBA/HFPOH	GC–MS–MS	0.00002
[48]	CBN, CBD, THC	1 *M* NaOH	L–L	–	GC–MS-EI	0.01–0.1
[6]	THC	MeOH	–	PSA	GC–MS-EI	0.1

Abbreviations: L–L: liquid–liquid extraction; PFPA: pentafluoropropionic acid anhydride; PFPOH: pentafluoropropanol; HFBA: heptafluorobutyric acid anhydride; HFPOH: hexafluoropropanol; TFAA: trifluoroacetic acid anhydride; SPE: solid-phase extraction; PSA: propionic acid anhydride.

Table 7
Reported concentrations of cannabis in hair

Reference	Compound	Number of positives	Concentration (ng/mg)
[43]	THC	298	0.06–7.63 (0.97)
	THC-COOH	298	0.06–3.87 (0.50)
[44]	THC	8	0.03–1.1
[47]	THC-COOH	>3000	(0.0007)
[6]	THC	104	0.009–16.70 (1.501)
[50]	THC	89	0.10–3.39 (0.64)
	CBD	306	0.03–3.00 (0.51)
	CBN	268	0.01–1.07 (0.16)
	THC-COOH	267	0.05–0.39 (0.10)
[25]	THC	102	0.4–6.2 (2.0)
	THC-COOH		1.7–5.0 (3.3)

Average concentrations in brackets.

cannabis measured in hair are very low, particularly for THC-COOH, which was seldom identified. To date, there is no consensus on positive cut-off values for cannabis. An international debate is needed to discuss the differences noticed between American laboratories, that reported THC-COOH in the low pg/mg range and some European laboratories, that mentioned concentrations in the low ng/mg range.

5. Amphetamine derivatives

Almost all the literature dealing with amphetamines in hair has come from Japanese researchers. In most cases, amphetamine (AP) and methamphetamine (MA) have been the target drugs. More recently, particular attention has been focused on methylenedioxy-amphetamine derivatives, like methylenedioxymethamphetamine (MDMA). The screening procedures listed in Table 1 are also used for amphetamine and its derivatives [4–6,51]. In 1995, Nakahara [53] published an excellent review on the detection of amphetamines in hair. Most techniques published before 1990 used acid or alkaline hydrolysis, or a combination of hydrochloric acid and methanol, followed by a purification step (LLE or SPE) and derivatization with trifluoroacetic anhydride (TFA).

A screening procedure for these compounds was developed by Röhrich and Kauert [52]. It allows the simultaneous determination of AP, methylenedioxyamphetamine (MDA), MDMA and methylene-dioxyethylamphetamine (MDEA), based on methanol sonication of 50–200 mg of hair for 5 h at 50°C in the presence of methaqualone, used as internal standard. According to the authors, the derivatization with TFA induces a more specific mass spectrometric information, but TFA-derivatives are less stable than the derivatives obtained with propionic acid anhydride (PSA). Compounds are identified by GC–MS-EI. The detection limit for all compounds was in the range of ~0.01 ng/mg, using 50–100 mg of hair for analysis, independently of the derivatization procedure applied. A total of 303 hair samples were tested, and 28 (9.2%) contained amphetamine derivatives, in the 0.02–6.52 ng/mg range.

When comparing four different procedures for AP, MDA and MDMA (methanol sonication, acid hydrolysis, alkaline hydrolysis and enzymatic hydrolysis) Kintz and Cirimele [54] demonstrated that best recoveries were observed after alkaline hydrolysis. However, it was not possible to determine which method performed best, based on recoveries, precision and practicability. Lower concentrations were observed after methanol sonication together with 'dirty' chromatograms.

