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REVIEW

The role of alternative specimens in toxicological analysis

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ABSTRACT: The use of alternative specimens in the field of toxicology was first described in 1979, when hair analysis was used to document chronic drug exposure. Since then, the use of these 'alternative' samples has gained tremendous importance in forensic toxicology, as well as in clinic toxicology, doping control and workplace drug testing. It is not surprising, therefore, that a large number of papers dealing with the determination of several classes of drugs in saliva, sweat, meconium and hair have been published ever since, owing to the fact that chromatographic equipment is becoming more and more sensitive, mass spectrometry (and tandem mass spectrometry) being the most widely used analytical tool, combined with gas or liquid chromatography. 'Alternative' specimens present a number of advantages over the 'traditional' samples normally used in toxicology (e.g. blood, urine and tissues), namely the fact that their collection is not invasive, their adulteration is difficult, and they may allow increased windows of detection for certain drugs. The main disadvantage of this kind of samples is that drugs are present in very low concentrations, and therefore high-sensitivity techniques are required to accomplish the analysis. This paper reviews a series of publications on the use of alternative specimens, with special focus on the main analytical and chromatographic problems that these samples present, as well on their advantages and disadvantages over traditional samples in documenting drug exposure. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: unconventional samples; analytical chromatography; therapeutic drug monitoring; toxicological analysis

INTRODUCTION

In the last decade, alternative or unconventional matrices have becoming more important in the field of toxicology, owing to the advantages that these specimens present when compared with 'conventional' samples used in laboratorial routine analysis. In general these samples present the advantage that collection is almost non-invasive and easy to perform. On the other hand, collection can also be achieved under close supervision, which prevents sample adulteration or substitution. Furthermore, some of these samples present larger detection windows, and therefore their range of analytical applications can be very wide.

Recent advances in analytical techniques have enabled the detection of drugs and metabolites at very low concentrations that were unthinkable a few years ago. In fact, LC/MS and LC/MS/MS techniques are increasing in popularity as confirmation techniques because of high sensitivity and specificity, and the ability to handle complex matrices. Also, LC/MS techniques do not require the time-consuming derivatization steps needed in GC/MS for a large number of compounds; however, ion suppression or enhancement due to complex matrices is a frequent analytical complication and must be addressed during method development and validation.

In fact, despite the analytical problems that liquid chromatography-based techniques can present, these are the state of art concerning analysis of alternative specimens because of their higher sensitivity, which is crucial if one takes into account the low amount of sample usually available in these situations.

Therefore, very low amounts of drugs of abuse or prescription drugs can be detected, for instance in the low picogram range for carboxy-THC, which is definite proof of cannabis consumption, using hair analysis, and for benzodiazepines, a single exposure to which in a drug-facilitated assault can be detected through hair analysis.

The first unconventional sample used was hair in the 1960s and 1970s to evaluate human exposure to toxic heavy metals, namely arsenic, lead and mercury (Hammer *et al.*, 1971; Kopito *et al.*, 1967). Since then, numerous papers dealing with the determination of various classes of compounds have been published in the scientific literature, normally concerning drugs of abuse and therapy. Nowadays other alternative samples such as oral fluid, meconium or sweat are being introduced and present a wide range of applications, e.g. in therapeutic drug monitoring, workplace drug testing and prenatal exposure to drugs of abuse.

This review will deal with the most used unconventional samples, with special focus on their advantages and disadvantages, collection procedures, classes of drugs that are analyzed and analytical methods.

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ALTERNATIVE SPECIMENS

The most commonly used unconventional samples are hair, oral fluid, sweat and meconium. The physiological and analytical properties of these samples, as well of other (less used) samples, including advantages and drawbacks of each and the main parameters that can affect their analysis, will be discussed below, in the light of existing literature on the topic.

Hair

Hair is a product of differentiated organs in the skin of mammals. It is constituted by proteins, mainly keratin (65–95%), water (15–35%), lipids (1–9%) and minerals (0.25–0.95%; Harkley and Henderson, 1989). A rich capillary system, which provides the growing hair with the necessary metabolic material, surrounds the hair follicle (Pragst and Balikova, 2006). It is estimated that the total number of hair follicles in adults is approximately 5 million. Hair grows at a rate of 0.6–1.4 cm per month, depending on the type of hair and anatomical site (Saitoh *et al.*, 1969).

The hair growth cycle is divided into the anagen (active growing), catagen (transition) and telogen (resting) stages. The proportions of anagen/telogen hair vary with anatomical site and this feature, together with variable growth rate, accounts for the observed differences in drug concentrations in hair collected from different regions. In fact, not only scalp hair can be used for analysis, and pubic hair, arm or leg hair and axillary hair have been suggested as alternative sources for drug detection when scalp hair is not available. However, care should be taken when interpreting the concentrations of drugs in these specimens, since various studies have found differences between pubic or axillary hair and scalp hair (Balabanova and Wolf, 1989; Offidani *et al.*, 1993; Han *et al.*, 2005). Indeed, the latter two studies have compared methadone and methamphetamine concentrations in hair from different anatomical sites, concluding that the highest values were found in axillary hair, followed by pubic hair and scalp hair. In contrast, in another study the highest morphine concentrations were found in pubic hair, followed by head hair and axillary hair (Mangin and Kintz, 1993). The significant differences of the drug concentrations in these studies can be explained not only by the totally different anagen/telogen ratio or growth rate, but also by a better blood circulation and a greater number of apocrine glands (Pragst *et al.*, 1998).

Beard hair is also a suitable specimen for analysis. This type of hair grows at about 0.27 mm per day, and therefore can be collected on a daily basis with an electric shaver.

Hair analysis is only useful if the measured drugs are a result of ingestion, rather than from other sources. Therefore, the mechanisms of incorporation of the drugs into the hair shaft must be addressed. It is generally accepted that drugs can enter the hair from three sources: (1) from the bloodstream during hair growth; (2) following excretion by sweat and sebum bathing the hair, usually after the hair emerges from the skin, and (3) from passive exposure from the hair to the drug, e.g. from smoke or dirty hands, followed by dissolution of the drug into the drug-free sweat. It is virtually impossible to distinguish between the presence of drugs derived from these two latter mechanisms and that proceeding from actual consumption, which is explained by the fact that the drugs are in an aqueous moiety, enhancing their incorporation. This is the reason why environmental exposure is sometimes called the 'stumbling block of hair testing' (Kidwell and Blank, 1996).

Incorporation of drugs is affected by the melanin content of the hair and by the substances' lipophilicity and basicity. For instance, the effect of melanin content of the hair on drug incorporation can be studied in individuals with gray hair, showing that the concentration of basic drugs in pigmented hair can be about 10-fold higher than in non-pigmented hair (Pragst and Balikova, 2006). In fact, it has been suggested that drugs bind to melanin, which explains the higher concentrations normally found in darker hair (Rollins *et al.*, 2003; Mieczkowski and Kruger, 2007).

Hair samples are best collected from the back of the head, the so-called vertex posterior. In fact, this is the region where hair grows with more homogeny, and also where the anagen/telogen ratio is higher, meaning that the number of hairs in active growth is larger. Hair should be cut as close as possible to the scalp with the aid of scissors, and the proximal zone (i.e. the zone which is closer to the root) should be clearly indicated if segmental analysis is to be performed. The sample can then be stored light and moisture protected at room temperature, for instance wrapped in aluminum foil.

Drugs are usually stable in regularly treated hair (without using aggressive cosmetic agents, such as oxidant dyes, bleaching or permanent wave), which makes them detectable for at least one year after intake (Pragst and Balikova, 2006).

Hair is usually exposed to several agents that may impair drug testing, such as shampoos, dust, sunlight and rain. In fact, there are several studies on the effect of cosmetic treatments on drug stability in hair. For example, Martins *et al.* (2007) have found that the concentrations of amphetamine-type stimulants decreased in bleached hair when compared with non-bleached hair, without influencing their enantiomeric ratios. Likewise, it has been shown that this treatment affects the stability and decreases hair concentrations of other drugs (Pötsch and Skoop, 1996; Yegles *et al.*, 2000). Cosmetic treatments can also produce analytical interferences that may hinder the detection of drugs. This is

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the case for minoxidil, whose TMS derivative prevents the detection of cocaine and metabolites (Zucchela *et al.*, 2007).

As stated above, one of the most important pitfalls in hair analysis is environmental contamination. Indeed, if adequate measures are not taken, the risk of reporting false positive results increases, which is unacceptable, especially if there are legal implications of drug consumption. Therefore, to minimize this effect it is strongly recommended that hair analysis procedures include a washing step. Several decontamination procedures are described in the literature, and these include organic solvents, aqueous buffers, water, soaps and combinations of these (Kintz *et al.*, 1995; Eser *et al.*, 1997; Girod and Staub, 2000; Skender *et al.*, 2002; Schaffer *et al.*, 2002; Villamor *et al.*, 2005). There is no general consensus regarding decontamination procedures, and it is assumed that the total elimination of deposited drug is not achieved even after laborious washing procedures. Several researchers propose criteria for differentiation between drug use and environmental contamination, namely the establishment of a concentration ratio between the last wash and the hair sample (Schaffer *et al.*, 2005; Tsanaclis and Wicks, 2007a).

Hair decontamination prior to analysis is not the only way to deal with environmental exposure. Therefore, the Society of Hair Testing also recommends the detection of drug metabolites and the use of metabolite to parent drug ratios to report positive results (Society of Hair Testing, 2004). In fact, as environmental contamination is not totally removed even using laborious washing techniques, only the detection of drug metabolites, i.e. proceeding from endogenous metabolism, guarantees that the drug that is being measured has been actively consumed. This is of particular importance in the case of drugs that are likely to be in the environment because of the way they are consumed, such as cannabis (THC-COOH should be detected) and cocaine (where at least one metabolite should be detected, with a concentration ratio to the parent drug of higher than 0.05).

The major practical advantage of hair testing compared with urine or blood testing for drugs is that it has a larger detection window (weeks to months, depending on the length of the hair shaft, against 2–4 days for most drugs). However, it is not advisable to rely only on hair analysis, since there are issues where it cannot provide adequate results, such as short-term information on an individual's drug use, for which blood and/or urine are better specimens. On the other hand, long-term histories are only accessible through hair analysis. Therefore, one can say that these tests complement each other.

The assessment of this 'chronic exposure' to drugs is achieved by segmental hair analysis. In fact, hair grows at approximately 1 cm per month, and it is possible to associate the drug distribution pattern in the analyzed segments with a period in the past, taking into account both variable hair growth rates and intra- and inter-individual differences. Furthermore, drugs are very stable within the hair matrix for long periods of time, providing that specimens are stored light- and moisture-protected. Another advantage of hair analysis when compared with blood or urine analysis is the collection procedure, because: (1) it is non-invasive and easy to perform; (2) the sample is not easy to adulterate by diluting with water (as can occur in urinalysis); and (3) in the case that there is a claim (sample switching, break in the chain of custody, etc.), it is possible to get an identical sample from the subject. Obviously, this latter is of great importance in the field of forensic toxicology.

However, hair analysis has several drawbacks, which sometimes are very difficult to handle in manageable proportions. The main problem in this type of analysis is the possibility of reporting false positive results due to environmental contamination of the hair, which can occur at any level. The fact that a drug is detected in a hair specimen does not necessarily mean that it was actively consumed. Therefore, hair specimens should be decontaminated prior to analysis, and specific metabolites of the drugs must be searched for. This may present a problem, because normally the metabolites are more polar drugs, and have less affinity for hair matrix constituents. This is the case, for example, for THC-COOH, the metabolite of THC (cannabis main constituent), which is found in hair in extremely low concentrations, usually in the low picogram range. To detect these low concentrations, mass spectrometric techniques are mandatory, using either gas or liquid chromatography.

In addition, as hair is quite a 'dirty' matrix, its constituents may interfere with chromatographic analysis, and therefore a sample cleanup step is normally required. In the development of new methods for drug detection in hair, special attention should be paid to the matrix effect, especially using liquid chromatographic methods, because they are more sensitive to ion suppression/enhancement effects.

Since the first report in the 1970s, hair analysis has aided toxicologists in several fields, such as in history and archaeology (Nakahara *et al.*, 1997; Báez *et al.*, 2000), in assessing consumption profiles of drugs and alcohol by the general (Jurado *et al.*, 1996; Hartwig *et al.*, 2003; Tsanaclis and Wicks, 2007b) or student populations (Kidwell *et al.*, 1997; Quintela *et al.*, 2000), driving licence renewals (Ricossa *et al.*, 2000), assessing intrauterine drug exposure (Chiarotti *et al.*, 1996; Ursitti *et al.*, 1997; Koren *et al.*, 2002; Garcia-Bournissen *et al.*, 2007), evaluating of compliance with drug substitution therapy (Moeller *et al.*, 1993; Kintz *et al.*, 1998; Lucas *et al.*,

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2000; Sabzevari *et al.*, 2004), in the workplace and pre-employment (Cairns *et al.*, 2004) and in post-mortem toxicology (Kintz, 2004). Another important application of hair analysis is in drug-facilitated crimes, in which the analytes must be detected after a single exposure, which is achieved due to the high sensitivity of LC/MS/MS (Negrusz and Gaensslen, 2003; Kintz, 2007).

Several classes of drugs can be detected in hair, such as biomarkers of alcohol consumption, cocaine and metabolites, opiates, cannabinoids, amphetamines and other designer drugs, GHB, benzodiazepines and hypnotics, antipsychotics, antidepressants, steroids, anaesthetics, antiparkinsonics and alkaloids (Table 1).

Hair analysis usually begins with a general screening by immunoassays, followed by a confirmation using chromatographic techniques. Gas chromatography coupled to mass spectrometry is by far the most widely used analytical tool for drug determination in hair specimens. Nevertheless, liquid chromatography–mass spectrometry (or tandem mass spectrometry) based methods are becoming more and more important in this field, owing to their better sensitivity for termolabile compounds, yielding lower limits of detection and quantitation, and the fact that time-consuming derivatization steps are not necessary to accomplish the analysis. However, before chromatographic analysis, the analytes must be (1) extracted from within the matrix (where they are bound to hair constituents) and (2) concentrated in a solvent which is compatible with the analytical instruments. There is no universal method to extract the analytes from the hair matrix, and it depends on the nature and chemical stability of the particular compound. Therefore, opioids and cocaine are best extracted using mild acidic hydrolysis (e.g. 0.05–0.5 M hydrochloric acid), to avoid conversion of heroin or 6-acetylmorphine to morphine and of cocaine to benzoylecgonine (Girod and Staub, 2000; Romano *et al.*, 2003; Cognard *et al.*, 2005; Cordero *et al.*, 2007; Tsanaclis and Wicks, 2007a). On the other hand, stable compounds like cannabinoids and amphetamines can be extracted using strong alkaline conditions (e.g. 1 M sodium hydroxide; Quintela *et al.*, 2000; Stanaszek and Piekoszewski, 2004; Villamor *et al.*, 2005; Martins *et al.*, 2005, 2006; Tsanaclis and Wicks, 2007a). Other extraction methods include buffer or solvent extraction (with, or without sonication; Paterson *et al.*, 2001; Scheidweiler and Huestis, 2004) and enzymatic hydrolysis (Vincent *et al.* 1999; Quintela *et al.*, 2000; Míguez-Framil *et al.*, 2007).

Following this extraction step, which is normally the most time-consuming step in hair analysis, a sample cleanup step is often required, to minimize any interference caused by endogenous compounds, which is particularly important in the case of liquid chromatography-based methods because of ion suppression/enhancement effects. This sample cleanup procedure is usually performed using liquid–liquid extraction (Sachs and Dressler, 2000; Stanaszek and Piekoszewski, 2004; Villamor *et al.*, 2005; Nakamura *et al.*, 2007) or solid-phase extraction (Girod and Staub, 2000; Scheidweiler and Huestis, 2004; Cognard *et al.*, 2005; Martins *et al.*, 2006; Moore *et al.*, 2006a,b; Lachenmeier *et al.*, 2006; Cordero and Paterson, 2007). However, solid-phase microextraction (Sporkert and Pragst, 2000; Lucas *et al.*, 2000; Musshoff *et al.*, 2002; Nadulski and Pragst, 2007), solid-phase dynamic extraction (Musshoff *et al.*, 2003) and supercritical fluid extraction (Cirimele *et al.*, 1995; Allen and Oliver, 2000; Brewer *et al.*, 2001) have also been described.

Saliva/oral fluid

Saliva is the excretion product originated from three pairs of major salivary glands (parotid, submandibular and sublingual), a great number of minor salivary glands, the oral mucosa and gingival crevices. As this excretion product is actually a fluid mixture, the term 'oral fluid' seems more appropriate to designate it, instead of 'saliva' or 'whole saliva' (Malamud, 1993). Water (99%) is the major oral fluid constituent, and other components such as proteins (mucins and digestion enzymes) and mineral salts are also present. Its pH is 6.8 in resting situations, but an increase in the salivary flow turns it more basic (approaching the plasma's pH) as a result of higher osmolarity (Kintz and Samyn, 2000). All these characteristics are influenced by a variety of factors, as the circadian rhythm, the type of the salivation stimulus, hormonal changes, stress, and therapeutic drugs (Aps and Martens, 2005). The total volume of oral fluid produced by an adult may be 1000 mL/day with typical flows of 0.05 mL/min while sleeping, 0.5 mL/min while spitting and 1–3 mL/min or more while chewing (Crouch, 2005).

Different mechanisms of drug transport are thought to occur, such as passive diffusion through the membrane, active processes against a concentration gradient, filtration through pores in the membrane and pinocytosis (Spihler, 2004). Most of the drugs enter oral fluid by a mechanism of passive diffusion, which is dependent on the particular physicochemical properties of the compound or class of compounds, such as molecular weight (a molecular weight of less than 500 Da favors diffusion), liposolubility, pH and pK_a, protein binding and ionization state (Paxton, 1979; Aps and Martens, 2005). Therefore, the concentrations of drugs in oral fluid represent the free non-ionized fraction in the blood plasma. In fact, the fraction of drug bound to saliva and plasma protein as a function of pK_a and pH can be predicted by the Henderson–Hasselbach equation (Spihler, 2004).