Recently, some minor modifications (inclusion of MDEA and N-methyl-benzodioxazolylbutanamine and change of the derivatization step) of a previously described procedure [51] allowed a complete screening [55] for AP, MA, MDA, MDMA, MDEA, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) and its metabolite, benzodioxazolyl butanamine

(BDB). Fig. 2 is a typical chromatogram obtained from a stimulants abuser. Briefly, after decontamination with dichloromethane, a 50-mg specimen was hydrolyzed with 1 ml 1 *M* NaOH in presence of the corresponding deuterated internal standards (one for each drug). After extraction with ethyl acetate, and evaporation to dryness in the presence of 2-propanol–HCl (99:1, v/v), the target compounds were derivatized with heptafluorobutyric acid anhydride (HFBA). Analytical parameters and results are presented in Table 8. Linearity was tested over the range 0.2–100.0 ng/mg. Limits of detection were in the range 0.02–0.05 ng/mg, with recoveries in the range 82–91%.

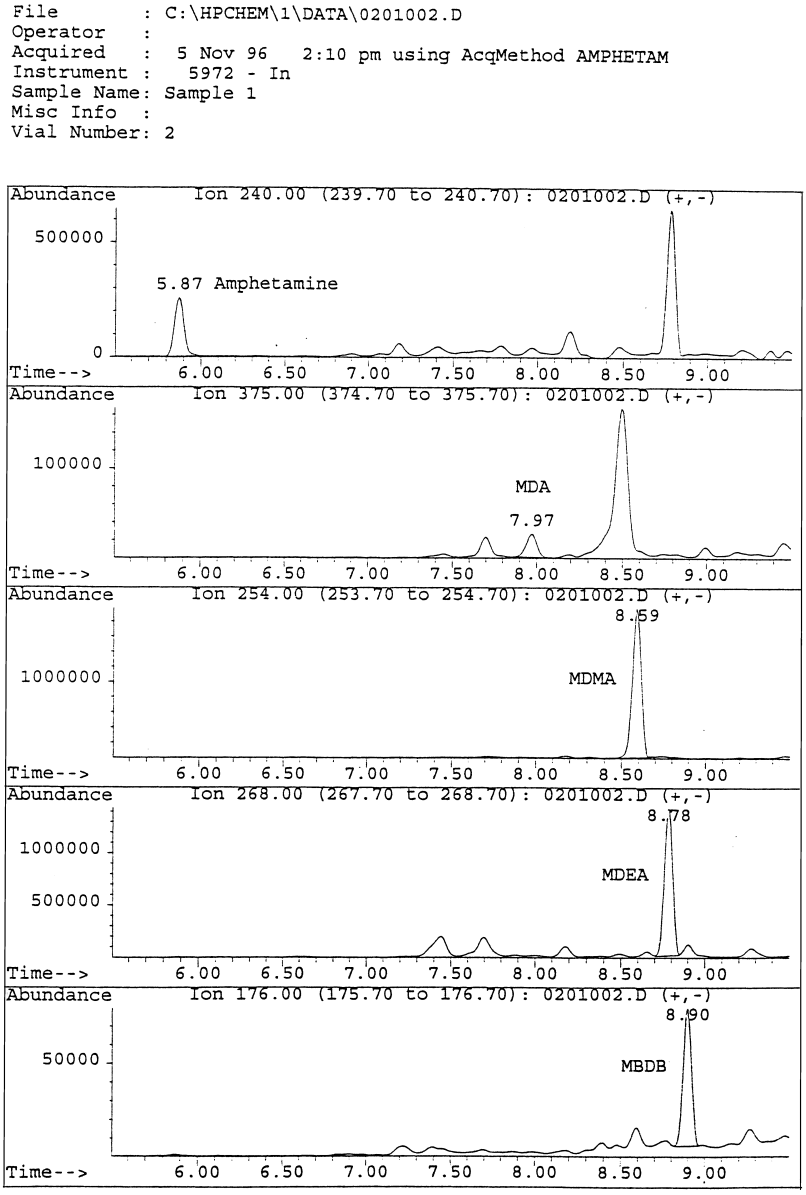

Fig. 2. Chromatogram obtained after alkaline extraction and derivatization with HFBA of a hair specimen from a stimulant abuser. Concentrations measured were 3.56, 4.88, 33.81, 39.32 and 3.09 ng/mg for AP, MDA, MDMA, MDEA and MBDB, respectively.