A variety of methods are available for oral fluid collection (Navazesh, 1993), including spitting, draining,

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Table 1. Hair analysis for several classes of compounds

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
Ethyl glucuronide, cocaethylene (COET)	50 mg of hair; no sample cleanup 50 mg of hair; SPE	LC/MS/MS	3 pg/mg for ethyl glucuronide 40 pg/mg for COET	Politi *et al.* (2007)
Fatty acid ethyl esters	30 mg of hair; liquid–liquid extraction procedure followed by HS-SPME	GC/MS	0.02 ng/mg; 0.2 ng/mg	De Giovanni *et al.* (2007)
Ethyl glucuronide	10–30 mg of hair; SPE	GC/MS	2 pg/mg; 4 pg/mg	Appenzeller *et al.* (2007)
Ethyl glucuronide	10 mg of hair; SPE	GC/MS/MS	0.01 ng/mL; 0.02 ng/mL	Paul *et al.* (2007)
Cocaine (COC), anhydroecgonine methylester (AEME), ecgonine methylester (EME), COET	50 mg of hair; SPE	GC/MS/MS	0.005 ng/mg; 0.05 ng/mg for COC and COET, 0.025 ng/mg; 0.05 ng/mg for EME 0.050 ng/mg; 0.10 ng/mg for AEME	Cognard *et al.* (2005)
COC, COET	50 mg of hair; SPME	GC/MS	0.08 ng/mg; 0.4 ng/mg for COC 0.02 ng/mg; 0.4 ng/mg for COET	Bermejo *et al.* (2006)
COC, benzoylecgonine (BE)	20–50 mg of hair; no sample-cleanup	LC/SACI-MS/MS-SRM LC/SACI-MS3-SRM	0.003 ng/mg; 0.01 ng/mg for COC 0.02 ng/mg; 0.04 ng/mg for BE	Cristoni *et al.* (2007)
COC, BE, COET and norcocaine	10 mg of hair; SPE	LC/MS/MS	25 pg/mg; 50 pg/mg	Moore *et al.* (2007a)
6-Monoacetylmorphine (MAM)	20–30 mg of hair; SPE	RIA and GC/MS	0.2 ng/mg; 0.5 ng/mg used RIA	Moeller and Mueller (1995)
Codeine (COD), morphine (MOR), hydrocodone, hydromorphone, MAM and oxycodone	10–50 mg of hair; SPE	GC/MS	0.32 ng/mg; 0.03 ng/mg for COD 0.15 ng/mg; 0.01 ng/mg for MOR 0.65 ng/mg; 0.06 ng/mg for hydrocodone 0.15 ng/mg; 0.01 ng/mg for hydromorphone 1.10 ng/mg; 0.1 ng/mg for MAM 0.14 ng/mg; 0.01 ng/mg for oxycodone	Jones *et al.* (2002)
MAM, MOR, COD	50 mg of hair; SPE	GC/MS	2 pg/μL for MAM 3 pg/μL for MOR and 5 pg/μL for COD	Acampora *et al.* (2003)
MAM, MOR, COD	10 mg of hair; SPE	RIA and LC/MS/MS	0.5 ng/10 mg	Hill *et al.* (2005)

Table 1. (*Continued*)

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
Heroin, MAM, MOR, COD, COC, BE and COET	50 mg of hair; SPE	ELISA and GC/MS	0.04 ng/mg; 0.21 ng/mg for heroin, 0.02 ng/mg; 0.15 ng/mg for MAM, 0.03 ng/mg; 0.11 ng/mg for MOR, 0.02 ng/mg; 0.04 ng/mg for COD, 0.01 ng/mg; 0.11 ng/mg for COC, 0.03 ng/mg; 0.26 ng/mg for BE, 0.05 ng/mg; 0.21 ng/mg for COET	Lachenmeier *et al.* (2006)
MAM, MOR, COD, hydrocodone	10 mg of hair; SPE	ELISA and GC/MS	50 pg/mg for all analytes	Moore *et al.* (2006a)
Opiates, amphetamines, cocaine and metabolites and diazepam and metabolite	10–50 mg of hair; SPE	GC/MS	0.1 ng/mg for amphetamines and 0.2 ng/mg for remaining drugs	Cordero and Paterson (2007)
Buprenorphine, COD, fentanyl, hydromorphone, methadone, MOR, oxycodone, oxymorphone, piritramide, tilidine, tramadol, and their metabolites bisnortilidine (BNTI), nortilidine (NTI), norfentanyl (NFE), and normorphine (NOMO)	50 mg of hair; methanol	LC/MS/MS	1.6 pg/mg; 5.6 pg/mg for buprenorphine, 12.7 pg/mg; 50.8 pg/mg for COD, 0.8 pg/mg; 2.6 pg/mg for fentanyl, 2.0 pg/mg; 6.6 pg/mg for hydromorphone, 8.9 pg/mg; 30.0 pg/mg for methadone, 6.1 pg/mg; 29.9 pg/mg for MOR, 12.0 pg/mg; 42.7 pg/mg for oxycodone, 1.2 pg/mg; 4.7 pg/mg for oxymorphone, 2.2 pg/mg; 9.1 pg/mg for piritramide, 2.4 pg/mg; 8.7 pg/mg for tilidine, 15.2 pg/mg; 57.8 pg/mg for tramadol, 17.4 pg/mg; 59.1 pg/mg for BNTI, 9.3 pg/mg; 32.2 pg/mg for NTI, 0.9 pg/mg; 3.5 pg/mg for NFE, and 3.4 pg/mg; 9.5 pg/mg for NOMO	Musshoff *et al.* (2007)
Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN)	10 mg of hair; SPDE	GC/MS	0.09; 0.44 ng/mg for THC, 0.09; 0.44 ng/mg for CBD, 0.12; 0.44 ng/mg for CBN	Musshoff *et al.* (2003)

Table 1. (*Continued*)

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), THC, CBN	15 mg of hair; SPE 50–100 mg of hair; methanol	GC/MS/MS; GC/MS; ELISA	0.1 pg/mg for THC-COOH 0.04 ng/mg for THC and CBN	Uhl and Sachs (2004)
THC, CBD, CBN	50 mg of hair; LLE	GC/MS	0.005 ng/mg for CBD, 0.002 ng/mg for CBN 0.006 ng/mg for THC	Kim *et al.* (2005)
THC, CBD, CBN, THC-COOH			review	Musshoff and Madea (2006)
THC-COOH	20 mg of hair; SPE	GC/MS	0.05 pg/mg	Moore *et al.* (2006b)
THC-COOH, THC	20 mg of hair; SPE	GC/MS; ELISA	1.0 pg/mg for THC and 0.1 pg/mg for THC-COOH	Huestis *et al.* (2007)
THC, CBD, CBN	100 mg of hair; HS-SPME	GC/MS	0.012; 0.037 ng/mg for THC, 0.013; 0.038 ng/mg for CBD, 0.016; 0.048 ng/mg for CBN	Nadulski and Pragst (2007)
THC, CBD, CBN	50 mg of hair; LLE	GC/MS	0.025 ng/mg; 0.06 ng/mg for CBN and CBD	Skopp *et al.* (2007)
THC, CBD, CBN	10 mg of hair; HS-SPME	GC/MS	0.07 ng/mg; 0.12 ng/mg for THC, CBD, CBN	Dizioli Rodrigues de Oliveira *et al.* (2007)
THC-COOH	25 mg of hair; LLE	GC/MS/MS	0.02 pg/mg; 0.05 pg/mg	Kim and In (2007)
Amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethamphetamine (MDEA)	50 mg of hair; LLE	GC/MS	0.045 ng/mg; 0.151 ng/mg for AP, 0.014 ng/mg; 0.048 ng/mg for MA, 0.013 ng/mg; 0.043 ng/mg for MDA, 0.017 ng/mg; 0.057 ng/mg for MDMA, 0.007 ng/mg; 0.023 ng/mg for MDEA	Villamor *et al.* (2005)
MA, MDMA, AP, MDA	5–19 mg of hair; methanol	GC/MS	0.125 ng/mg; 0.5 ng/mg	Han *et al.* (2006)
MA, AP	1 piece of hair; SPME	GC/MS	0.02 ng/0.08 mg/vial; 0.05 ng/0.08 mg/vial for MA 0.05 ng/0.08 mg/vial; 0.10 ng/0.08 mg/vial for AP	Nishida *et al.* (2006b)
MDMA, MDA, MA, AP	methanol	HPLC-fluorescence detection	0.25 ng/mg for MDMA, 0.15 ng/mg for MDA, 0.25 ng/mg for MA, 0.19 ng/mg for AP	Nakamura *et al.* (2006)
MDMA, MDA	50 mg of hair; LLE	GC/MS	—	Liu *et al.* (2006)
MA, MDMA, AP, MDA, MDEA, N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)	20 mg of hair; LLE	LC-MS/MS	1.1 pg/mg; 2.4 pg/mg for MA, 2.1 pg/mg; 4.8 pg/mg for MDMA, 6.1 pg/mg; 14.7 pg/mg for AP, 6.3 pg/mg; 15.7 pg/mg for MDA, 1.4 pg/mg; 3.2 pg/mg for MDEA, 0.3 pg/mg; 0.7 pg/mg for MBDB	Chèze *et al.* (2007a)

Table 1. (*Continued*)

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
MA, MOR, COD, Ketamine	10 mg of hair; LLE	CSEI-Sweep-MEKC and LC-MS	50 pg/mg for MA and ketamine, 100 pg/mg hair for COD and 200 pg/mg hair for MOR	Lin *et al.* (2007)
MDMA, AP, MDA	20 mg of hair; methanol	LC-MS/MS	0.1 ng/mg; 0.2 ng/mg for MDMA, AP, MDA	Kłys *et al.* (2007)
GHB	10 mg of hair; buffer solution and SPE	GC/MS and LC/MS	—	Kalasinsky *et al.* (2001)
GHB	5 mg of hair; LLE	GC/MS/MS	—	Goullé *et al.* (2003)
GHB	5 mg of hair; LLE	GC/MS	0.1 pg/mg	Kintz *et al.* (2005d)
Diazepam, nordiazepam, oxazepam, alprazolam, OH-alprazolam, nitrazepam, 7-aminonitrazepam, flunitrazepam, 7-aminoflunitrazepam, clonazepam, and 7-aminoclonazepam	10–30 mg of hair; LLE	LC-MS/MS	0.025–0.125 ng/mg	Kronstrand *et al.* (2002)
Bromazepam, clonazepam, 7-aminoclonazepam, hydroxy bromazepam	20 mg of hair; LLE	LC-MS/MS	1–2 pg/mg; 5 pg/mg for bromazepam, 0.5 pg/mg; 2 pg/mg for clonazepam, 2 pg/mg; <10 pg/mg for 7-aminoclonazepam	Chèze *et al.* (2004)
Zopiclone	20 mg of hair; LLE	LC-MS/MS	0.3 pg/mg	Villain *et al.* (2004)
Alprazolam, 7-aminoclonazepam, 7-aminoflunitrazepam, bromazepam, clobazam, diazepam, lorazepam, lormetazepam, midazolam, nordiazepam, oxazepam, temazepam, tetrazepam, triazolam, zaleplon and zolpidem	20 mg of hair; LLE	LC-MS/MS	0.5–2 pg/mg	Villain *et al.* (2005)
Tetrazepam	20 mg of hair; LLE	LC-MS/MS	1.5 pg/mg; 5 pg/mg	Concheiro *et al.* (2005b)
26 benzodiazepines and metabolites, zolpidem and zopiclone	20 mg of hair; LLE	LC-MS/MS	0.5–10 pg/mg	Laloup *et al.* (2005a)
7-Aminoflunitrazepam, flunitrazepam, oxazepam, lorazepam, chlordiazepoxide, temazepam, diazepam, nordiazepam, nitrazepam	30 mg of hair; monobasic phosphate buffer/SPE	ELISA/ LC/MS/MS	2 ng/mg for ELISA 0.03–0.62 ng/30 mg; 0.05–1.02 ng/30 mg for LC/MS/MS	Miller *et al.* (2006)
Diazepam and others benzodiazepines	30 mg of hair; MIP/MISPE and SPE	MISPE and LC/MS/MS	0.09 ng/mg; 0.14 ng/mg for diazepam 0.03–0.78 ng/mg; 0.06–1.32 ng/mg for others benzodiazepines	Ariffin *et al.* (2007)
Alprazolam α-hydroxyalprazolam	10 mg of hair	GC/MS	7 ng/mg; 20 ng/mg	García-Algar *et al.* (2007)
Carbamazepine, amitriptyline, doxepin, trihexyphenidyl, chlorpromazine, chlorprothixene, trifluoperazine, clozapine and haloperidol	10–20 mg of hair; methanol	GC-MS	0.1–0.5 ng/mg	Shen *et al.* (2002)

Table 1. (*Continued*)

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
Clozapine, flupentixol, haloperidol, penfluridol, thioridazine, zuclopenthixol	50 mg of hair; SPE	LC/MS/MS	0.017 ng/mg; 0.051 ng/mg for clozapine, 0.011 ng/mg; 0.031 ng/mg for flupentixol, 0.013 ng/mg; 0.039 ng/mg for haloperidol, 0.012 ng/mg; 0.036 ng/mg for penfluridol, 0.014 ng/mg; 0.042 ng/mg for thioridazine, 0.008 ng/mg; 0.024 ng/mg for zuclopenthixol	Weinmann *et al.* (2002)
Trimeprazine	20 mg of hair; LLE	LC/MS/MS	2 pg/mg	Kintz *et al.* (2006)
Clozapine	50 mg of hair; methanol	LC/MS/MS	—	Thieme *et al.* (2007)
Risperidone, sertraline, paroxetine, trimipramine, mirtazapine, and their metabolites	100–200 mg of hair; methanol	LC/MS/MS	—	Doherty *et al.* (2007)
Clozapine	5–15 mg of hair; LLE	LC/MS/MS	—	Kronstrand *et al.* (2007)
Amitriptyline, nortriptyline, dothiepin, doxepin, imipramine, trimipramine, desipramine, mianserin, haloperidol, chlorpromazine, diazepam, flunitrazepam, nitrazepam, oxazepam, temazepam and thioridazine	20–100 mg of hair; LLE	GC-MS and HPLC-UV	—	Couper and McIntyre (1995)
Clomipramine, norclomipramine	50 mg of hair; SPE	LC/MS	0.5; 0.5 ng/mg for clomipramine, norclomipramine	Kłys *et al.* (2005)
Methyltestosterone, nandrolone, boldenone, fluoxymesterolone, COC, BE	50 mg of hair; SPE and methanol	GC/MS/MS	10 pg/mg for methyltestosterone, nandrolone, boldenone, fluoxymesterolone 0.1 ng/mg for COC, BE	Gambelunghe *et al.* (2007)
Midazolam, 1-hydroxymidazolam, propofol	50 mg of hair; LLE (midazolam, 11-hidroxymidazolam), buffer solution (propofol)	GC/MS and HPLC/DAD	—	Cirimele *et al.* (2002)
Fentanyl, alfentanil and sufentanil	50 mg of hair; LLE	GC/MS/MS	1 pg/mg	Kintz *et al.* (2005c)
Fentanyl	10 mg of hair; phosphate buffer and SPE	ELISA and GC/MS	20 pg/mg with ELISA 5 pg/mg with GC/MS	Moore *et al.* (2007b)
Selegiline, desmethylselegiline, MA, AP	5 mg of hair; methanol and SPE	LC/MS	0.01 ng/mg; 0.04 ng/mg for selegiline, desmethylselegiline and MA. 0.05 ng/mg; 0.2 ng/mg for AP	Nishida *et al.* (2006a)
Ibogaine and noribogaine	50 mg of hair; LLE	LC-MS/MS	5 pg/mg; 10 pg/mg for ibogaine 5 pg/mg; 25 pg/mg for noribogaine	Chèze *et al.* (2007b)

LOD, limit of detection; LOQ, limit of quantitation; CSEI-Sweep-MEKC, cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography; ELISA, enzyme-linked immunosorbent assay; GC/MS/MS, gas chromatography with tandem mass spectrometry detection; GC/MS, gas chromatography with mass spectrometry detection; HS-SPME, headspace solid-phase microextraction; LC/SACI-MS/MS-SRM, liquid chromatography/surface-activated chemical ionization tandem mass spectrometry single reaction monitoring; LC//MS/MS, liquid chromatography with tandem mass spectrometric detection; LC/SACI-MS3-SRM, liquid chromatography/surface-activated chemical ionization multiple collisional stage mass spectrometry single reaction monitoring; LLE, liquid–liquid extraction; MISPE, molecularly imprinted solid-phase extraction; RIA, radioimmunoassay; SPDE, solid-phase dynamic extraction; —, no information available.

suction and collection on various types of absorbent swabs.

Several techniques may be used to collect stimulated saliva. The simplest involves tongue, cheek or lip movements without any external stimulus (Mucklow *et al.*, 1978). Chewing paraffin wax, Parafilm, teflon, rubber bands, gum base and chewing gum are usually referred to as mechanical methods of stimulating saliva production. Likewise, a lemon juice drop or citric acid can be placed in the mouth to provide a gustatory stimulus for saliva production (Crouch *et al.*, 2004). However, the stimulation of saliva production may present several problems which can compromise drug-testing accuracy. For instance, some drugs and/or metabolites have been shown to be absorbed by Parafilm, and citric acid stimulation changes saliva pH and may alter drug concentrations. On the other hand, citric acid and cotton have also been shown to alter immunoassay drug test results (Crouch, 2005).

A variety of commercial collection devices that promote easy, quick and reproducible collection are available. In general, these devices consist of a sorbent material that becomes saturated in the mouth of the donor, and after removal the oral fluid is recovered by applying pressure or by centrifugation. Examples of commercially available devices include Orasure® (Epitope, Inc., Beaverton, OR, USA), Omni-SAL® (Cozart Biosciences Ltd, Abington, UK), Salivette® (Sarstedt AG, Rommelsdorf, Germany), Drugwipe® (Securetec, Ottobrunn, Germany), Intercept® ORALscreen™ and Quantisal™ (Immunalysis Corp., Pomona, USA; Spihler, 2004; Samyn *et al.*, 2007). Care should be taken when using these collection devices, because deficient recovery of drugs from oral fluid in the absorbent and adsorption of the drug on device components are likely to occur (Lenander-Lumikari *et al.*, 1995; O'Neal *et al.*, 2000; Samyn *et al.*, 2007).