Table 8
Analytical parameters and results for a general screening procedure for amphetamine derivatives

Compound	Ions monitored (m/z)	Linearity (r)	Precision (at 2 ng/mg, %)	Concentration[a] (ng/mg)
Amphetamine	91, 118, 240	0.998	6.9	2.3–20.6 (n=5)
Methamphetamine	169, 210, 254	0.995	8.4	–
MDA	135, 240, 375	0.994	9.1	0.4–8.0 (n=13)
MDMA	210, 254, 389	0.996	10.2	0.3–42.7 (n=14)
MDEA	240, 268, 403	0.997	13.0	0.6–69.3 (n=6)
MBDB	176, 268, 403	0.994	8.7	1.41–3.09 (n=2)
BDB	135, 176, 389	0.996	9.4	0.21 (n=1)

[a] Number of positive cases in brackets.

In addition to screening, various procedures have been proposed for single compounds, like methoxyphenamine [56,57] or benzaphetamine [58].

Since the first identification of MDMA in human hair by Moeller et al. [59], this compound, particularly in Europe, is one of the most frequently identified. Therefore, it has to be included in all screening procedures.

To date, the only international inter-laboratory comparison concerning the quantitative determination of amphetamine and related compounds showed unsatisfactory reproducibility in hair testing [54].

6. Benzodiazepines

Surprisingly, until 1995 the chromatographic analysis of benzodiazepines, the most used class of drugs in the world, was not described in the literature. Only one paper reported their detection by RIA [60].

Acid or alkaline hydrolysis [61,62] were found unsuitable to extract the target drugs from the hair matrix, leading to decomposed compounds, including benzophenones. Methanol incubation can be used, but the chromatograms obtained look often 'dirty'. This can be avoided by the use of GC–MS–MS, as mentioned by Uhl [46] for flunitrazepam. Large series of results were obtained using incubation in buffer, like Söerensen buffer [62–64] or a mixture of β-glucuronidase/arylsulfatase at pH 4.0 [65]. In most cases, GC–MS in either the EI or NCI mode was used; however, to detect diazepam, nitrazepam and oxazepam [61], midazolam [66] or alprazolam [67], HPLC–UV, GC with electron capture detection (ECD) or HPLC–DAD, respectively, were employed.

Table 9 summarizes most procedures devoted to the analysis of benzodiazepines in hair. Concentrations of individual benzodiazepines tested in hair are presented in Table 10. Benzodiazepine concentrations are generally low, so GC–MS-NCI represents the state-of-the-art method for testing for benzodiazepines in human hair, due to the high electrophilic character of the analytes. GC–MS-NCI was also successfully used to determine alprazolam in rat hair [68].

Table 9
Analytical procedures for benzodiazepines testing in human hair

Ref.	Hydrolysis	Extraction	Derivatization	Analysis
[46]	Methanol, overnight	–	–	GC–MS–MS
[61]	Methanol, overnight	Chlorobutane	–	HPLC–UV
[62]	Söerensen buffer, overnight	Ether–chloroform	BSTFA	GC–MS-NCI
[63]	Söerensen buffer, 2 h	Ether–chloroform	HFBA	GC–MS-NCI
[64]	Söerensen buffer, 2 h	Ether–chloroform	BSTFA	GC–MS-NCI
[65]	β-Glucuronidase/arylsulfatase	SPE-C_{18}	–	GC–MS-EI
[66]	Methanol, overnight	SPE-Bond Elut	–	GC–ECD
[67]	0.1 M HCl, overnight	SPE, C_{18}	–	HPLC–DAD