One of the advantages of saliva testing is that the sample is collected under direct supervision without loss of privacy. In consequence, the risk of an invalid specimen being provided or sample adulteration and/or substitution (which are likely to occur in urine analysis) is reduced. In addition, monitoring of oral fluid may be especially advantageous and important when multiple serial samples are needed or when drug concentrations in children are required (Kim *et al.*, 2002). Other advantages are that, in principle, saliva drug concentrations can be related to plasma free-drug concentrations and to the pharmacological effects of drugs.

On the other hand, drugs that are ingested orally as well as those that can be smoked may be detected in high concentrations in oral fluid following recent use, due to residual amounts of drug remaining in the oral cavity. Therefore, and for these substances, results may not be accurate because the drug concentration found in the oral fluid may not reflect the blood-drug concentration.

Another disadvantage of studying oral fluid is that people are sometimes unable to produce sufficient amounts of material for analysis. Moreover, an important feature of urine testing is the accuracy of the on-site tests to detect drugs of abuse in fresh samples. Unfortunately, this is not the current situation for oral fluid testing (Grönholm and Lillsunde, 2001; Kintz *et al.*, 2005a). In addition, oral fluid contains several macromolecules (mucopolysaccharides and mucoproteins), which make it less easily pipetted than for instance urine, and may not be available from all individuals at all times, since there are drugs that can inhibit saliva secretion and cause dry mouth. Furthermore, and because drug concentration on this sample depends on plasma drug concentrations, drugs that have a short plasma half-life and are cleared rapidly from the body are detectable in saliva for a short time only, which represents a potential disadvantage over hair, sweat or urine. In fact, saliva and blood have the shortest detection windows (Spihler, 2004).

Because of the above-mentioned advantages, oral fluid testing is an analytical tool used in therapeutic drug monitoring of various drugs (Horning *et al.* 1977; Bennett *et al.*, 2003; Quintela *et al.* 2005; Dams *et al.*, 2007), pharmacokinetic studies (Schepers *et al.*, 2003; Huestis and Cone, 2004; Huestis, 2005; Drummer, 2005; Kauert *et al.*, 2007), and detection of illicit drugs in impaired driving (Samyn *et al.*, 2002b; Kintz *et al.*, 2005a; Toennes *et al.*, 2005; Wylie *et al.*, 2005a,b; Laloup *et al.*, 2006; Concheiro *et al.*, 2007; Drummer *et al.*, 2007; Pehrsson *et al.*, 2007).

There is no doubt that one of the most impacting applications of oral fluid testing is in the assessment of drug-impaired driving, enabled not only by the development of several on-site collection devices, but also its easy and non-invasive sample collection procedure. In addition, the premise of a good correlation between oral fluid levels and blood levels means that oral fluid levels may be used to assess the degree of impairment of a driver.

Drugs of abuse are by far the most frequently detected substances in oral fluid specimens, because of their implications in workplace medicine and motor vehicle driving. Therefore, methods are described to detect opiates, cannabinoids, amphetamines, cocaine, benzodiazepines and other substances such as ketamine, GHB, antibiotics, analgesics, cyanides and other tobacco compounds, and sildenafil (Table 2).

One issue that is gaining popularity within law enforcement and traffic regulation agencies is that the initial testing of oral fluid for drugs can be made in the field, by means of on-site collecting devices. Several devices are commercially available for this purpose, including instruments that provide an electronic readout (e.g. Dräger DrugTest® and Orasure Uplink®, Cozart Rapiscan® and Drugread® hand photometer) and

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Table 2. Classes of compounds detected in oral fluid

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
COD, MOR, MAM, dihydrocodeine and metabolites	1 mL, SPE	GC/MS	10 ng/mL	Speckl *et al.* (1999)
COC, BE, EME, AEME, MOR, MAM, COD, AP, MDA, MDMA, MDEA, MBDB, ephedrine, THC, CBN, CBD, 11-hydroxy-D-9-tetrahydrocannabinol (OH-THC) and THC-COOH	SPE	GC/MS	—	Samyn and van Haeren (2000)
COD, MOR, MAM, hydrocodone, hydromorphone, and oxycodone	0.25–1 mL; SPE	GC/MS	2 ng/mL; 2 ng/mL for MOR and COD 2 ng/mL; 3 ng/mL for MAM, hydromorphone and oxycodone 3 ng/mL; 10 ng/mL for hydrocodone	Jones *et al.* (2002)
AP, MA, MDA, MDMA, MDEA, MOR, COD, COC, BE	200 µL; SPE	LC/MS/MS	1.07 ng/mL; 5.5 ng/mL for MDA 0.71 ng/mL; 8.5 ng/mL for MDMA 0.22–1.07 ng/mL; 2 ng/mL for other compounds	Mortier *et al.* (2002)
MOR, COD, dihydrocodeine, diacetylmorphine and MAM	0.025–0.5 mL; buffer solution and SPE	EIA, GC/MS	5 ng/mL; 5 ng/mL for all compounds	Cooper *et al.* (2005a)
COD, MOR, MAM	1 mL; LLE	GC/MS	0.7 ng/mL; 2.3 ng/mL for COD, 2.0 ng/mL; 6.7 ng/mL for MOR, 0.6 ng/mL; 2 ng/mL for MAM	Campora *et al.* (2006)
MOR, COD, MAM, acetylcodeine, and heroin	0.5 mL; methanol and ammonium acetate	LC–MS–MS	1 µg/mL; 2 µg/mL for MAM, COD and acetylcodeine 2 µg/mL; 6 µg/mL for MOR 6 µg/mL; 10 µg/mL for heroin	Phillips and Allen (2006)
THC	25–200 µL; LLE	Intercept MICRO-PLATE Enzyme Immunoassay and GC–MS–MS	0.37 ng/mL with Inmunoassay 0.2 mL with GC/MS/MS	Niedbala *et al.* (2001)
THC	200 µL; LLE	LC/MS	2 ng/mL; 2 ng/mL	Concheiro *et al.* (2004)
THC	100–500 µL; LLE	LC/MS/MS	0.5–0.1 ng/mL	Laloup *et al.* (2005b)
THC-COOH, THC	1 mL; LLE	ELISA and GC/MS	4 ng/mL for ELISA 2 pg/mL for GC/MS	Moore *et al.* (2006c)
11-nor-9-carboxytetrahydrocannabinol	100 µL; SPE	GC/MS/MS	10 pg/mL; 10 pg/mL	Day *et al.* (2006)
2-carboxy-tetrahydrocannabinol, THC, CBN, CBD	1 mL; SPE	GC/MS	0.5 ng/mL for THC and CBN 1 ng/mL for CBD and 2-carboxy-tetrahydrocannabinol	Moore *et al.* (2006d)
THC, THC-COOH	500 µL; LLE	LC-MS-TOF	0.05 ng/mL; 0.1 ng/mL for THC 0.2 ng/mL; 0.5 ng/mL for THC-COOH	Quintela *et al.* (2007)
MDMA, MDEA, AP	50 µL; methanol	LC–MS–MS	1–5 ng/mL	Samyn *et al.* (2002c)

Table 2. (*Continued*)

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
MDMA, MDA, MDEA, AP, MA, and ephedrine	50 µL; methanol	LC–MS–MS	0.15 µg/mL; 0.5 µg/mL for MDEA, 0.20 µg/mL; 0.5 µg/mL for MDMA, 0.5 µg/mL; 1 µg/mL for MDA and ephedrine, 0.2 µg/mL; 0.5 µg/mL for MA, 0.5 µg/mL; 0.5 µg/mL for AP	Wood *et al.* (2003)
MDMA, MDA, MDEA and MBDB	1 mL; LLE	HPLC with fluorescence detection	2 ng/mL; 10 ng/mL for all compounds	Concheiro *et al.* (2005a)
MDMA, MDA, MDEA, AP, AM, 3-hydroxy-4-methoxy-methamphetamine (HMMA) and 3-hydroxy-4-methoxy-amphetamine (HMA)	400 µL; SPE	GC/MS	2.5 ng/mL; 5 ng/mL for AP, MDA, MDMA, MDEA 1 ng/mL; 5 ng/mL for MA 5 ng/mL; 25 ng/mL for HMA 2.5 ng/mL; 25 ng/mL HMMA	Scheidweiler and Huestis (2006)
MA, AP	—	GC/MS	50 ng/mL; 2.5 ng/mL for MA 2.5 ng/mL for AP	Huestis and Cone (2007)
MDMA, MDA, MDEA, AP, MA	50 µL; LLE	GC/MS	5 ng/mL for MDA 25 ng/mL for the other compounds	Peters *et al.* (2007)
AP, MA, MDMA, MDA, MDEA and MBDB	0.5–1 mL; buffer solution and SPE	Cozart® RapiScan system and GC-MS	2 ng/mL; 5 ng/mL for AP, 1 ng/mL; 2 ng/mL for MA, 5 ng/mL; 5 ng/mL for MDA, 1 ng/mL; 5 ng/mL for MDMA, 2 ng/mL; 5 ng/mL for MDEA, and 1 ng/mL; 5 ng/mL for MBDB	Wilson *et al.* (2007)
AP	250 µL; buffer solution and SPE	Immunoassay; GC/MS	25 µg/L with inmunoassay —	Engblom *et al.* (2007)
COC, BE, COET	100 µL; SPE	LC/MS/TOF	1 µg/L; 10 µg/L for all compounds	Clauwaert *et al.* (2004)
COC, BE	—	GC/MS	1 ng/mL; 8 ng/mL	Jufer *et al.* (2006)
Flunitrazepam, 7-aminoflunitrazepam	1 mL; SPE	GC/MS	0.5 µg/mL; 10 µg/mL for flunitrazepam 10 µg/mL; 15 µg/mL for 7-aminoflunitrazepam	Samyn *et al.* (2002a)
Lorazepam	500 µL; LLE	LC-MS-MS	0.05 ng/mL; 1 ng/mL	Kintz *et al.* (2004)
Alprazolam, 7-aminoclonazepam, 7-aminoflunitrazepam, bromazepam, clobazam, diazepam, lorazepam, lormetazepam, midazolam, nordiazepam, oxazepam, temazepam, tetrazepam, triazolam, zaleplon, zopiclone and zolpidem	500 µL; LLE	LC-MS-MS	0.1–0.2 ng/mL	Kintz *et al.* (2005b)

Table 2. (*Continued*)

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
Midazolam, bromazepam, tetrazepam, alprazolam, lorazepam, triazolam, flunitrazepam, diazepam and lormetazepam	500 µL; LLE	LC-MS	0.2 ng/mL; 0.5 ng/mL for alprazolam, lorazepam and bromazepam, 0.1 ng/mL; 0.2 ng/mL for diazepam, flunitrazepam, lormetazepam, midazolam, tetrazepam and triazolam	Quintela *et al.* (2005)
Tetrazepam	500 µL; LLE	LC-MS-MS	0.03 ng/mL; 0.1 ng/mL	Concheiro *et al.* (2005b)
Oxazepam, temazepam, nordazepam, midazolam, OH-midazolam, alprazolam, OH-alprazolam, OH-Ethylflurazepam, lorazepam, lormetazepam, diazepam, zolpidem, zopiclone, nitrazepam	1.0–0.5 mL; LLE	EMIT and LC-MS-MS	10 ng/mL for EMIT 3.9 ng/mL; 13.1 ng/mL for Oxazepam, 2.4 ng/mL; 8.1 ng/mL for temazepam, 2.3 ng/mL; 7.6 ng/mL for nordazepam, 0.7 ng/mL; 2.3 ng/mL for midazolam, 0.3 ng/mL; 1.1 ng/mL for OH-midazolam, 0.3 ng/mL; 1.2 ng/mL alprazolam, 0.2 ng/mL; 0.5 ng/mL OH-alprazolam, 0.8 ng/mL; 2.7 ng/mL for OH-Ethylflurazepam, 0.3 ng/mL; 1.2 ng/mL for lorazepam, 0.4 ng/mL; 1.3 ng/mL for lormetazepam, 2.1 ng/mL; 6.9 ng/mL for diazepam, 3.0 ng/mL; 10.0 ng/mL for zolpidem, 3.9 ng/mL; 13.0 ng/mL for zopiclone, 0.0 ng/mL; 0.1 ng/mL for nitazepam	Smink *et al.* (2006)
Midazolam, 1′-hydroxymidazolam and 4-hydroxymidazolam	1 mL; LLE	LC-MS	0.025 ng/mL; 0.05 ng/mL	Link *et al.* (2007)
Temazepam, Oxazepam, Nordiazepam, Tetrazepam, Diazepam	500 µL; LLE	LC-MS-MS	0.20 ng/mL for temazepam and tetrazepam, 0.05 ng/mL for oxazepam, nordiazepam and diazepam	Laloup *et al.* (2007)
Diazepam, oxazepam, temazepam, nordiazepam, lorazepam, chlordiazepoxide, alprazolam, OH-hydroxyalprazolam, desalkylflurazepam, hydroxyethylflurazepam, clonazepam, 7-aminoclonazepam, flunitrazepam, and 7-aminoflunitrazepam.	0.4 mL; SPE	LC-MS-MS	0.02 ng/mL; 0.1 ng/mL for flunitrazepam and alprazolam 0.05 ng/mL; 0.1 ng/mL for oxazepam, diazepam, lorazepam, desalkylflurazepam, clonazepam, temazepam, hydroxyethylflurazepam and nordiazepam. 0.5 ng/mL; 1 ng/mL for 7-aminoclonazepam and chlordiazepoxide 0.2 ng/mL; 0.5 ng/mL for OH-alprazolam 0.1 ng/mL; 0.5 ng/mL for 7-aminoflunitrazepam	Ngwa *et al.* (2007)

Table 2. (*Continued*)

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
Ketamine	1 mL; SPE	LC-MS-MS	20 ng/mL; 45 ng/mL	Cheng *et al.* (2007)
GHB	1 mL; LLE	GC-MS	0.1 µg/mL; 0.1 µg/mL	Brenneisen *et al.* (2004)
GHB	100 µL; LLE		0.2 µg/mL	Abanades *et al.* (2007)
Metronidazole and spiramycin I	500 µL; LLE	LC-MS-MS	50 ng/mL for metronidazole 15 ng/mL for spiramycin I	Sagan *et al.* (2005)
Propoxyphene	0.25 mL; SPE	ELISA; GC/MS	40 ng/mL with ELISA 2 ng/mL; 5 ng/mL with GC/MS	Rana *et al.* (2006)
Meperidine, tramadol and oxycodone	0.25 mL; SPE	ELISA; GC/MS	25 ng/mL for oxycodone and 50 ng/mL for meperidine and tramadol with ELISA 10 ng/mL for all compounds with GC/MS	Moore *et al.* (2006c)
Nicotine and cotinine	2 mL; LLE	GC/MS	0.60 ng/mL; 0.011 µg/mL for nicotine and cotinine	Toraño *et al.* (2003)
Nicotine, cotinine, norcotinine, and *trans*-3-hydroxycotinine	0.5 mL; SPE	GC/MS	5 ng/mL; 5 ng/mL	Kim *et al.* (2005)
Cyanide, thiocyanate	0.5 mL; LLE	GC/MS	0.026 µg/mL	Paul and Smith (2006)
Sildenafil	3 mL; LLE	LC/MS	0.2 ng/mL; 0.5 ng/mL	Tracqui and Ludes (2003)

EIA, Cozart microplate enzyme immunoassay; EMIT, enzyme multiplied immunoassay technique; LC-MS-TOF, liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry.

hand-held cartridges that require visual identification, i.e. providing a visual readout (e.g. DrugWipe®, iScreen OFD™, OralScreen®, Oratect®, SalivaScreen™). The main advantage of these devices is that they provide a preliminary drug result within minutes without the need for sophisticated laboratory screening equipment. However, at the moment there is no objective way to assess its performance, because there is a lack of consistency in their specifications. For example, for some of them, cut-off concentrations are used to define their detectability, whereas for others concentrations are given when drugs can be detected (Drummer, 2006). The apparent sensitivity is often not defined in terms of consistency of detection in oral fluid specimens.

Taking these facts into account, the results provided by these devices must be confirmed in the laboratory. Laboratorial techniques include screening procedures by ELISA-based immunoassays, which in general are adequate for opioids (Barnes *et al.*, 2003; Kacinko *et al.*, 2004; Lachenmeier *et al.*, 2006), methadone (Cooper *et al.*, 2005b), cocaine (Kolbrich *et al.*, 2003; Kim *et al.*, 2003; Cooper *et al.*, 2004; Lachenmeier *et al.*, 2006), amphetamines (Kupiec *et al.*, 2002; Laloup *et al.*, 2005c; Cooper *et al.*, 2006), and buprenorphine (De Giovanni *et al.*, 2005) and provide a reliable means to screen oral fluid. Cannabis can be more difficult, particularly if the immunoassay has little cross-reactivity to THC. Nevertheless enzyme immunoassay has been successfully used for this drug (Niedbala *et al.*, 2001; Moore *et al.* 2006c). The same applies for benzodiazepines despite their low concentrations in oral fluid (Kemp *et al.*, 2002; Smink *et al.*, 2006).

Concerning sample preparation techniques, analytes can be extracted by liquid–liquid extraction (Campora *et al.*, 2006; Quintela *et al.*, 2007; Pujadas *et al.*, 2007), solid-phase extraction with different types of extraction cartridges (Mortier *et al.*, 2002; Jones *et al.*, 2002; Wood *et al.*, 2005; Ngwa *et al.*, 2007), or even solid-phase microextraction (Pragst, 2007).

The introduction of LC-MS as a routine laboratory technique has enabled the benefits of high-performance liquid chromatography (HPLC) separation techniques to be linked to the high sensitivity and specificity of MS (Drummer, 2006), which has assisted in the development of drug testing in oral fluid due to the relatively small sample volumes that can be obtained. In fact, concerning oral fluid analysis, sample volume may present a serious problem if several analyses are to be performed.

Mass-spectrometry-based methods have been also used to screen for a range of drugs (Allen *et al.*, 2005), some allowing the quantitation of several drugs (Gunnar *et al.*, 2005; Campora *et al.*, 2006; Scheidweiler and Huestis, 2006; Peters *et al.*, 2007; Concheiro *et al.*, 2007; Cone *et al.*, 2007). By the use of GC-MS or LC-MS including tandem mass spectrometry (MS-MS), low

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analytical limits can be achieved, even using sample volumes as low as 0.1 mL.

The main analytical problem in oral fluid testing is due to the nature of the sampling devices. Indeed, these systems contain several stabilizers and preservatives that are able to impair precise and accurate analyses. This issue is of particular importance when LC-MS/MS is used, as ion suppression or enhancement effects are usually observed. Therefore, care must be taken when developing new analytical procedures and the possibility that these effects affect the precision and accuracy of the assay must be documented. Several papers addressing this issue have been published (Dams *et al.*, 2003; Annesley, 2003).

Another point of concern in oral fluid testing is drug recovery from the collection device, which may have an undesirable effect on the accuracy of the assay. In fact, if the analytes are not fully recovered from the device, the drugs' concentration on the sample may be underestimated, with consequences for their oral fluid to plasma ratio.

There have been published some recovery studies using several collection devices, suggesting that desorption of drugs may limit the usefulness of some materials. For instance, Salivette® has poor recovery for THC but is reasonable for codeine, whereas the Cozart® collector has good recovery for THC (Drummer, 2006). For these reasons, more information on drug recovery and stability is required, particularly for those drugs that are likely to be measured in oral fluid.

Sweat

Sweat is a clear, hypotonic solution produced by two types of glands: eccrine and apocrine located in epidermis (Sato *et al.*, 1989). Water (99%) is its main constituent, as well as high concentrations of sodium and chloride. Low levels of potassium, glucose, lactic and piruvic acids and urea are also present. The sweat from apocrine glands also contains proteins and fatty acids.

Sweat acts physiologically by regulating body temperature, since its evaporation from the skin surface reduces the excess heat. Sweating is increased by nervousness, exercise, stress and nausea and decreased by cold. Sweat excretion is also affected by other factors, such as ambient temperature, relative humidity, body location (in general, sweat glands are distributed over the entire body, except for the lips, nipples and external genital organs), hormonal imbalances, overactive thyroid gland and the sympathetic nervous system, and certain foods and medications.

Between 300 and 700 mL/day of sweat is produced over the whole body, whereas 2–4 L/h may be produced by extensive exercise (Kintz and Samyn, 2000). Several mechanisms of incorporation of drugs into sweat have been suggested, including passive diffusion from blood into sweat glands and transdermal passage of drugs across the skin. Non-ionized basic drugs diffuse into sweat and become ionized as a result of its lower pH (5.8) as compared with blood (7.4) (Huestis *et al.*, 1999). Generally, parent drugs are found in sweat, rather than their polar metabolites, which usually predominate in urine (Follador *et al.*, 2004). As sweat glands are associated with hair, it is thought to be a major contributor to drug appearance in hair, as stated above.

The analysis of drugs in sweat is rarely performed because it is extremely difficult to estimate sweat volume and evaluate drug concentrations, and also to collect adequate quantities. Initially, sweat collection devices consisted of an occlusive bandage formed by one to three layers of filter paper or pieces of cotton, gauze or towel (Kintz and Samyn, 2000). Heat or chemicals (e.g. pilocarpine) were used to increase sweat production. However, this kind of patch was time-consuming to apply, uncomfortably large, prone to detachment and yielded a small volume of sweat for analysis. In addition, it was found to alter the steady-state pH of the skin, the types of bacteria that colonize the skin and the transport characteristics of the skin, producing skin irritation after approximately 24 h (Huestis *et al.*, 1999).

To overcome these difficulties, non-occlusive sweat collection devices have been developed, consisting of an adhesive layer on a thin transparent film of surgical dressing to which a rectangular absorbent pad is attached. Non-volatile substances from the environment cannot penetrate the transparent film, which is a semipermeable membrane over the pad that allows oxygen, water and carbon dioxide to pass through the patch, leaving the skin underneath healthy (Kintz and Samyn, 2000). During wearing of the patch, as sweat saturates the pad and slowly concentrates it, drugs present in sweat are retained, while water evaporates from the patch. Using this non-occlusive design it is not possible to quantitate the concentrations of analytes in sweat, since the whole volume of secreted sweat is not known. Nevertheless, the wear period may be extended (usually a wear period of seven days is used) without skin irritation. Therefore, a cumulative record of drug exposure can be obtained (Huestis *et al.*, 1999).

In addition, the sweat patches have a unique identification number, which aids with chain of custody and identification. On the other hand, the patch's design makes sample adulteration difficult, because attempts to remove it before the end of the collection period or tamper with it are readily visible to personnel trained to monitor the sweat patch.

Prior to attaching the patch, skin is cleaned with an alcohol wipe, both to remove external contamination from drugs in the environment and to improve patch adherence. Kidwell and Smith (2001) have shown that inappropriate cleansing of the skin prior to patch placement can result in contamination of the patch. Careful

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preparation of the skin prior to application of a collection device helps to reduce the possibility of bacterial growth and previous skin contamination.

Several papers have been published on sweat testing, including both chromatographic (using mass spectrometry) and immunoassay techniques, particularly for the determination of drugs of abuse. The main detected drugs are opiates, buprenorphine, amphetamines and designer drugs, cocaine and metabolites, and GHB. Prescription drugs, such as benzodiazepines and antipsychotics, have also been detected. A summary of the main detected drugs can be seen on Table 3.

In general, sweat testing has several advantages over blood and urine, including non-invasive collection, reduced opportunity for sample adulteration, and in some cases, longer detection windows than for plasma or urine. Since the patches are worn for several days, sweat analysis provides cumulative measure of drug exposure, detecting both parent drugs and metabolites. However, as stated above, there is a lack of information concerning dose–response relationships, and analytes are present in low concentrations. In addition, care should be taken during application and removal of the patch, because of the risk of contamination. Another drawback of sweat analysis is related to the impossibility of knowing accurately the volume of sample that was collected. In fact, there is considerably inter-individual variability, due to large variations in sweat production, which makes it difficult to quantitate the amount of sweat that is secreted over a period of time. Therefore, sweat testing is considered a qualitative monitoring method.

Meconium

Meconium is the first faecal matter passed by a neonate and it is identified most commonly by its dark green/black color and a lack of the odor of regular feces. It is a highly complex matrix consisting of water, mucopolysaccharides, bile salts, bile acids, epithelial cells and other lipids, as well as the residue of swallowed amniotic fluid (Gourley *et al.*, 1990; Ostrea *et al.*, 1994; Kwong and Ryan, 1997; Moore *et al.*, 1998; Chan *et al.*, 2004; Gareri *et al.*, 2006).

Despite being variably reported to form from within the first trimester (Browne *et al.*, 1992) to as late as five months of gestation (Kintz and Samyn, 2000), it is generally accepted that meconium begins to form at approximately 12 weeks of gestation, because it is at this time that fetal swallowing of amniotic fluid begins (Gareri *et al.*, 2006). The formation of meconium has been evidenced at this stage by the presence of cocaine found in the meconium of early gestational fetuses. Fetal swallowing is thought to be the mechanism by which drugs are concentrated in the meconium; as the fetus releases urine into the amniotic fluid, any excreted compounds and metabolites are then swallowed and ultimately deposited into the meconium (Browne *et al.*, 1992; Ostrea *et al.*, 2006). In addition to this mechanism, fetal exposure is a product of maternal consumption, metabolism and elimination, placental transfer and metabolism, and also fetal metabolism (Chan *et al.*, 2004).

Some authors state that meconium collection from a newborn is more successful than urine collection (Maynard *et al.*, 1991), which makes meconium the specimen of choice for detecting *in utero* drug exposure. Furthermore, drug concentrations in meconium generally are higher than in urine because of its accumulation over several months of gestation (Ostrea *et al.*, 2001; Bar-Oz *et al.*, 2003; Eyler *et al.*, 2005). These factors make meconium an optimal matrix for identifying *in utero* exposure as it is considered a preserved record of the ultimate exposure by the fetus.

Furthermore, it has been suggested that meconium is a more sensitive matrix to analyze for neurotoxicants in the environment when compared with other specimens, because of its wide window of exposure to these compounds. In fact, there is an ongoing study which compares the analysis of various matrices (maternal blood, maternal hair, infant hair, cord blood and meconium) to detect exposure to various pesticides. Preliminary results among 750 mother/infant dyads shows a significantly higher percentage of exposure by meconium analysis (Ostrea *et al.*, 2006).

One issue that should be taken into account when analyzing meconium is the possibility of urine contamination, which is likely to occur when a neonate has been exposed to a drug near-term and evacuates drug-contained urine into a meconium-soiled diaper, leading to an increase in the sensitivity of meconium screening due to the augmentation of drug levels in the specimen. However, this contamination can interfere with the development of dose–response relationships with regard to the level of drugs present in meconium. Indeed, in this case, the drugs have not been metabolized to the same degree as meconium-deposited drugs, and therefore the expected relationships between drugs and metabolites will be different in meconium.

Meconium collection is easy and non-invasive, and it is achieved by scraping the contents (0.5 g minimum) of the soiled diaper into a specimen collection container, for which contributes its thick and viscous nature. Studies indicate that drugs are stable in this specimen for up to 2 weeks at room temperature and for at least 1 year if frozen (Kintz and Samyn, 2000).

One major advantage of meconium is a relatively wide window for sample collection. Indeed, meconium contents can provide a history of a fetal swallowing and bile excretion, representing a window of prenatal drug exposure of about 20 weeks prepartum (Moore *et al.*, 1998; Kintz and Samyn, 2000). It represents, therefore,

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Table 3. Compounds detectable in sweat samples

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
Heroin, COC and metabolites	SPE	GC/MS	1 ng/patch for all compounds	Cone *et al.* (1994)
Heroin, MAM, MOR	Extraction with acetonitrile	GC/MS	0.5 ng/patch for heroin, 1 ng/patch for MOR and MAM	Kintz *et al.* (1997)
Metadone, heroin, MAM, MOR, COD	Extraction with buffer (methanol and sodium acetate)	ELISA and GC/MS	10 ng/mL with ELISA 5 ng/mL with GC/MS	Huestis *et al.* (2000)
COC, BE, EME, heroin, MAM, and MOR	Extraction with buffer	RIA and ELISA	25 ng/patch for COC and BE, 50 ng/patch for EME using ELISA, 2.5 ng/patch for COC, 50 ng/patch BE, 100 ng/patch EME using RIA, 1.7–2.5 ng/patch; 7.3–25.7 ng/patch heroin, MAM and MOR with ELISA	Moody and Cheever (2001)
COD, norcodeine, MOR, normorphine, and MAM	SPE	GC/MS	2.5 ng/patch; 2.5 ng/patch for COD and MOR, 5 ng/patch; 5 ng/patch for other analytes	Schwilke *et al.* (2006)
Methadone, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP), COC, BE, EME, MOR, COD, MAM	LLE	GC/MS	20 ng/patch; 50 ng/patch	Fucci *et al.* (2007)
Buprenorphine	—	—	—	Chawarski *et al.* (2007)
MDA, MDMA, MDEA, *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)	Review			Kintz and Samyn (1999)
MDMA, HMMA	No extraction	Drugwipe	—	Pacifíci *et al.* (2001)
MDMA, HMMA, MDA	No extraction/phosphate potassium buffer and SPE	Drugwipe, ELISA and GC/MS	1.4 ng/patch for MDMA with ELISA 0.96 ng/patch; 3.2 ng/patch for MDMA, 0.72 ng/patch; 2.4 ng/patch for MDA, and 0.96 ng/patch; 3.2 ng/patch for HMMA	Pichini *et al.* (2003)
AM, MA, MDA, MDMA, MDEA, 3-hydroxy-4-methoxymethamphetamine (HMMA), 3-hydroxy-4-methoxyamphetamine (HMA)	SPE	GC/MS	2.5 ng/patch; 2.5 ng/patch for AM, MA, MDEA and MDMA, 5 ng/patch; 5 ng/patch for MDA, HMMA and HMA	De Martinis *et al.* (2007)
COC, COD, BE, EME and other metabolites	SPE	GC/MS	1.25 ng/patch; 2.5 ng/patch for COC, BE, EME, 1.25–5.0 ng/patch for all other analytes	Huestis *et al.* (1999)
COC, COET	SPME	GC/MS	12.5 ng/patch	Follador *et al.* (2004)
COC, BE, and EME	Extraction with acetate/methanol buffer	GC/MS	4 ng/mL for COC, 2 ng/mL for BE, and 2 ng/mL for EME	Liberty *et al.* (2004)
COC, BE, and EME	SPE	RIA and GC/MS	4 ng/patch	Moody *et al.* (2004)
COC, BE and EME	SPE	GC/MS	2 ng/patch	Uemura *et al.* (2004)
GHB	Extraction with acetonitrile	GC/MS	—	Abanades *et al.* (2007)
Diazepam, nordiazepam, oxazepam	Extraction with methanol	GC/MS	0.01 ng/patch for diazepam 0.01 ng/patch for nordiazepam 0.005 ng/patch for oxazepam	Kintz *et al.* (1996)
Clozapine	Extraction with methanol	GC/MS	1 ng/patch	Cirimele *et al.* (2000)

a repository for many compounds that the fetus has been exposed to during gestation, including a wide variety of licit and illicit drugs, food additives and heavy metals (Bielawski *et al.*, 2005).

After birth, meconium is excreted by the neonate several times a day for the first 1–5 days postpartum. Studies using zinc coproporphyrin (a meconium-specific bile pigment) as a marker have determined that meconium is fully evacuated by 125 h post-natally (Gourley *et al.*, 1990). Despite these findings, viable analysis appears to be optimal via collection within 72 h, since in the later stages of meconium excretion a matrix of meconium and feces is produced. For instance, analysis of meconium for cocaine and opiates has demonstrated positive results upon third post-natal day sample collection (Ostrea *et al.*, 1998). In fact, this window of collection covers 99% of term infants, which pass their first formed stool by 48 h. On the other hand, in extremely low birth weight infants, which is of particular interest in the drug-exposed neonatal population, median age of first stool is 3 days, with 90% of infants passing their first stool by day 12 (Verna and Dhanireddy, 1993). For these reasons, the possibility of sample collection beyond 48 h post-natally is a remarkable advantage of this matrix (Ostrea, 2001). In general, it can be assumed that, in a general neonatal population, meconium can be reliably collected for drug analysis within the first three post-natal days.

As meconium is a very complex matrix, its analysis is often complicated by the presence of large amounts of interferences, resulting in lower sensitivity as compared with the urine, which is a much cleaner specimen.

Meconium analyses usually require a thorough, preliminary clean-up procedure, using liquid–liquid or solid-phase extraction, prior to any analytical assays. This step is very important, especially in GC-MS assays, where sensitivity and specificity are greatly influenced by background noise. Moore *et al.* (1995) compared several extraction techniques including methanol, acidified water, phosphate buffer with methanol, and glacial acetic acid and diphenylamine in acetone, the latter yielding the best sensitivity. The choice of extraction solvents is based on the physical–chemical characteristics of the drug. For example, Moriya *et al.* (1994) have used chloroform–isopropanol (3:1) to extract benzoylecgonine, methamphetamine, morphine and phencyclidine. However, for practical reasons, pure or buffered methanol has been the most widely used extraction medium (Kintz and Samyn, 2000).

Taking into account both matrix complexity and the implications of a positive result, strict criteria for the identification of substances in meconium are needed, since many matrix constituents may elute together with the compounds of interest. Therefore, except for the cases where the molecular ions are detected in the mass spectrum, the presence of breakdown ion masses alone may not be sufficient to identify unambiguously the compounds, unless specific ratios of target ion to qualifiers are also used.

Enzyme multiplied immunoassay test (EMIT) is the most commonly employed screening assay, but fluorescence polarization immunoassay (FPIA), radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) have also been utilized (Moore *et al.*, 1998; Gareri *et al.*, 2006). All these provide high sensitivity; for example, cut-off values for cocaine and metabolites are less than 50 ng/mg (Ostrea, 2001; Bar-Oz *et al.*, 2003; Moore *et al.*, 1995).

Meconium is particularly useful in documenting *in utero* drug exposure (Moore *et al.*, 1998; Gareri *et al.*, 2006; Lozano *et al.*, 2007; Gray and Huestes, 2007), and a number of papers have been published on the determination of several substances, such as cocaine and metabolites, opiates, cannabinoids, amphetamines, biomarkers of ethanol consumption, pesticides, phtalates, benzodiazepines, tobacco compounds and pollutants and metals, see Table 4.

Other

As stated above, sensitive and specific analytical techniques have enabled drug testing in other alternative and more complex matrices, with special focus on the early detection of exposure. This is the case for umbilical cord tissue and umbilical cord blood, amniotic fluid and vernix caseosa, which may be used to document *in utero* drug exposure.

Other alternative specimens, despite being scarcely used routinely in toxicology, do exist, for example pericardial fluid and skeletal muscle, mainly used in post-mortem situations (Garriott, 1991; Moriya and Hashimoto, 1999).