Table 10
Reported concentrations of several benzodiazepines

Ref.	Compound	Number of positive samples	Concentrations (ng/mg)	Mean (ng/mg)
[62]	Nordiazepamoxazepam	13	0.25–18.9	4.16
		5	0.11–0.50	0.28
[63]	Flunitrazepam	14	0.031--0.129	0.060
	7-Aminoflunitrazepam	26	0.003--0.161	0.046
[64]	Lorazepam	4	0.031--0.049	0.040
[65]	Nordiazepamoxazepam	15	0.01–2.2	0.31
	Lormetazepamlorazepam	20	0.1–1.8	0.49
	Diazepam	15	0.02–3.4	1.71
	7-Aminoflunitrazepam	8	0.02–905	2.02
		3	4.1–29.1	17.09
		1	4.9	–
[67]	Alprazolam	1	0.3	–

The use of deuterated internal standards is highly recommended when commercially available, to enhance the accuracy and precision of the method. The recent introduction of deuterated flunitrazepam and 7-aminoflunitrazepam allowed a rise of about 5% in precision of our procedure. Fig. 3 shows a typical

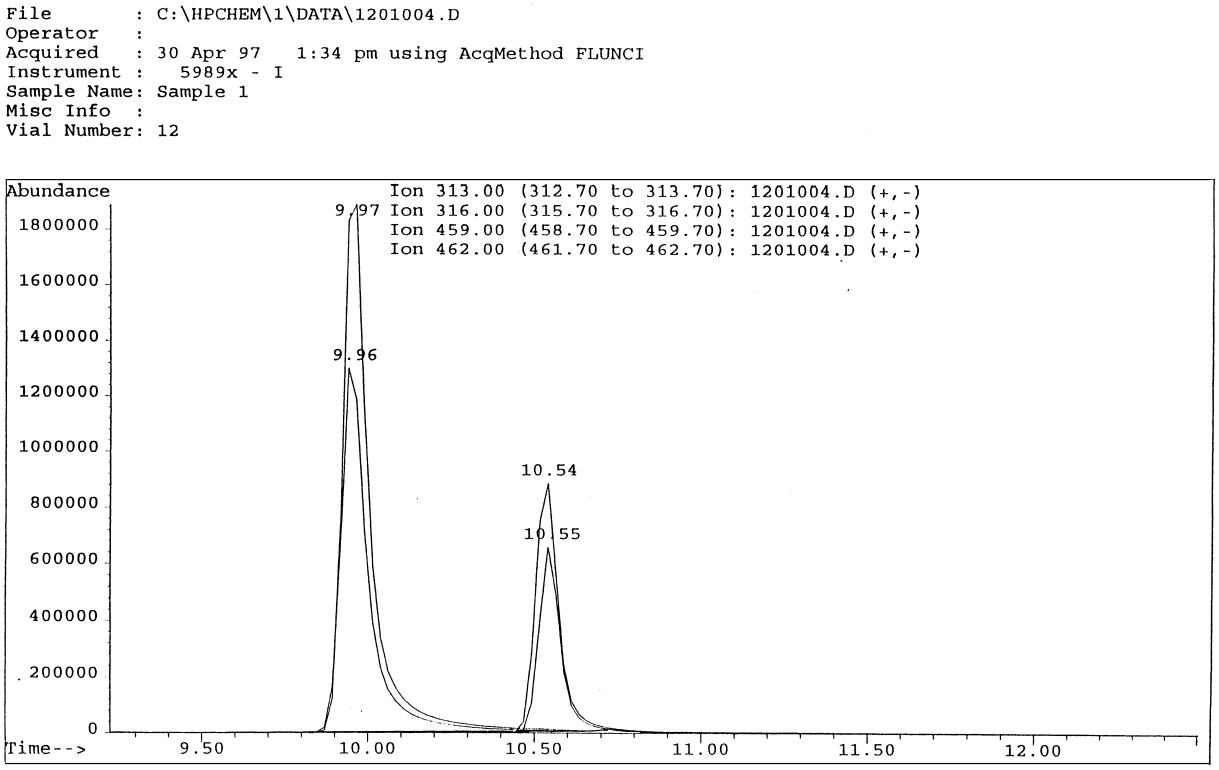

Fig. 3. Chromatogram obtained after extraction of a hair specimen from a street drug addict using heroin and flunitrazepam. Concentrations were 57 and 84 pg/mg for flunitrazepam (t_R: 10.55 min) and 7-aminoflunitrazepam (t_R: 9.97 min), respectively.

chromatogram obtained from the hair of a drug addict using heroin in combination with flunitrazepam.