Umbilical cord tissue and umbilical cord blood. Umbilical cord tissue is a new alternative specimen, easily and non-invasively collected at birth, and may reflect a long window of drug detection, similarly to meconium. However, using the umbilical cord it is possible to perform the analyses immediately after birth, in contrast to meconium analysis, which is inevitably delayed for up to three days. Several substances have been detected in this specimen, such as amphetamines, opiates, cannabinoids and cocaine (Montgomery *et al.*, 2006), suggesting that it is more sensitive for detecting amphetamines than, for instance, meconium. Nonetheless, the interpretation of results is difficult, due to the few studies available to date (Gray and Huestis, 2007). The same goes for umbilical cord blood, which has been used to detect cotinine, antidepressants and cocaine (Moore *et al.*, 1993; Dempsey *et al.*, 1998; Pichini *et al.*, 2000; Hostetter *et al.*, 2000; Hendrick *et al.*, 2003). It is expected, however, that the window of detection in

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Table 4. Classes of compounds determined in meconium

Compound(s)	Sample preparation	Detection mode	LOD; LOQ	References
m- and *p*-Hydroxybenzoylecgonine	1 g; SPE	LC/MS	0.0015 and 0.0045 μg/g; 0.005 μg/g for *p*-hydroxybenzoylecgonine 0.0004 and 0.0013 μg/g; 0.005 μg/g for *m*-hydroxybenzoylecgonine	Pichini *et al.* (2005)
COC, BE, COD, MOR, MAM	0.5 g; LLE and SPE	GC/MS	30 ng/g; 40 ng/g for COC, BE and COD, 20 ng/g; 40 ng/g for MOR and MAM	López *et al.* (2007)
Pesticides	Review			Margariti *et al.* (2007)
Methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP)	1 g; LLE	EMIT-ETS™ and GC/MS	10 ng/g; 25 ng/g for methadone, 25 ng/g; 25 ng/g for EDDP, and 10 ng/g; 25 ng/g for EMDP	ElSohly *et al.* (2001)
Oxycodone	0.5 g; SPE	ELISA and GC/MS	100 ng/g with ELISA 50 ng/g with GC/MS	Le *et al.* (2005)
COD, MOR, Hydrocodone, MAM Hydromorphone, and Oxycodone	1 g; LLE	LC/MS/MS	1 ng/mL; 2 ng/mL	Coles *et al.* (2007)
Cannabinoids	Review			Staub (1999)
11-nor-9-Carboxy-Δ9-tetrahidrocannabinol and 11-hydroxy-Δ9-tetrahidrocannabinol	1 g; SPE	GC/MS	5 ng/g; 10 ng/g	Coles *et al.* (2005)
THC, THC-COOH, 11-hydroxy-Δ-tetrahydrocannabinol (THC-OH)	1 g; LLE	GC/MS	7 mg/g; 20 μg/g for all compounds	Marchei *et al.* (2006)
11-nor-9-Carboxy-Δ9-tetrahidrocannabinol and 11-Hydroxy-Δ9-tetrahidrocannabinol	1 g; SPE	EMIT and GC/MS	40 ng/g with EMIT 5 ng/g; 10 ng/g with GC/MS	Marin *et al.* (2007)
AP, MOR	500 mg no extraction and SPE	FPIA and HPLC/PDA	1.35 μg/g for AP, 0.35 μg/g for MOR with FPIA 0.5 μg/g with HPLC/PDA	Franssen *et al.* (1994)
Fatty acids ethyl esters	0.5–1 g; LLE and SPE	GC/MS	50 ng/g	Moore *et al.* (2003)
Fatty acid ethyl esters	Review			Caprara *et al.* (2007)
Fatty acids ethyl esters	1 g; LLE	GC/FID and GC/MS	2 pmo	Bearer *et al.* (2005)
Several classes of pesticides	0.5 g; SPE	GC/MS	0.10–4.15 μg/g	Bielawski *et al.* (2005)
Paraquat	0.5 g; LLE	GC/MS	0.0156 μg/g	Posecion *et al.* (2007)
Several classes of phtalates	0.5 g; LLE and SPE	LC/MS/MS	0.3–2.3 ng/mL	Kato *et al.* (2006)
Alprazolam	1 g; LLE	GC/MS	7 ng/mg; 20 ng/mg	García-Algar *et al.* (2007)
Nicotine, cotinine and *trans*-3-hydroxycotinine (OH-cotinine)	0.5 g; SPE	HPLC	20 pmol/L	Köhler *et al.* (2007)
Lead, cadmium, zinc, copper, iron	0.8–1 g; digestion acid	AAS	0.045 μg/mL for Pb, 0.009 μg/mL for Cd, 0.037 μg/mL for Zn, 0.080 μg/mL for Cu, 0.014 μg/mL for Fe	Turker *et al.* (2006)
Pollutants and neurotoxicants	—	—	—	Ostrea *et al.* (2006)
Polychlorinated biphenyls (PBCs), organochlorine pesticides	2–5 g; SPE	micro-EROD bioassay, GC/ECD	0.02–0.08 μg/kg for PCBs 0.04–0.10 μg/kg for organochlorine pesticides	Zhao *et al.* (2007)
Mercury	0.5–1 g	AAS	9.4 μg/g	Unuvar *et al.* (2007)

AAS, atomic absorption spectrophotometer; FPIA, fluorescence polarization immunoassay; GC/ECD, gas chromatography with electron capture detector; GC/FID, gas chromatography with flame ionization detector; HPLC/PDA, high-performance liquid chromatography with photodiode array detector.

cord blood will be quite short, as occurs with maternal blood specimens, being only useful for determining acute exposure to drugs of abuse in the period immediately previous to delivery (Lozano *et al.*, 2007; Gray and Huestis, 2007).

Amniotic fluid. This sample consists of a filtrate of maternal blood. Drugs can enter amniotic fluid by diffusion across the placenta and from excretion of fetal urine in the latter stages of gestation (Szeto, 1993; Gray and Huestis, 2007). Amniotic fluid acts as a fetal excretion reservoir, accumulating drugs throughout gestation. The fetus is potentially re-exposed to drugs excreted in urine due to continuous swallowing of amniotic fluid. Another possible route of exposure via amniotic fluid is transdermal diffusion, early in pregnancy when the skin is poorly developed and late in pregnancy when the production of vernix caseosa takes place.

The major disadvantage of amniotic fluid is sample collection. In fact, this sample can only be non-invasively collected at birth or as excess specimen from another necessary medical procedure (e.g. amniocentesis). In general, this sample is not collected for monitoring *in utero* drug exposure alone (Gray and Huestis, 2007; Lozano *et al.*, 2007). Few papers have been published on drug detection in amniotic fluid (Moore *et al.*, 1992, 1993; Ripple *et al.*, 1992; Jain *et al.*, 1993).

Vernix caseosa. Vernix caseosa is a thick, white lipid and cell mixture that covers the fetus starting at about 24 weeks of gestational age. This coating prevents direct contact of the forming fetal skin with amniotic fluid. This sample can be easily removed from a newborn's skin with gauze prior to the first bath (Gray and Huestis, 2007).

The mechanism of drug deposition into vernix caseosa is unknown, but it is possible that the drug is deposited from amniotic fluid. The only paper using this matrix was published by Moore *et al.* (1996), in which cocaine and metabolites were detected using solid-phase extraction and chromatographic analysis by GC/MS (Moore *et al.*, 1996).

Furthermore, the amount of vernix caseosa available for collection is limited, particularly in post-mortem situations, which makes weighing specimens, and therefore quantitative measurements, difficult (Gray and Huestis, 2007).

CONCLUSIONS AND PERSPECTIVES

The use of alternative specimens is gaining a tremendous importance in the field of toxicology, which is in part due to their non-invasive collection procedures. Analytical equipment is becoming more and more sensitive and specific, which enables both drug detection and quantitation in very low amounts, and analyses where concentrations are expected to be low, such as in hair and oral fluid. In fact, these are the most widely used unconventional specimens, particularly because of their potential use in forensic situations. Nevertheless, documentation of *in utero* drug exposure has a major appeal nowadays, where the fetus may be exposed during pregnancy to toxic substances, for instance drugs of abuse or pollutants. In these situations, meconium plays an important role, since it is a repository for many compounds to which the fetus has been exposed during gestation. While the main drawbacks are adequately dealt with in cases of hair and oral fluid analyses, further studies are needed with respect to the other matrices, particularly to aid the interpretation of results.

Concerning the complexity of these alternative matrices, it is generally mandatory that the samples are thoroughly cleaned up before chromatographic analysis can be performed, not only to overcome ion suppression/enhancement issues (which can compromise the methods' precision and accuracy), but also to improve the detection limits. In fact, the latter is a very important issue when analyzing these unconventional samples, since in most situations the amount of sample available is small.

The improvements seen in analytical technology have led to the enhancement of the methods' sensitivity and accuracy, providing better scientific understanding and improved test results interpretation. This analytical instrumentation is becoming accessible to most toxicological laboratories worldwide, and therefore it is expected that further applications of these unconventional matrices will appear in the near future.

REFERENCES

Abanades S, Farré M, Segura M, Pichini S, Pastor A, Pacifici R, Pellegrini M and de la Torre R. Disposition of gamma-hydroxybutyric acid in conventional and nonconventional biologic fluids after single drug administration: issues in methodology and drug monitoring. *Therapeutic Drug Monitoring* 2007; **29**: 64–70.

Acampora A, Della Casa E, Martone G and Miraglia N. Determination of opiates in biological samples through the analysis of heptafluorobutyric derivatives by gas chromatography/negative ion chemical ionization mass spectrometry. *Journal of Mass Spectrometry* 2003; **38**: 1007–1111.

Allen DL and Oliver JS. The use of supercritical fluid extraction for the determination of amphetamines in hair. *Forensic Science International* 2000; **107**: 191–199.

Allen KR, Azad R, Field HP and Blake DK. Replacement of immunoassay by LC tandem mass spectrometry for the routine measurement of drugs of abuse in oral fluid. *Annals of Clinical Biochemistry* 2005; **42**: 277–284.

Annesley T. Ion suppression in mass spectrometry. *Clinical Chemistry* 2003; **49**: 1041–1044.

Appenzeller BM, Agirman R, Neuberg P, Yegles M and Wennig R. Segmental determination of ethyl glucuronide in hair: a pilot study. *Forensic Science International* 2007; **173**: 87–92.

Aps JKM and Martens LC. The physiology of saliva and transfer of drugs into saliva. *Forensic Science International* 2005; **150**: 119–131.

Ariffin MM, Miller EI, Cormack PA and Anderson RA. Molecularly imprinted solid-phase extraction of diazepam and its metabolites from hair samples. *Analytical Chemistry* 2007; **79**: 256–262.

Báez H, Castro MM, Benavente MA, Kintz P, Cirimele V, Camarco C and Thomas C. Drugs in prehistory: Chemicals analysis of ancient human hair. *Forensic Science International* 2000; **108**: 173–179.

Balabanova S and Wolf HU. Methadone concentrations in human hair of the head, axillary. *Zietschrift für Rechtsmedische* 1989; **102**: 293–296.

Barnes AJ, Kim I, Schepers R, Moolchan ET, Wilson L, Cooper G, Reid C, Hand C and Huestis MA. Sensitivity, specificity, and efficiency in detecting opiates in oral fluid with the Cozart Opiate Microplate EIA and GCMS following controlled codeine administration. *Journal of Analytical Toxicology* 2003; **27**: 402–407.

Bar-Oz B, Klein J, Karaskov T and Koren G. Comparison of meconium and neonatal hair analysis for detection of gestational exposure to drugs of abuse. *Archive of Diseases of Childhood and Fetus Neonatal Edition* 2003; **88**: F98–F100.

Bearer CF, Santiago LM, O'Riordan MA, Buck K, Lee SC and Singer LT. Fatty Acid ethyl esters: quantitative biomarkers for maternal alcohol consumption. *Journal of Pediatrics* 2005; **146**: 824–830.

Bennett GA, Davies E and Thomas P. Is oral fluid analysis as accurate as urinalysis in detecting drug use in a treatment setting? *Drug Alcohol Dependancy* 2003; **72**: 265–269.

Bermejo AM, López P, Alvarez I, Tabernero MJ and Fernández P. Solid-phase microextraction for the determination of cocaine and cocaethylene in human hair by gas chromatography–mass spectrometry. *Forensic Science International* 2006; **156**: 2–8.

Bielawski D, Ostrea Jr E, Posecion Jr N, Corrion M and Seagraves J. Detection of several classes of pesticides and metabolites in meconium by gas chromatography–mass spectrometry. *Chromatographia*. 2005; **62**: 623–629.

Brenneisen R, Elsohly MA, Murphy TP, Passarelli J, Russmann S, Salamone SJ and Watson DE. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *Journal of Analytical Toxicology* 2004; **28**: 625–630.

Brewer WE, Galipo RC, Sellers KW and Morgan SL. Analysis of cocaine, benzoylecgonine, codeine, and morphine in hair by supercritical fluid extraction with carbon dioxide modified with methanol. *Analytical Chemistry* 2001; **73**: 2371–2376.

Browne SP, Tebbett IR, Moore CM, Dusick A, Covert R and Yee GT. Analysis of meconium for cocaine in neonates. *Journal of Chromatography* 1992; **575**: 158–161.

Cairns T, Hill V, Schaffer M and Thistle W. Levels of cocaine and its metabolites in washed hair of demonstrated cocaine users and workplace subjects. *Forensic Science International* 2004; **145**: 175–181.

Campora P, Bermejo AM, Tabernero MJ and Fernandez P. Use of gas chromatography/mass spectrometry with positive chemical ionization for the determination of opiates in human oral fluid. *Rapid Communications in Mass Spectrometry* 2006; **20**: 1288–1292.

Caprara DL, Nash K, Greenbaum R, Robet J and Koren G. Novel approaches to diagnosis of fetal alcohol spectrum disorder. *Neuroscience and Biobehavior Review* 2007; **31**: 254–260.

Chan D, Klein J and Koren G. New methods for neonatal drug screening. *NeoReviews* 2004; **4**: e1–e8.

Chawarski MC, Fiellin DA, O'Connor PG, Bernard M and Schottenfeld RS. Utility of sweat patch testing for drug use monitoring in outpatient treatment for opiate dependence. *Journal of Substance Abuse and Treatment* 2007; **33**: 411–415.

Cheng WC, Ng KM, Chan KK, Mok VK and Cheung BK. Roadside detection of impairment under the influence of ketamine–evaluation of ketamine impairment symptoms with reference to its concentration in oral fluid and urine. *Forensic Science International* 2007; **170**: 51–58.

Chèze M, Villain M and Pépin G. Determination of bromazepam, clonazepam and metabolites after a single intake in urine and hair by LC-MS/MS. Application to forensic cases of drug facilitated crimes. *Forensic Science International* 2004; **145**: 123–130.

Chèze M, Deveaux M, Martin C, Lhermitte M and Pépin G. Simultaneous analysis of six amphetamines and analogues in hair, blood and urine by LC-ESI-MS/MS. Application to the determination of MDMA after low ecstasy intake. *Forensic Science International* 2007a; **170**: 100–104.

Chèze M, Lenoan A, Deveaux M and Pépin G. Determination of ibogaine and noribogaine in biological fluids and hair by LC-MS/MS after Tabernanthe iboga abuse: Iboga alkaloids distribution in a drowning death case. *Forensic Science International* 2008; **176**: 58–66.

Chiarotti M, Strano-Rossi S, Offidani C and Fiori A. Evaluation of cocaine use during pregnancy through toxicological analysis of hair. *Journal of Analytical Toxicology* 1996; **20**: 555–558.

Cirimele V, Kintz P, Majdalani R and Mangin P. Supercritical fluid extraction of drugs in drug addict hair. *Journal of Chromatography B* 1995; **673**: 173–181.

Cirimele V, Kintz P, Gosselin O and Ludes B. Clozapine dose–concentration relationships in plasma, hair and sweat specimens of schizophrenic patients. *Forensic Science International* 2000; **107**: 289–300.

Cirimele V, Kintz P, Doray S and Ludes B. Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis. *International Journal of Legal Medicine* 2002; **116**: 54–57.

Clauwaert K, Decaestecker T, Mortier K, Lambert W, Deforce D, Van Peteghem C and Van Bocxlaer J. The determination of cocaine, benzoylecgonine, and cocaethylene in small-volume oral fluid samples by liquid chromatography–quadrupole–time-of-flight mass spectrometry. *Journal of Analytical Toxicology* 2004; **28**: 655–659.

Cognard E, Rudaz S, Bouchonnet S and Staub C. Analysis of cocaine and three of its metabolites in hair by gas chromatography–mass spectrometry using ion-trap detection for CI/MS/MS. *Journal of Chromatography B* 2005; **826**: 17–25.

Coles R, Clements TT, Nelson GJ, McMillin GA and Urry FM. Simultaneous analysis of the Δ9-THC metabolites 11-nor-9-carboxy-Δ9-THC and 11-hydroxy-Δ9-THC in meconium by GC–MS. *Journal of Analytical Toxicology* 2005; **29**: 522–527.

Coles R, Kushnir MM, Nelson GJ, McMillin GA and Urry FM. Simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, oxycodone, and 6-acetylmorphine in urine, serum, plasma, whole blood, and meconium by LC–MS–MS. *Journal of Analytical Toxicology* 2007; **31**: 1–16.

Concheiro M, de Castro A, Quintela O, Cruz A and Lopez-Rivadulla M. Development and validation of a method for the quantitation of Delta9tetrahydrocannabinol in oral fluid by liquid chromatography electrospray–mass-spectrometry. *J Chromatogr B* 2004; **810**: 319–324.

Concheiro M, de Casto A, Quintela O, Lopez-Rivadulla M and Cruz A. Determination of MDMA, MDA, MDEA and MBDB in oral fluid using high performance liquid chromatography with native fluorescence detection. *Forensic Science International* 2005a; **150**: 221–226.

Concheiro M, Villain M, Bouchet S, Ludes B, Lopez-Rivadulla M and Kintz P. Windows of detection of tetrazepam in urine, oral fluid, beard, and hair, with a special focus on drug-facilitated crimes. *Therapeutic Drug Monitoring* 2005b; **27**: 565–570.

Concheiro M, de Castro A, Quintela O, Cruz A and Lopez-Rivadulla M. Confirmation by LC-MS of drugs in oral fluid obtained from roadside testing. *Forensic Science International* 2007; **170**: 156–162.

Cone EJ, Hillsgrove MJ, Jenkins AJ, Keenan RM and Darwin WD. Sweat testing for heroin, cocaine, and metabolites. *Journal of Analytical Toxicology* 1994; **18**: 298–305.

Cone EJ, Clarke J and Tsanaclis L. Prevalence and disposition of drugs of abuse and opioid treatment drugs in oral fluid. *Journal of Analytical Toxicology* 2007; **31**: 424–433.

Cooper G, Wilson L, Reid C, Baldwin D, Hand C and Spieher V. Validation of the Cozart microplate EIA for cocaine and metabolites in oral fluid. *Journal of Analytical Toxicology* 2004; **28**: 498–503.