Quite recently, Cirimele et al. [69] proposed a screening procedure for eight forensic benzodiazepines (nordiazepam, oxazepam, bromazepam, diazepam, lorazepam, flunitrazepam, alprazolam and triazolam), based on Söerensen buffer incubation, purification with diethyl ether–chloroform, derivatization by silylation, and detection by GC–MS-NCI.

7. Hallucinogens

Nakahara et al. [70] found LSD in hair from two out of seventeen self-reported LSD users at concentrations of 8–17 pg/mg. The hair sample was extracted with 2 ml methanol–5 *M* HCl (20:1, v/v) under ultrasonication for 1 h. After neutralization with 28% NH_4OH and evaporation, the residue was extracted with dichloromethane from alkalinized solution (0.1 *M* NaOH). LSD was determined as its TMS-derivative.

Phencyclidine (PCP) was one of first drugs that could be detected in hair [1]. Recently Kidwell published a method based on tandem mass spectrometry [71] and Sakamoto et al. [72] additionally detected the metabolites 4-phenyl-4-piperidinocyclohexanol (PPC) and 1-(1-phenylcyclohexyl)-4-hydroxy-piperidine (PCHP) in rat hair. They extracted with methanol–5 *M* HCl (20:1, v/v) under ultrasonication followed by a clean-up with Bond Elut Certify. The metabolites were detected using GC–MS after derivatisation with N,O-bis-(trimethylsilyl) acetamide. PCP was detected in rat hair after doses of 0.05 mg/kg.

8. Barbiturates, antiepileptics, neuroleptics, psychostimulants and antidepressants

More than 50 pharmaceuticals and drugs of abuse have been detected to date in human hair. The extraction methods and detection techniques of most of these drugs were summarized by Tracqui [73]. Amobarbital, phenobarbital and secobarbital are the barbiturates which have been detected in hair. Because of its use as antiepileptic drug, phenobarbital is the most investigated drug of this group. A dose–concentration relationship for phenobarbital was investigated [74], but the correlation coefficient (<0.7) was poor.

The antiepileptic drug carbamazepine and its metabolites carbamazepine-10,11-epoxide and acridine were detected in hair by GC–MS [75], but no dose–concentration relation could be detected. In 1997 Saris et al. [76] published an HPLC method to detect carbamazepine, carbamazepine-10,11-epoxide, and carbamazepine-diol after digesting 50 mg of hair with 1 *M* NaOH for 20 h at 37°C. In the hair of one patient they found a decreasing concentration from the root to the hair tip.

The neuroleptics chlorpromazine, clozapine and haloperidol were detected in hair using the procedures listed in Table 11.

The psychostimulant, nicotine, was investigated in hair by many researchers in order to find a way of distinguishing between smokers and nonsmokers. However, because of possible external contamination and passive inhalation it is still difficult to establish concentration limits to solve this problem, even when the metabolite cotinine is determined. Modern determination procedures are summarized in Table 12 together with the procedures for caffeine and fenfluramine.

Table 11
Procedures for detecting neuroleptics

Analyte	Preparation	Extraction	Analysis	Reference
Chlorpromazine	NaOH (2 *M* 1 ml, 30 min, 80°C)	*n*-Hexane–isoamyl alcohol (98.5:1.5, v/v)	HPLC–ECD	[77]
Clozapine	MeOH		GC–MS	[78]
Haloperidol	NaOH (2.5 *M*, 30 min, 80°C)	*n*-Hexane–isoamyl alcohol (98.5:1.5, v/v)	HPLC–ECD	[79]

Table 12
Analytical procedures for psychostimulants

Analyte	Preparation	Extraction	Detection	Reference
Caffeine Theophyline Theobromine	1 *M* NaOH (1 ml, 30 min, 100°C)	$CHCl_3$	HPLC–DAD	[80]
Fenfluramine	NaOH (60 min, 100°C)	$CHCl_3$–2-propanol–*n*-heptane (50:17:33, v/v)	GC–MS	[81]
Nicotine	NaOH (60 min, 100°C)	Diethyl ether	GC–MS	[82]

Table 13
Analytical procedures for psychostimulants

Compound	Preparation	Extraction	Detection	Reference
Amitriptyline	1 *M* NaOH (1 ml, 30 min, 100°C)	*n*-Heptane–isoamylalcohol (98.5:1.5, v/v)	GC–MS	[83]
Clomipramine	1 *M* NaOH (1 ml, 60 min, 100°C)	$CHCl_3$–2-propanol –*n*-heptane	GC–MS	[84]
Amitriptyline Nortriptyline Imipramine Desipramine Dothiepin Nordothiepin	NaOH (1 ml, 30 min, 70°C) vs. 0.1 *M* HCl (18 h, 55°C) vs. MeOH (18 h, 55°C) vs. 10 g/l Subtilisin (18 h, 55°C)	*n*-Heptane–butanol (95:5, v/v)	HPLC–UV	[85]

Amitriptyline, clomipramine and imipramine could be determined in hair including their metabolites nortriptyline and desipramine, respectively, as shown in Table 13.

9. Cardiovascular drugs, anti-infection drugs

Cardiovascular drugs like atenolol, betaxolol, propranolol and sotalol, were extracted from the hair matrix with diethyl ether–CH_2Cl_2 (80:20, v/v) after digestion with 1 *M* NaOH for 10 min at 100°C and analysed by HPLC–UV [81,86]. Ofloxacin, temofloxacin and fluoroquinoline were extracted with CH_3Cl after a similar preparation with NaOH (1 *M*, 30 min, 80°C) and analysed by HPLC–fluorometry [87–89].

10. Conclusion

Although there is still controversy on how to interpret the results, particularly concerning external contamination, cosmetic treatments, ethnical bias or drug incorporation, pure analytical work on hair analysis has reached a sort of plateau, having solved almost all the analytical problems. Conferences on hair analysis in Genoa [90], Strasbourg [91], Tampa [92], and Abu Dhabi [93] between 1992 and 1996 indicate the increasing role of this method for the investigation of drug abuse.

Although GC–MS is the method of choice in practice, GC–MS–MS or LC–MS are today used in several laboratories, even for routine cases, particularly to target low dosage compounds, like THC-COOH, fentanyl, flunitrazepam or buprenorphine.

Electrophoretic/electrokinetic analytical strategies [94], chiral separation [95] or application of ion mobility spectrometry [96] constitute the latest developments that have been applied to drug testing in hair. Today, quality assurance is a major issue of drug testing in hair. Since 1990, the National Institute of Standards and Technology (Gaithersburg, MD, USA) has developed inter-laboratory comparisons, recently followed by the new Society of Hair Testing (Strasbourg, France).

11. List of abbreviations

AEME	Androhydroecgonine methyl ester
AP	Amphetamine
BDB	Benzodioxazolyl butanamine
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
CBD	Cannabidiol
CBN	Cannabinol
CI	Chemical ionization
DAD	Diode array detection
EI	Electron impact
FT-IR	Fourier transform infrared spectroscopy
HFBA	Heptafluorobutyric acid anhydride
HPLC	High-performance liquid chromatography
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
GC–MS–MS	Gas chromatography–tandem mass spectrometry
LLE	Liquid–liquid extraction
MA	Methamphetamine
MAM	6-O-Acetylmorphine
MBDB	N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine
MDMA	Methylenedioxymethamphetmine
MDEA	Methylenedioxyethylamphetamine
NCI	Negative chemical ionization
PSA	Propionic acid anhydride
RIA	Radioimmunoassay
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction
TFA	Trifluoroacetic acid anhydride
THC	Tetrahydrocannabinol
TMCS	Trimethylchlorosilane

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