Cooper G, Wilson G, Reid C, Baldwin D, Hand C and Spiehler V. Validation of the Cozart microplate EIA for analysis of opiates in oral fluid. *Forensic Science International* 2005a; **154**: 240–246.

Cooper G, Wilson L, Reid C, Baldwin D, Hand C and Spiehler V. Comparison of GC-MS and EIA results for the analysis of methadone in oral fluid. *Journal of Forensic Science* 2005b; **50**: 928–932.

Cooper G, Wilson L, Reid C, Hand C and Spiehler V. Validation of the cozart amphetamine microplate EIA for the analysis of amphetamines in oral fluid. *Forensic Science International* 2006; **159**: 104–112.

Cordero R and Paterson S. Simultaneous quantification of opiates, amphetamines, cocaine and metabolites and diazepam and metabolite in a single hair sample using GC–MS. *Journal of Chromatography B* 2007; **850**: 423–431.

Couper FJ, McIntyre IM and Drummer OH. Extraction of psychotropic drugs from human scalp hair. *Journal of Forensic Science* 1995; **40**: 83–86.
Cristoni S, Basso E, Gerthoux P, Mocarelli P, Gonella E, Brambilla M, Crotti S and Bernardi R. Surface-activated chemical ionization ion trap mass spectrometry for the analysis of cocaine and benzoylecgonine in hair after extraction and sample dilution. *Rapid Communications in Mass Spectrometry* 2007; **21**: 2515–2553.
Crouch DJ. Oral fluid collection: The neglected variable in oral fluid testing. *Forensic Science International* 2005; **150**: 165–173.
Crouch DJ, Day J and Baudys J. Evaluation of saliva/oral fluid as an alternate drug testing specimen—final report. National Institute of Standards and Technology (NISTR 7139. Gaithersburg, MD), July 2004; 1–70.
Dams R, Huestis MA, Lambert WE and Murphy CM. Matrix effect in bio-analysis of illicit drugs with LC–MS/MS: influence of ionization type, sample preparation, and biofluid. *Journal of the American Society of Mass Spectrometry* 2003; **14**: 1290–1294.
Dams R, Choo RE, Lambert WE, Jones H and Huestis MA. Oral fluid as an alternative matrix to monitor opiate and cocaine use in substance-abuse treatment patients. *Drug Alcohol Dependancy* 2007; **87**: 258–267.
Day D, Kuntz DJ, Feldman M and Presley L. Detection of THCA in oral fluid by GC-MS-MS. *Journal of Analytical Toxicology* 2006; **30**: 645–50.
De Giovanni N, Fucci N, Scarlata S and Donzelli G. Buprenorphine detection in biological samples. *Clinical Chemistry Laboratory Medicine* 2005; **43**: 1377–1379.
De Giovanni N, Donaldio G and Chiarotti M. The reliability of fatty acid ethyl esters (FAEE) as biological markers for the diagnosis of alcohol abuse. *Journal of Analytical Toxicology* 2007; **31**: 93–97.
De Martinis BS, Barnes AJ, Scheidweiler KB and Huestis MA. Development and validation of a disk solid phase extraction and gas chromatography–mass spectrometry method for MDMA, MDA, HMMA, HMA, MDEA, methamphetamine and amphetamine in sweat. *Journal of Chromatography B* 2007; **852**: 450–458.
Dempsey DA, Partridge JC, Jones RT and Rowbotham MC. Cocaine, nicotine, caffeine, and metabolite plasma concentrations in neonates. *Journal of Analytical Toxicology* 1998; **22**: 220–224.
Dizioli Rodrigues de Oliveira C, Yonamine M and Lúcia de Moraes Moreau R. Headspace solid-phase microextraction of cannabinoids in human head hair samples. *Journal of Separation Science* 2007; **30**: 128–134.
Doherty B, Rodriguez V, Leslie JC, McClean S and Smyth WF. An electrospray ionisation tandem mass spectrometric investigation of selected psychoactive pharmaceuticals and its application in drug and metabolite profiling by liquid chromatography/electrospray ionisation tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 2007; **21**: 2031–2038.
Drummer OH. Review: Pharmacokinetics of illicit drugs in oral fluid. *Forensic Science International* 2005; **150**: 133–142.
Drummer OH. Drug testing in oral fluid. *Clinical Biochemistry Revies* 2006; **27**: 147–159.
Drummer OH, Gerostamoulos D, Chu M, Swann P, Boorman M and Cairns I. Drugs in oral fluid in randomly selected drivers. *Forensic Science International* 2007; **170**: 105–110.
ElSohly MA, Feng S and Murphy TP. Analysis of methadone and its metabolites in meconium by enzyme immunoassay (EMIT®) and GC–MS. *Journal of Analytical Toxicology* 2001; **25**: 40–44.
Engblom C, Gunnar T, Rantanen A and Lillsunde P. Driving under the influence of drugs-amphetamine concentrations in oral fluid and whole blood samples. *Journal of Analytical Toxicology* 2007; **31**: 276–280.
Eser HP, Pötsch L, Skopp G and Moeller MR. Influence of sample preparation on analytical results: drug analysis (GC/MS) of hair snippets versus hair powder using various extraction methods. *Forensic Science International* 1997; **84**: 271–279.
Eyler FD, Behnke M, Wobie K, Garvan CW and Tebbett I. Relative ability of biologic specimens and interviews to detect prenatal cocaine use. *Neurotoxicology and Teratology* 2005; **27**: 677–687.
Follador MJD, Yonamine M and Silva RL. Detection of cocaine and cocaethylene in sweat by solid-phase microextraction and gas chromatography/mass spectrometry. *Journal of Chromatography B* 2004; **811**: 37–40.
Franssen RM, Stolk LM, van den Brand W and Smit BJ. Analysis of morphine and amphetamine in meconium with immunoassay and HPLC-diode-array detection. *Journal of Analytical Toxicology* 1994; **18**: 294–295.
Fucci N, De Giovanni N and Scarlata S. Sweat testing in addicts under methadone treatment: an Italian experience. *Forensic Science International* 2008; **174**: 107–110.
Gambelunghe C, Sommavilla M, Ferranti C, Rossi R, Aroni K, Manes N and Bacci M. Analysis of anabolic steroids in hair by GC/MS/MS. *Biomedical Chromatography* 2007; **21**: 369–375.
García-Algar O, López-Vílchez Ma, Martín I, Mur A, Pellegrini M, Pacifici R, Rossi S and Pichini S. Confirmation of gestational exposure to alprazolam by analysis of biological matrices in a newborn with neonatal sepsis. *Clinical Toxicology* 2007; **45**: 295–298.
Garcia-Bournissen F, Rokach B, Karaskov T and Koren G. Cocaine detection in maternal and neonatal hair: implications to fetal toxicology. *Therapeutic Drug Monitoring* 2007; **29**: 71–76.
Gareri J, Klein J and Koren G. Drugs of abuse testing in meconium. *Clinica Chimica Acta* 2006; **366**: 101–111.
Garriott JC. Skeletal muscle as an alternative specimen for alcohol and drug analysis. *Journal of Forensic Science* 1991; **36**: 60–69.
Girod C and Staub C. Analysis of drugs of abuse in hair by automated solid-phase extraction, GC/EI/MS and GC ion trap/CI/MS. *Forensic Science International* 2000; **107**: 261–271.
Goullé JP, Chèze M and Pépin G. Determination of endogenous levels of GHB in human hair. Are there possibilities for the identification of GHB administration through hair analysis in cases of drugfacilitated sexual assault? *Journal of Analytical Toxicology* 2003; **27**: 574–580.
Gourley GR, Kreamer B and Arend R. Excremental studies in human neonates. Identification of zinc coproporphyrin as a marker for meconium. *Gastroenterology* 1990; **99**: 1705–1709.
Gray T and Huestis M. Bioanalytical procedures for monitoring in utero drug exposure. *Analytical and Bioanalytical Chemistry* 2007; **388**: 1455–1465.
Grönholm M and Lillsunde P. A comparison between on-site immunoassay drug-testing devices and laboratory results. *Forensic Science International* 2001; **121**: 37–46.
Gunnar T, Ariniemi K and Lillsunde P. Validated toxicological determination of 30 drugs of abuse as optimized derivatives in oral fluid by long column fast gas chromatography/electron impact mass spectrometry. *Journal of Mass Spectrometry* 2005; **40**: 739–753.
Hammer DI, Finklea JF, Hendricks RH, Shy CM and Horton RJ. Hair traces metal levels and environmental exposure. *American Journal of Epidemiology* 1971; **93**: 84–92.
Han E, Yang W, Lee J, Park Y, Kim E, Lim M and Chung H. Correlation of methamphetamine results and concentrations between head, axillary, and pubic hair. *Forensic Science International* 2005; **147**: 21–24.
Han E, Park Y, Yang W, Lee J, Lee S, Kim E, Lim M and Chung H. The study of metabolite-to-parent drug ratios of methamphetamine and methylenedioxymethamphetamine in hair. *Forensic Science International* 2006; **161**: 124–129.
Harkley MR and Henderson GL. Hair analysis for drugs of abuse. In *Advances in Analytical Toxicology*, Baselt RC (ed.). Year Book Medical: Chicago, IL, 1989; 298–329.
Hartwig S, Auwärter V and Pragst. Effect of hair care and hair cosmetics on the concentrations of fatty acid ethyl esters in hair as markers of chronically elevated alcohol consumption. *Forensic Science International* 2003; **131**: 90–97.
Hendrick V, Stowe ZN, Altshuler LL, Hwang S, Lee E and Haynes D. Placental passage of antidepressant medications. *American Journal of Psychiatry* 2003; **160**: 993–996.
Hill V, Cairns T, Cheng CC and Schaffer M. Multiple aspects of hair analysis for opiates: methodology, clinical and workplace populations, codeine, and poppy seed ingestion. *Journal of Analytical Toxicology* 2005; **29**: 696–703.
Horning MG, Brown L, Nowlin J, Lertratanangkoon K, Kellaway P and Zion TE. Use of saliva in therapeutic drug monitoring. *Clinical Chemistry* 1977; **23**: 157–164.
Hostetter A, Ritchie JC and Stowe ZN. Amniotic fluid and umbilical cord blood concentrations of antidepressants in three women. *Biological Psychiatry* 2000; **48**: 1032–1034.

Huestis MA. Pharmacokinetics and metabolism of the plant cannabinoids, delta9-tetrahydrocannabinol, cannabidiol and cannabinol. *Handbook of Experimental Pharmacology* 2005; **168**: 657–90.
Huestis MA and Cone EJ. Relationship of Delta 9-tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis. *Journal of Analytical Toxicology* 2004; **28**: 394–399.
Huestis MS and Cone EJ. Methamphetamine disposition in oral fluid, plasma, and urine. *Annals of the New York Academy of Science* 2007; **1098**: 104–21.
Huestis MA, Oyler JM, Cone EJ, Wstadik AT, Schoendorfer D and Joseph Jr RE. Sweat testing for cocaine, codeine and metabolites by gas chromatography–mass spectrometry. *Journal of Chromatography B* 1999; **733**: 247–264.
Huestis MA, Cone EJ, Wong CJ, Umbricht A and Preston KL. Monitoring opiate use in substance abuse treatment patients with sweat and urine drug testing. *Journal of Analytical Toxicology* 2000; **24**: 509–521.
Huestis MA, Gustafson RA, Moolchan ET, Barnes A, Bourland JA, Sweeney SA, Hayes EF, Carpenter PM and Smith ML. Cannabinoid concentrations in hair from documented cannabis users. *Forensic Science International* 2007; **169**: 129–136.
Jain L, Meyer W, Moore C, Tebbett I, Gauthier D and Vidyasagar D. Detection of fetal cocaine exposure by analysis of amniotic fluid. *Obstetrics and Gynecolology* 1993; **81**: 787–790.
Jones J, Tomlinson K and Moore C. The simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, 6-acetylmorphine, and oxycodone in hair and oral fluid. *Journal of Analytical Toxicology* 2002; **26**: 171–175.
Jufer R, Walsh SL, Cone EJ and Sampson-Cone A. Effect of repeated cocaine administration on detection times in oral fluid and urine. *Journal of Analytical Toxicology* 2006; **30**: 458–462.
Jurado C, Menéndez M, Repetto M, Kintz P, Cirimele V and Mangin P. Hair testing for cannabis in Spain and France: Is there difference in consumption? *Journal of Analytical Toxicology* 1996; **20**: 111–115.
Kacinko SL, Barnes AJ, Kim I, *et al.* Performance characteristics of the Cozart RapiScan oral fluid drug testing system for opiates in comparison to ELISA and GC/MS following controlled codeine administration. *Forensic Science International* 2004; **141**: 41–48.
Kalasinsky KS, Dixon MM, Schmunk GA and Kish SJ. Blood, brain, and hair GHB concentrations following fatal ingestion. *Journal of Forensic Science* 2001; **46**: 728–730.
Kato K, Silva MJ, Needham LL and Calafat AM. Quantifying phthalate metabolites in human meconium and semen using automated off-line solid-phase extraction coupled with on-line SPE and isotope-dilution high-performance liquid chromatography-tandem mass spectrometry. *Analytical Chemistry* 2006; **78**: 6651–6655.
Kauert GF, Ramaekers JG, Schneider E, Moeller MR and Toennes SW. Pharmacokinetic properties of delta9-tetrahydrocannabinol in serum and oral fluid. *Journal of Analytical Toxicology* 2007; **31**: 288–93.
Kemp P, Sneed G, Kupiec T and Spiehler V. Validation of a microtiter plate ELISA for screening of post-mortem blood for opiates and benzodiazepines. *Journal of Analytical Toxicology* 2002; **26**: 504–512.
Kidwell DA, Blanco MA and Smith FP. Cocaine detection in a university population by hair analysis and skin swab testing. *Forensic Science International* 1997; **84**: 75–86.
Kidwell DA and Blank DL. Environmental exposure—the stumbling block of hair testing. In *Drug Testing in Hair*, Kintz P (ed.). CRC Press: Boca Raton, FL, 1996; 17–68.
Kidwell DA and Smith FP. Susceptibility of PharmChek™ drugs of abuse patch to environmental contamination. *Forensic Science International* 2001; **116**: 89–106.
Kim I, Barnes AJ, Oyler JM, Schepers R, Joseph Jr RE, Cone EJ, Lafko D, Moolchan ETRE and Huestis MA. Plasma and oral fluid pharmacokinetics and pharmacodynamics after oral codeine administration. *Clinical Chemistry* 2002; **48**: 1486–1496.
Kim I, Barnes AJ, Schepers R, Moolchan ET, Wilson L, Cooper G, Reid C, Hand C and Huestis M. Sensitivity and specificity of the Cozart microplate EIA cocaine oral fluid at proposed screening and confirmation cutoffs. *Clinical Chemistry* 2003; **49**: 1498–1503.
Kim I, Darwin W and Huestis MA. Simultaneous determination of nicotine, cotinine, norcotinine, and *trans*-3-hydroxycotinine in human oral fluid using solid phase extraction and gas chromatography–mass spectrometry. *Journal of Chromatography B*, 2005: **814**: 233–240.
Kim JY and In MK. Determination of 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid in hair using gas chromatography/tandem mass spectrometry in negative ion chemical ionization mode. *Rapid Communications in Mass Spectrometry* 2007; **21**: 1339–1342.
Kim JY, Suh SI, In MK, Paeng KJ and Chung BC. Simultaneous determination of cannabidiol, cannabinol, and delta9-tetrahydrocannabinol in human hair by gas chromatography–mass spectrometry. *Archives of Pharmaceutical Reseasrch* 2005; **28**: 1086–1091.
Kintz P. Value of hair analysis in post-mortem toxicology. *Forensic Science International* 2004; **142**: 127–134.
Kintz P. Bioanalytical procedures for detection of chemical agents in hair in the case of drug-facilitated crimes. *Analytical and Bioanalytical Chemistry* 2007; **388**: 1467–1474.
Kintz P and Samyn N. Determination of 'Ecstasy' components in alternative biological specimens. *Journal of Chromatography B* 1999; **733**: 137–143.
Kintz P and Samyn N. Unconventional samples and alternative matrices. In *Handbook of Analytical Separations*, Vol. 2, Bogusz MJ (ed.). Elsevier Science: Amsterdam, 2000; 459–488.
Kintz P, Cirimele V, Tracqui A and Mangin P. Simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioximethamphetamine in human hair by gas chromatography–mass spectrometry. *Journal of Chromatography B* 1995; **670**: 162–166.
Kintz P, Tracqui A and Mangin P. Sweat testing for benzodiazepines. *Journal of Forensic Science* 1996; **41**: 851–854.
Kintz P, Brenneisen R, Bundeli P and Mangin P. Sweat testing for heroin and metabolites in a heroin maintenance program. *Clinical Chemistry* 1997; **43**: 736–739.
Kintz P, Bundeli P, Brenneisen R and Ludes B. Dose–concentration relationships in hair from subjects in a controlled heroin-maintenance program. *Journal of Analytical Toxicology* 1998; **22**: 231–236.
Kintz P, Villain M, Cirimele V, Pepin G and Ludes B. Windows of detection of lorazepam in urine, oral fluid and hair, with a special focus on drug-facilitated crimes. *Forensic Science International* 2004; **145**: 131–135.
Kintz P, Bernhard W, Villain M, Gasser M, Aebi B and Cirimele V. Detection of cannabis use in drivers with the drugwipe device and by GC–MS after Intercept® device collection. *Journal of Analytical Toxicology* 2005a; **28**: 724–727.
Kintz P, Villain M, Concheiro M and Cirimele V. Screening and confirmatory method for benzodiazepines and hypnotics in oral fluid by LC-MS/MS. *Forensic Science International* 2005b; **150**: 213–220.
Kintz P, Villain M, Dumestre V and Cirimele V. Evidence of addiction by anesthesiologists as documented by hair analysis. *Forensic Sci International* 2005c; **153**: 81–84.
Kintz P, Villain M, Pélissier AL, Cirimele V and Leonetti G. Unusually high concentrations in a fatal GHB case. *Journal of Analytical Toxicology* 2005d; **29**: 582–585.
Kintz P, Villain M and Cirimele V. Determination of trimeprazine-facilitated sedation in children by hair analysis. *Journal of Analytical Toxicology* 2006; **30**: 400–402.
Kłys M, Scisłowski M, Rojek S and Kołodziej J. A fatal clomipramine intoxication case of a chronic alcoholic patient: application of postmortem hair analysis method of clomipramine and ethyl glucuronide using LC/APCI/MS. *Legal Medicine* 2005; **7**: 319–325.
Kłys M, Rojek S, Woźniak K and Rzepecka-Woźniak E. Fatality due to the use of a designer drug MDMA (Ecstasy). *Legal Medicine* 2007; **9**: 185–191.
Köhler E, Avenarius S, Rabsilber A, Gerloff C and Jorch G. Assessment of prenatal tobacco smoke exposure by determining nicotine and its metabolites in meconium. *Human Experimental Toxicology* 2007; **26**: 535–544.
Kolbrich EA, Kim I, Barnes AJ, *et al.* Cozart RapiScan oral fluid drug testing system: an evaluation of sensitivity, specificity, and efficiency for cocaine detection compared with ELISA and GC-MS following controlled cocaine administration. *Journal of Analytical Toxicology* 2003; **27**: 407–411.

Kopito L, Byers RK and Shwachman H. Lead in hair of children with chronic lead poisoning. *New England Journal of Medicine* 1967; **276**: 949–953.
Koren G, Chan D, Klein J and Karaskov T. Estimation of fetal exposure to drugs of abuse, environmental tobacco smoke, and ethanol. *Therapeutic Drug Monitoring* 2002; **24**: 23–25.
Kronstrand R, Nyström I, Josefsson M and Hodgins S. Segmental ion spray LC-MS-MS analysis of benzodiazepines in hair of psychiatric patients. *Journal of Analytical Toxicology* 2002; **26**: 479–484.
Kronstrand R, Roman M, Hedman M, Ahlner J and Dizdar N. Dose–hair concentration relationship and pigmentation effects in patients on low-dose clozapine. *Forensic Science Medicine and Pathology* 2007; **3**: 107–114.
Kupiec T, DeCicco L, Spiehler V, Sneed G and Kemp P. Choice of an ELISA assay for screening post-mortem blood for amphetamine and/or methamphetamine. *Journal of Analytical Toxicology* 2002; **26**: 513–518.
Kwong TC and Ryan RM. Detection of intrauterine illicit drug exposure by newborn drug testing. National academy of clinical biochemistry. *Clinical Chemistry* 1997; **43**: 235–242.
Lachenmeier K, Musshoff F and Madea B. Determination of opiates and cocaine in hair using automated enzyme immunoassay screening methodologies followed by gas chromatographic-mass spectrometric (GC-MS) confirmation. *Forensic Science International* 2006; **159**: 189–199.
Laloup M, Ramirez Fernandez MM, De Boeck G, Wood M, Maes V and Samyn N. Validation of a liquid chromatography–tandem mass spectrometry method for the simultaneous determination of 26 benzodiazepines and metabolites, zolpidem and zopiclone, in blood, urine, and hair. *Journal of Analytical Toxicology* 2005a; **29**: 616–626.
Laloup M, Ramirez Fernandez MM, Wood M, De boeck G, Maes V and Samyn N. Quantitative analysis of delta9-tetrahydrocannabinol in preserved oral fluid by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A* 2005b; **1082**: 15–24.
Laloup M, Tilman G, Maes V, *et al.* Validation of an ELISA-based screening assay for the detection of amphetamine, MDMA and MDA in blood and oral fluid. *Forensic Science International* 2005c; **153**: 29–37.
Laloup M, Ramirez Fernandez MM, Wood M, De Boeck G, Maes V and Samyn N. Correlation of Delta9-tetrahydrocannabinol concentrations determined by LC-MS-MS in oral fluid and plasma from impaired drivers and evaluation of the on-site Drager DrugTest. *Forensic Science International* 2006; **161**: 175–179.
Laloup M, Fernandez MM, Wood M, Maes V, De Boeck G, Vanbeckevoort Y and Samyn N. Detection of diazepam in urine, hair and preserved oral fluid samples with LC-MS-MS after single and repeated administration of Myolastan and Valium. *Analytical and Bioanalytical Chemistry* 2007; **388**: 1545–1556.
Le NL, Reiter A, Tomlinson K, Jones J and Moore C. The detection of oxycodone in meconium specimens. *Journal of Analytical Toxicology* 2005; **29**: 54–57.
Lenander-Lumikari M, Johansson I, Vilja P and Samaranayake LP. Newer saliva collection methods and saliva composition: a study of two Salivette kits. *Oral Disease* 1995; **1**: 86–91.
Liberty HJ, Johnson BD and Fortner N. Detecting cocaine use through sweat testing: multilevel modeling of sweat patch length-of-wear data. *Journal of Analytical Toxicology* 2004; **28**: 667–683.
Lin YH, Lee MR, Lee RJ, Ko WK and Wu SM. Hair analysis for methamphetamine, ketamine, morphine and codeine by cation -selective exhaustive injection and sweeping micellar electrokinetic chromatography. *Journal of Chromatography A*. 2007; **1145**: 234–240.
Link B, Haschke M, Wenk M and Krahenbuhl S. Determination of midazolam and its hydroxy metabolites in human plasma and oral fluid by liquid chromatography/electrospray ionization ion trap tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 2007; **21**: 1531–1540.
Liu RH, Liu HC and Lin DL. Distribution of methylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA) in postmortem and antemortem specimens. *Journal of Analytical Toxicology* 2006; **30**: 545–550.
López P, Bermejo AM, Tabernero MJ, Fernández P and Alvarez I. Determination of cocaine and heroin with their respective metabolites in meconium by gas chromatography–mass spectrometry. *Journal of Applied Toxicology* 2007; **27**: 464–471.
Lozano J, García-Algar O, Vall O, de la Torre R, Scaravelli G and Pichini S. Biological matrices for the evaluation of in utero exposure to drugs of abuse. *Therapeutic Drug Monitoring* 2007; **29**: 711–734.
Lucas ACS, Bermejo AM, Tabernero MJ, Fernández P and Strano-Rossi S. Use of solid-phase microextraction (SPME) for the determination of methadone and EDDP in human hair by CG–MS. *Forensci Science International* 2000; **107**: 225–232.
Malamud D. Guidelines for saliva nomenclature and collection. *Annals of the New York Academy of Sciences* 1993; **694**: xi–xii.
Mangin P and Kintz P. Variability of opiates concentrations in human hair according to their anatomical origin: head, axillary and pubic regions, *Forensic Science International* 1993; **63**: 77–83.
Marchei E, Pellegrini M, Pacifici R, Palmi L, Lozano J, Garcia-Algar O and Pichini S. Quantification of Delta9-tetrahydrocannabinol and its major metabolites in meconium by gas chromatographic-mass spectrometric assay: assay validation and preliminary results of the 'meconium project'. *Therapeutic Drug Monitoring* 2006; **28**: 700–706.
Margariti MG, Tsakalof AK and Tsatsakis AM. Analytical methods of biological monitoring for exposure to pesticides: recent update. *Therapeutic Drug Monitoring* 2007; **29**: 150–163.
Marin SJ, Coles R, Urry FM and McMillin GA. Confirmation of cannabinoids in meconium using two-dimensional gas chromatography with mass spectrometry detection. *Journal of Chromatography B* 2007; **858**: 59–64.
Martins L, Yegles M, Chung H and Wennig R. Simultaneous determination of amphetamine and congeners in hair specimens by negative chemical ionization gas chromatography–mass spectrometry. *Journal of Chromatography B* 2005; **825**: 57–62.
Martins LF, Yegles M, Chung H and Wennig R. Sensitive, rapid and validated gas chromatography/negative ion chemical ionization–mass spectrometry assay including derivatization with a novel chiral agent for the enantioselective quantification of amphetamine-type stimulants in hair. *Journal of Chromatography B* 2006; **842**: 98–105.
Martins LF, Yegles M, Thieme D and Wenning R. Influence of bleaching on the enantiomeric disposition of amphetamine-type stimulants in hair. *Forensic Science International* 2008; **176**: 38–41.
Maynard EC, Amoruso LP and Oh W. Meconium for drug testing. *American Journal of Diseases in Children* 1991; **145**: 650–652.
Mieczkowski T and Kruger M. Interpreting the color effect of melanin on cocaine and benzoylecgonine assays for hair analysis: brown and black samples compared. *Journal of Forensic Legal Medicine* 2007; **14**: 7–15.
Míguez-Framil M, Moreda-Piñeiro A, Bermejo-Barrera P, López P, Tabernero MJ and Bermejo AM. Improvements on enzymatic hydrolysis of human hair for illicit drug determination by gas chromatography/mass spectrometry. *Analytical Chemistry* 2007; **79**: 8564–8570.
Miller EI, Wylie FM and Oliver JS. Detection of benzodiazepines in hair using ELISA and LC-ESI-MS-MS. *Journal of Analytical Toxicology* 2006; **30**: 441–448.
Moeller MR and Mueller C. The detection of 6-monoacetylmorphine in urine, serum and hair by GC/MS and RIA. *Forensic Science International* 1995; **70**: 125–133.
Moeller MR, Fey P and Wennig R. Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program. *Forensic Science International* 1993; **63**: 185–206.
Montgomery D, Plate C, Alder SC, Jones M, Jones J and Christensen RD. Testing for fetal exposure to illicit drugs using umbilical cord tissue vs meconium. *Journal of Perinatology* 2006; **26**: 11–14.
Moody DE and Cheever ML. Evaluation of immunoassays for semiquantitative detection of cocaine and metabolites or heroin and metabolites in extracts of sweat patches. *Journal of Analytical Toxicology* 2001; **25**: 190–197.
Moody DE, Spanbauer AC, Taccogno JL and Smith EK. Comparative analysis of sweat patches for cocaine (and metabolites) by radioimmunoassay and gas chromatography–positive ion chemical ionization–mass spectrometry. *Journal of Analytical Toxicology* 2004; **28**: 83–93.
Moore C, Browne S, Tebbett I, Negrusz A, Meyer W and Jain L. Determination of cocaine and benzoylecgonine in human amniotic

fluid using high flow solid-phase extraction columns and HPLC. *Forensic Science International* 1992; **56**: 177–181.
Moore CM, Brown S, Negrusz A, Tebbett I, Meyer W and Jain L. Determination of cocaine and its major metabolite, benzoylecgonine, in amniotic fluid, umbilical cord blood, umbilical cord tissue, and neonatal urine: a case study. *Journal of Analytical Toxicology* 1993; **17**: 62.
Moore C, Lewis D and Leikin J. False-positive and false-negative rates in meconium drug testing. *Clinical Chemistry* 1995; **41**: 1614–1616.
Moore C, Dempsey D, Deitermann D, Lewis D and Leikin J. Fetal cocaine exposure: analysis of vernix caseosa. *Journal of Analytical Toxicology* 1996; **20**: 509–511.
Moore C, Negrusz A and Lewis D. Determination of drugs of abuse in meconium. *Journal of Chromatography B* 1998; **713**: 137–146.
Moore C, Jones J, Lewis D and Buchi K. Prevalence of fatty acid ethyl esters in meconium specimens. *Clinical Chemistry* 2003; **49**: 133–136.
Moore C, Feldman M, Harrison E, Rana S, Coulter C, Kuntz D, Agrawal A, Vincent M and Soares J. Disposition of hydrocodone in hair. *Journal of Analytical Toxicology* 2006a; **30**: 353–359.
Moore C, Rana S, Coulter C, Feyerherm F and Prest H. Application ot two-dimensional gas chromatography with electron capture chemical ionization mass spectrometry to the detection of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in hair. *Journal of Analytical Toxicology* 2006b; **30**: 171–177.
Moore C, Ross W, Coulter C, Adams L, Rana S, Vincent M and Soares J. Detection of the marijuana metabolite 11-nor-Delta9-tetrahydrocannabinol-9-carboxylic acid in oral fluid specimens and its contribution to positive results in screening assays. *Journal of Analytical Toxicology* 2006c; **30**: 413–418.
Moore C, Coulter C and Crompton K. Determination of cocaine, benzoylecgonine, cocaethylene and norcocaine in human hair using solid-phase extraction and liquid chromatography with tandem mass spectrometric detection. *Journal of Chromatography B* 2007a; **859**: 208–212.
Moore C, Marinetti L, Coulter C and Crompton K. Analysis of pain management drugs, specifically fentanyl, in hair: application to forensic specimens. *Forensic Science International* 2008; **176**: 47–50.
Moore C, Rana S and Coulter C. Determination of meperidine, tramadol and oxycodone in human oral fluid using solid phase extraction and gas chromatography–mass spectrometry. *Journal of Chromatography B*, 2007c; **850**: 370–375.
Moore C, Rana S and Coulter C. Simultaneous identification of 2-carboxy-tetrahydrocannabinol, tetrahydrocannabinol, cannabinol and cannabidiol in oral fluid. *Journal of Chromatography B* 2007d; **852**: 459–464.
Moriya F, Chan K-M, Noguchi TT and Wu PYK. Testing for drugs of abuse in meconium of newborn infants. *Journal of Analytical Toxicology* 1994; **18**: 41–45.
Moriya F and Hashimoto Y. Pericardial fluid as an alternative specimen to blood for post-mortem toxicological analyses. *Legal Medicine* 1999; **1**: 86–94.
Mortier KA, Maudens KE, Lambert WE, Clauwaert KM, Van Bocxlaer JF, Deforce DL, Van Peteghem CH and De Leenheer AP. Simultaneous, quantitative determination of opiates, amphetamines, cocaine and benzoylecgonine in oral fluid by liquid chromatography quadrupole-time-of-flight mass spectrometry. *Journal of Chromatography B* 2002; **779**: 321–330.
Mucklow JC, Bending MR, Kahn GC and Dollery CT. Drug Concentration in Saliva. *Clinical and Pharmacological Therapy* 1978; **24**: 563–570.
Musshoff F and Madea B. Review of biologic matrices (urine, blood, hair) as indicators of recent or ongoing cannabis use. *Therapeutic Drug Monitoring* 2006; **28**: 55–63.
Musshoff F, Junker HP, Lachenmeier DW, Kroener L and Madea B. Fully automated determination of cannabinoids in hair samples using headspace solid-phasemicroextraction and gas chromatography-mass spectrometry. *Journal of Analytical Toxicology* 2002; **26**: 1–6.
Musshoff F, Lachenmeier DW, Kroener L and Madea B. Automated headspace solid-phase dynamic extraction for the determination of cannabinoids in hair samples. *Forensic Science International* 2003, **133**: 32–38.
Musshoff F, Lachenmeier K, Trafkowski J, Madea B, Nauck F and Stamer U. Determination of opioid analgesics in hair samples using liquid chromatography/tandem mass spectrometry and application to patients under palliative care. *Therapeutic Drug Monitoring* 2007; **29**: 655–661.
Nadulski T and Pragst F. Simple and sensitive determination of Delta (9)-tetrahydrocannabinol, cannabidiol and cannabinol in hair by combined silylation, headspace solid phase microextraction and gas chromatography–mass spectrometry. *Journal of Chromatography B* 2007; **846**: 78–85.
Nakahara Y, Takahashi K, Sakamoto T, Tanaka A, Hill VA and Baumgartner A. Hair analysis for drugs of abuse XVII. Simultaneous detection of PCP, PCHP, and PCPdiol in human hair for confirmation of PCP use. *Journal of Analytical Toxicology* 1997; **21**: 356–362.
Nakamura S, Tomita M, Wada M, Chung H, Kuroda N and Nakashima K. Quantification of MDMA and MDA in abusers' hair samples by semi-micro column HPLC with fluorescence detection. *Biomedical Chromatography* 2006; **20**: 622–627.
Nakamura S, Wada M, Crabtree BL, Reeves PM, Montgomery JH, Byrd HJ, Harada S, Kuroda N and Nakashima K. A sensitive semimicro column HPLC method with peroxyoxalate chemiluminescence detection and column switching for determination of MDMA-related compounds in hair. *Analytical and Bioanalytical Chemistry* 2007; **387**: 1983–1990.
Navazesh M. Methods for collecting saliva. *Annals of the New York Academy of Sciences* 1993; **694**: 72–77.
Negrusz A and Gaensslen RE. Analytical developments in toxicological investigation of drug-facilitated sexual assault. *Analytical and Bioanalytical Chemistry* 2003; **376**: 1192–1197.
Ngwa G, Frich D, Blum K and Newland G. Simultaneous analysis of 14 benzodiazepines in oral fluid by solid-phase extraction and LC-MS-MS. *Journal of Analytical Toxicology* 2007; **31**: 369–376.
Niedbala RS, Kardos KW, Fritch DF, Kardos S, Fries T, Waga J, Robb J and Conne EJ. Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. *Journal of Analytical Toxicology* 2001; **25**: 289–303.
Nishida K, Itoh S, Inoue N, Kudo K and Ikeda N. High-performance liquid chromatographic–mass spectrometric determination of methamphetamine and amphetamine enantiomers, desmethylselegiline and selegiline, in hair samples of long-term methamphetamine abusers or selegiline users. *Journal of Analytical Toxicology* 2006a; **30**: 232–237.
Nishida M, Yashiki M, Namera A and Kimura K. Single hair analysis of methamphetamine and amphetamine by solid phase microextraction coupled with in matrix derivatization. *Journal of Chromatography B* 2006b; **842**: 106–110.
Offidani C, Strano Rossi S and Chiarotti M. Drug distribution in the head, axillary and pubic hair of chronic addicts. *Forensic Science International* 1993; **63**: 105–108.
O'Neal CL, Crouch DL, Rollins DE and Fatah AA. The effects of collection method on oral fluid codeine concentrations, *Journal of Analytical Toxicology* 2000; **24**: 536–542.
Ostrea Jr EM. Understanding drug testing in the neonate and the role of meconium analysis. *Journal of Perinatal and Neonatal Nursing* 2001; **14**: 61–82.
Ostrea Jr EM, Romero A, Knapp DK, Ostrea AR, Lucena JE and Utarnachitt RB. Postmortem drug analysis of meconium in early-gestation human fetuses exposed to cocaine: clinical implications. *Journal of Pediatrics* 1994; **124**: 477–479.
Ostrea Jr EM, Parks PM and Brady MJ. Rapid isolation and detection of drugs in meconium of infants of drug-dependent mothers. *Clinical Chemistry* 1998; **34**: 2372–2373.
Ostrea EM Jr, Knapp DK, Tannenbaum L, Ostrea AR, Romero A, Salari V and Ager J. Estimates of illicit drug use during pregnancy by maternal interview, hair analysis, and meconium analysis. *Journal of Pediatrics* 2001; **138**: 344–348.
Ostrea Jr EM, Bielawski DM and Posecion Jr NC. Meconium analysis to detect fetal exposure to neurotoxicants. *Archive of Diseases in Children* 2006; **91**: 628–629.
Pacifici R, Farré M, Pichini S, Ortuño J, Roset P, Zuccaro P, Segura J and de la Torre R. Sweat testing of MDMA with the Drugwipe® analytical device: a controlled study with two volunteers. *Journal of Analytical Toxicology* 2001; **25**: 144–146.

Paterson S, McLachlan-Troup N, Cordero R, Dohnal M and Carman S. Qualitative screening for drugs of abuse in hair using GC-MS. *Journal of Analytical Toxicology* 2001; **25**: 203–208.
Paul BD and Smith ML. Cyanide and thiocyanate in human saliva by gas chromatography–mass spectrometry. *Journal of Analytical Toxicology* 2006; **30**: 511–515.
Paul R, Kingston R, Tsanaclis L, Berry A and Guwy A. Do drug users use less alcohol than non-drug users? A comparison of ethyl glucuronide concentrations in hair between the two groups in medico-legal cases. *Forensic Science International* 2007; in press.
Paxton JW. Measurement of drugs in saliva: a review. *Methods and Findings in. Experimental Clinical Pharmacology* 1979; **1**: 11–21.
Pehrsson A, Gunnar T, Engblom C, Seppa H, Jama A and Lillsunde P. Roadside oral fluid testing: comparison of the results of Drugwipe 5 and Drugwipe benzodiazepines on-site tests with laboratory confirmation results of oral fluid and whole blood. *Forensic Science International* 2007; in press.
Peters FT, Samyn N, Kraemer T, Riedel WJ and Maurer HH. Negative-ion chemical ionization gas chromatography–mass spectrometry assay for enantioselective measurement of amphetamines in oral fluid: application to a controlled study with MDMA and driving under the influence cases. *Clinical Chemistry* 2007; **53**: 702–710.
Phillips SG and Allen KR. Acetylcodeine as a marker of illicit heroin abuse in oral fluid samples. *Journal of Analytical Toxicology* 2006; **30**: 370–374.
Pichini S, Basagaña XB, Pacifici R, Garcia O, Puig C, Vall O, Harris J, Zuccaro P, Segura J and Sunyer J. Cord serum cotinine as a biomarker of fetal exposure to cigarette smoke at the end of pregnancy. *Environmental Health Perspectives* 2000; **108**: 1079–1083.
Pichini S, Navarro M, Pacifici R, Zuccaro P, Ortuño J, Farré M, Roset P, Segura J and de la Torre R. Usefulness of sweat testing for the detection of MDMA after a single-dose administration. *Journal of Analytical Toxicology* 2003; **27**: 294–303.
Pichini S, Marchei E, Pacifici R, Pellegrini M, Lozano J and Garcia-Algar O. Application of a validated high-performance liquid chromatography–mass spectrometry assay to the analysis of *m*- and *p*-hydroxybenzoylecgonine in meconium. *Journal of Chromatography B* 2005; **820**: 151–156.
Politi L, Zucchella A, Morini L, Stramesi C and Polettini A. Markers of chronic alcohol use in hair: comparison of ethyl glucuronide and cocaethylene in cocaine users. *Forensic Science International* 2007; **172**: 23–27.
Posecion NC, Ostrea EM and Bielawski DM. Quantitative determination of paraquat in meconium by sodium borohydride–nickel chloride chemical reduction and gas chromatography/mass spectrometry (GC/MS). *Journal of Chromatography B*; 2007: in press.
Pötsch L and Skoop G. Stability of opiates in hair fibers after exposure to cosmetic treatment. *Forensic Science International* 1996; **81**: 95–102.
Pragst F. Application of solid-phase microextraction in analytical toxicology. *Analytical and Bioanalytical Chemistry* 2007; **388**: 1393–1414.
Pragst F and Balikova M. State of the art in hair analysis for detection of drug and alcohol abuse. *Clinica Chimica Acta* 2006; **370**: 17–49.
Pragst F, Rothe M, Spiegel K and Sporkert F. Illegal and therapeutic drug concentrations in hair segments—a timetable of drug exposure. *Forensic Science Review* 1998; **10**: 81–111.
Pujadas M, Pichini S, Civit E, Santamarina E, Perez K and de la Torre R. A simple and reliable procedure for the determination of psychoactive drugs in oral fluid by gas chromatography–mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 2007; **44**: 594–601.
Quintela O, Bermejo AM, Tabernero MJ, Strano-Rossi S, Chiarotti M and Lucas ACS. Evaluation of cocaine, amphetamines and cannabis use in university students through hair analysis: preliminary results. *Forensic Science International* 2000; **107**: 273–279.
Quintela O, Cruz A, Castro A, Concheiro M and Lopez-Rivadulla M. Liquid chromatography–electrospray ionisation mass spectrometry for the determination of nine selected benzodiazepines in human plasma and oral fluid. *Journal of Chromatography B* 2005; **825**: 63–71.
Quintela O, Andrenyak DM, Hoggan AM and Crouch DJ. A validated method for the detection of Delta 9-tetrahydrocannabinol and 11-nor-9-carboxy-Delta 9-tetrahydrocannabinol in oral fluid samples by liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry. *Journal of Analytical Toxicology* 2007; **31**: 157–164.
Rana S, Moore C, Vincent M, Coulter C, Agrawal A and Soares J. Determination of propoxyphene in oral fluid. *Journal of Analytical Toxicology* 2006; **30**: 516–518.
Ricossa MA, Bernini M and De Ferrari F. Hair analysis for driving licence in cocaine and heroin users. An epidemiological study. *Forensic Science International* 2000; **107**: 301–308.
Ripple MG, Goldberger BA, Caplan YH, Blitzer MG and Schwartz S. Detection of cocaine and its metabolites in human amniotic fluid. *Journal of Analytical Toxicology* 1992; **16**: 328–331.
Rollins DE, Wilkins DG, Krueger GG, Augsburger MP, Mizuno A, O'Neal C, Borges CR and Slawson MH. The effect of hair color on the incorporation of codeine into human hair. *Journal of Analytical Toxicology* 2003; **27**: 545–551.
Romano G, Barbera N, Spadano G and Valenti V. Determination of drugs of abuse in hair: evaluation of external heroin contamination and risk of false positives. *Forensic Science International* 2003; **131**: 98–102.
Sabzevari O, Abdi Kh, Amini M and Shafice A. Application of a simple and sensitive GC-MS method for determination of morphine in the hair of opium abusers. *Analytical and Bioanalytical Chemistry* 2004; **379**: 120–124.
Sachs H and Dressler U. Detection of THCCOOH in hair by MSD-NCI after HPLC clean-up. *Forensic Science International* 2000; **107**: 239–247.
Sagan C, Salvador A, Dubreuil D, Poulet PP, Duffaut D and Brumpt I. Simultaneous determination of metronidazole and spiramycin I in human plasma, saliva and gingival crevicular fluid by LC-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis* 2005; **38**: 298–306.
Saitoh M, *et al.* Rate of hair growth. In *Advances in Biology of Skin*, Montagna W and Dobson RL (eds). Pergamon Press: Oxford, 1969; 183–201.
Samyn N and van Hieren C. On-site testing of saliva and sweat with Drugwipe and determination of concentrations of drugs of abuse in saliva, plasma and urine of suspected users. *International Journal of Legal Medicine* 2000; **113**: 150–154.
Samyn N, De Boeck G, Cirimele V, Verstraete A and Kintz P. Detection of flunitrazepam and 7-aminoflunitrazepam in oral fluid after controlled administration of rohypnol. *Journal of Analytical Toxicology* 2002a; **26**: 211–215.
Samyn N, De Boeck G and Verstraete AG. The use of oral fluid and sweat wipes for the detection of drugs of abuse in drivers. *Journal of Forensic Science* 2002b; **47**: 1380–1387.
Samyn N, De Boeck G, Wood M, Lamers CT, De Waard D, Brookhuis KA, Verstraete AG and Riedel WJ. Plasma, oral fluid and sweat wipe ecstasy concentrations in controlled and real life conditions. *Forensic Science International* 2002c; **128**: 90–97.
Samyn N, Laloup M and De Boeck G. Bioanalytical procedures for determination of drugs of abuse in oral fluid. *Analytical and Bioanalytical Chemistry* 2007; **388**: 1437–1453.
Sato K, Kang WH, Saga K and Sato KT. Biology of sweat glands and their disorders. I. Normal sweat gland function. *Journal of the American Academy of Dermatology* 1989; **20**: 537–563.
Schaffer MI, Wang W and Irving J. An evaluation of two wash procedures for the differentiation of external contamination versus ingestion in the analysis of human hair samples for cocaine. *Journal of Analytical Toxicology* 2002; **26**: 485–488.
Schaffer M, Hill V and Cairns T. Hair analysis for cocaine: The requirement for effective wash procedures and effects of drug concentration and hair porosity in contamination and decontamination. *Journal of Analytical Toxicology* 2005; **29**: 319–326.
Scheidweiler KB and Huestis MA. Simultaneous quantification of opiates, cocaine, and metabolites in hair by LC-APCI-MS/MS. *Analytical Chemistry* 2004; **76**: 4358–4363.
Scheidweiler KB and Huestis MA. A validated gas chromatographic-electron impact ionization mass spectrometric method for methylenedioxymethamphetamine (MDMA), methamphetamine and metabolites in oral fluid. *Journal of Chromatography B* 2006; **835**: 90–99.

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Schepers RJ, Oyler JM, Joseph RE Jr, Cone EJ, Moolchan ET and Huestis MA. Methamphetamine and amphetamine pharmacokinetics in oral fluid and plasma after controlled oral methamphetamine administration to human volunteers. *Clinical Chemistry* 2003; **49**: 121–132.

Schwilke E, Barnes AJ, Kacinko SL, Cone EJ, Moolchan ET and Huestis MA. Opioid disposition in human sweat after controlled oral codeine administration. *Clinical Chemistry* 2006; **52**: 1539–1545.

Shen M, Xiang P, Wu H, Shen B and Huang Z. Detection of antidepressant and antipsychotic drugs in human hair. *Forensic Science International* 2002; **126**: 153–161.

Skender L, Karačić V, Brčić I and Bagarić A. Quantitative determination of amphetamines, cocaine, and opiates in human hair by gas chromatography/mass spectrometry. *Forensic Science International* 2002; **125**: 120–126.

Skopp G, Strohbeck-Kuehner P, Mann K and Hermann D. Deposition of cannabinoids in hair after long-term use of cannabis. *Forensic Science International* 2007; **170**: 46–50.

Smink BE, Mathijssen MP, Lusthof KJ, de Gier JJ, Egberts AC and Uges DR. Comparison of urine and oral fluid as matrices for screening of thirty-three benzodiazepines and benzodiazepine-like substances using immunoassay and LC-MS(-MS). *Journal of Analytical Toxicology* 2006; **30**: 478–485.

Society of Hair Testing. Recommendations for hair testing in forensic cases. *Forensic Science International* 2004; **145**: 83–84.

Speckl IM, Hallbach J, Guder WG, Meyer LV and Zilker T. Opiate detection in saliva and urine—a prospective comparison by gas chromatography–mass spectrometry. *Journal of Toxicology: Clinical Toxicology* 1999; **37**: 441–445.

Spihler V. *Drugs in Saliva. Clarke's Analysis of Drugs and Poisons*. Pharmaceutical Press: London, 2004; 109.

Sporkert F and Pragst F. Use of headspace solid-phase microextraction (HS-SPME) in hair analysis for organic compounds. *Forensic Science International* 2000; **107**: 129–148.

Stanaszek R and Piekoszewski W. Simultaneous determination of eight underivatized amphetamines in hair by high performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). *Journal of Analytical Toxicology* 2004; **28**: 77–85.

Staub C. Chromatographic procedures for determination of cannabinoids in biological samples, with special attention to blood and alternative matrices like hair, saliva, sweat and meconium. *Journal of Chromatography B* 1999; **733**: 119–126.

Szeto H. Kinetics of drug transfer to the fetus. *Clinical Obstetrics and Gynecology* 1993; **36**: 246–254.

Thieme D and Sachs H. Examination of a long-term clozapine administration by high resolution segmental hair analysis. *Forensic Science International* 2007; **166**: 110–114.

Toennes SW, Kauert GF, Steinmeyer S and Moeller MR. Driving under the influence of drugs—evaluation of analytical data of drugs in oral fluid, serum and urine, and correlation with impairment symptoms. *Forensic Science International* 2005; **152**: 149–155.

Toraño JS and van Kan HJ. Simultaneous determination of the tobacco smoke uptake parameters nicotine, cotinine and thiocyanate in urine, saliva and hair, using gas chromatography–mass spectrometry for characterisation of smoking status of recently exposed subjects. *Analyst* 2003; **128**: 838–843.

Tracqui A and Ludes B. HPLC-MS for the determination of sildenafil citrate (Viagra) in biological fluids. Application to the salivary excretion of sildenafil after oral intake. *Journal of Analytical Toxicology* 2003; **27**: 88–94.

Tsanaclis L and Wicks JFC. Differentiation between drug use and environmental contamination when testing for drugs in hair. *Forensic Science International* 2008; **176**: 19–22.

Tsanaclis L and Wicks JFC. Patterns in drug use in the United Kingdom as revealed through analysis of hair in large population sample. *Forensic Science International* 2007b; **170**: 121–128.

Turker G, Ergen K, Karakoc Y, Arisoy AE and BarutCu UB. Concentrations of toxic metals and trace elements in the meconium of newborns from an industrial city. *Biology of the Neonate* 2006; **89**: 244–250.

Uemura N, Nath RP, Harkey MR, Henderson GL, Mendelson J and Jones RT. Cocaine levels in sweat collection patches vary by location of patch placement and decline over time. *Journal of Analytical Toxicology* 2004; **28**: 253–259.

Uhl M and Sachs H. Cannabinoids in hair: strategy to prove marijuana/hashish consumption. *Forensic Science International* 2004; **145**: 143–147.

Unuvar E, Ahmadov H, Kiziler AR, Aydemir B, Toprak S, Ulker V and Ark C. Mercury levels in cord blood and meconium of healthy newborns and venous blood of their mothers: clinical, prospective cohort study. *Science of the Total Environment* 2007; **374**: 60–70.

Ursitti F, Klein J and Koren G. Clinical utilization of the neonatal hair test for cocaine: a four-year experience in Toronto. *Biology of the Neonate* 1997; **72**: 345–351.

Verna A and Dhanireddy R. Time of first stool in extremely low birth weight (< or = 1000 grams) infants. *Journal of Pediatrics* 1993; **122**: 626–629.

Villain M, Chèze M, Tracqui A, Ludes B and Kintz P. Testing for zopiclone in hair application to drug-facilitated crimes. *Forensic Science International* 2004; **145**: 117–121.

Villain M, Concheiro M, Cirimele V and Kintz P. Screening method for benzodiazepines and hypnotics in hair at pg/mg level by liquid chromatography–mass spectrometry/mass spectrometry. *Journal of Chromatography B* 2005; **825**: 72–78.

Villamor JL, Bermejo AM, Fernández P and Tabernero MJ. A new GC-MS method for the determination of five amphetamines in human hair. *Journal of Analytical Toxicology* 2005; **29**: 135–139.

Vincent F, Bessard J, Vacheron J, Mallaret M and Bessard G. Determination of buprenorphine and norbuprenorphine in urine and hair by gas chromatography–mass spectrometry. *Journal of Analytical Toxicology* 1999; **23**: 270–279.

Weinmann W, Müller C, Vogt S and Frei A. LC-MS-MS analysis of the neuroleptics clozapine, flupentixol, haloperidol, penfluridol, thioridazine, and zuclopenthixol in hair obtained from psychiatric patients. *Journal of Analytical Toxicology* 2002; **26**: 303–307.

Wilson L, Jehanli A, Hand C, Cooper G and Smith R. Evaluation of a rapid oral fluid point-of-care test for MDMA. *Journal of Analytical Toxicology* 2007; **31**: 98–104.

Wood M, De Boeck G, Samyn N, Morris M, Cooper DP, Maes RA and De Bruijn EA. Development of a rapid and sensitive method for the quantitation of amphetamines in human plasma and oral fluid by LC-MS-MS. *Journal of Analytical Toxicology* 2003; **27**: 78–87.

Wood M, Laloup M, Ramirez Fernandez MM, Jenkins KM, Young MS, Ramaekers JG, De Boeck G and Samyn N. Quantitative analysis of multiple illicit drugs in preserved oral fluid by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Forensic Science International* 2005; **150**: 227–238.

Wylie FM, Torrance H, Seymour A, Buttress S and Oliver JS. Drugs in oral fluid Part I. Validation of an analytical procedure for licit and illicit drugs in oral fluid. *Forensic Science International* 2005a; **150**: 191–198.

Wylie FM, Torrance H, Seymour A, Buttress S and Oliver JS. Drugs in oral fluid Part II. Investigation of drugs in drivers. *Forensic Science International* 2005b; **150**: 199–204.

Yegles M, Marson Y and Wenning R. Influence of bleaching on stability of benzodiazepines in hair. *Forensic Science International* 2000; **107**: 87–92.

Zhao G, Xu Y, Li W, Han G and Ling B. Prenatal exposures to persistent organic pollutants as measured in cord blood and meconium from three localities of Zhejiang, China. *Science of the Total Environment* 2007; **377**: 179–191.

Zucchela A, Stramesi C, Politi L, Morini L and Polettini A. Treatments against hair loss may hinder cocaine and metabolites detection. *Forensic Science and Medical Pathology* 2007; **3**: 93–100.