Int J Legal Med (1999) 112 : 213–221

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REVIEW

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Perspiration versus saliva – basic aspects concerning their use in roadside drug testing

Received: 13 August 1997 / Received in revised form: 25 August 1998

Abstract Various aspects concerning the practical application and forensic interpretation of data obtained by saliva drug testing and drug monitoring from the skin surface are discussed. Basic information on the composition of saliva and skin secretions and their particular transport mechanisms, as far as known, are given. For drugs of abuse secretion into saliva is suggested to be by passive diffusion and to depend on lipid solubility, pKa, plasma protein binding and on the pH of saliva. Drug molecules from blood are considered to reach the skin surface by various routes such as by sweat and sebum as well as by inter- and/or transcellular diffusion. The role of the stratum corneum as a temporary drug reservoir exceeding positive drug findings in urine is outlined. Current data on opioids, cocaine metabolites, cannabinoids and amphetamines detected in saliva and on the skin surface are reviewed. Aspects of collection, processing and analysis of the samples for implementation in roadside testing are addressed. The requirement of test sensitivity covering the broad concentration ranges and the importance of test specificity bearing in mind that the parent drug is the main analyte present in those specimens is stressed. Theoretical and practical findings on frequently abused drugs are discussed with regard to the possibilities and limitations of drug monitoring from saliva and perspiration to support a suspicion of actual or recent drug administration.

Key words Roadside testing · Actual/recent drug administration · Drug monitoring · Saliva · Perspiration

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Introduction

The use of illicit drugs presents a vast number of associated dangers to public health especially with regard to road traffic. The rigorous prosecution of drunken drivers in Germany has resulted in a decrease in alcohol-related accidents since the 1990s [54]. Alcoholic *fetor oris* already indicates alcohol consumption and as intoxication increases, neuropsychiatric symptoms can be recognized. The breath alcohol concentration can be easily determined on-site [43] but the situation is different for drug-impaired drivers as specific symptoms are less obvious and can be difficult to diagnose without the support of monitoring biological specimens [43]. Even in the last decade, politicians and police authorities started to take a high interest in the identification of an impaired driver more easily by means of roadside screening tests and called upon the forensic scientists to offer non-invasive sampling techniques and suitable screening methods for on-site drug testing.

In the meantime unconventional specimens for the detection of drugs of abuse have become very popular due to the commercial availability of devices for routine collection as well as of simple test kits. Therefore, perspiration and saliva were strongly promoted and promised to offer excellent possibilities to clarify suspicion and to detect recent or actual drug administration. However, considering the physiology and biochemistry of drug excretion in these biological materials as well as analytical data, considerable information is already present to elucidate these demands and to comment upon the hitherto existing attempts concerning roadside drug testing.

Biological data on drug monitoring in saliva

Saliva is an aqueous glandular fluid with a low protein content. Saliva production occurs in 2 stages: firstly, a fluid isotonic to blood is released by the acinus cells of the salivary glands, which is subsequently rendered hypotonic

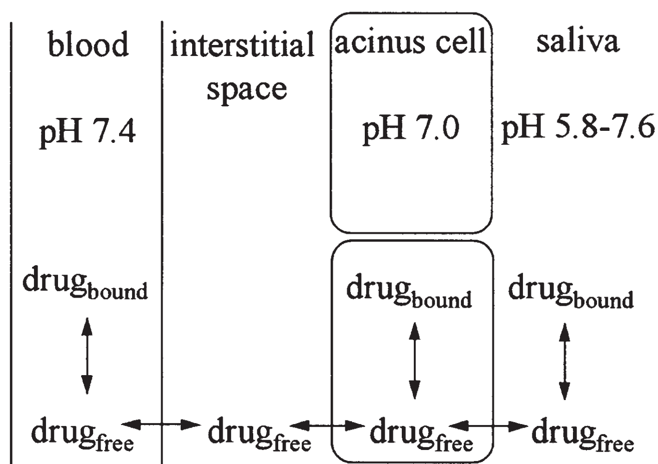


Fig. 1 Passive diffusion of drugs from blood into saliva modified according to [25]

by reabsorption of sodium ions [24]. The saliva/plasma drug concentration ratio (S/P) primarily depends on the transfer mechanisms by which drugs pass from blood into saliva. For drugs of abuse, the possible route is suggested to be passive diffusion (Fig. 1) [25]. Passive diffusion is governed by:

- the molecular mass
- the spatial configuration of the drug molecule
- its binding properties in blood and saliva
- its lipid solubility and degree of ionization.

Therefore, the main analyte in saliva is predominantly the parent drug [55, 65] and drug concentrations might be dependent on the pH value in this body fluid. The relationship between the saliva/plasma drug concentration ratio, pKa, pH and binding is expressed by the equations of Rasmussen/Matin [45]. The pH value of saliva ranges from 5.8 to 7.6 while the pH value of blood remains fairly constant. For example, if the low binding of cocaine (pKa 8.6) to plasma and saliva components is not considered, a decrease in pH value from 7.6 to 5.8 will result in an increase in saliva/plasma drug concentration ratio from 0.65 to 37.5 [50]. The concentration of benzoylecgonine containing both acidic and basic functional groups (pKa_1 2.25, pKa_2 11.2) is thought to be unaffected by the saliva pH [56]. In addition to the physico-chemical properties of the drug molecule, its concentration in the saliva is dependent on the salivary flow rate, which is under physiological control and strongly influenced by external and emotional stimuli [25]. A circadian rhythm of salivary flow rate had been observed [24]. Other factors influencing the saliva/plasma ratio of a drug are arterial-venous fluctuations [23, 24], reabsorption of the drug in the buccal cavity as well as the elimination kinetics of the particular drug and the effects of additional drugs present.

Mixed saliva is only one component of the material collected from the oral cavity, which always contains epithelial cells, crevicular fluid and, possibly, food or drug remnants. This fact is linked to some additional and important factors in saliva testing. For example, the concentration of tetrahydrocannabinol, a substance which is largely protein-bound in blood [73] is expected to be very low in saliva, based on the passive diffusion model. The surprisingly high amounts of tetrahydrocannabinol found in saliva samples imply that drug molecules are sequestered in the mucosa of the oral cavity during smoking [52]. This also applies to orally and nasally administered or inhaled drugs which are transiently stored in the oral cavity [6, 11].

Due to large differences within individuals, salivary excretion is generally of little quantitative importance, although a relatively constant saliva/plasma drug concentration ratio for some drugs e.g. theophylline, phenytoin, carbamazepine, diazepam, primidone, quinidine, ethanol and caffeine has initiated some interest in the use of saliva for therapeutic drug monitoring [25]. The salivary excretion of illegal drugs has been studied far less systematically. An overview on drugs of abuse detected in saliva was given by Schramm et al. [55] and current data on opioids, cocaine, cannabinoids and amphetamines are briefly reviewed below.

Findings on illegal drugs detected in saliva

Opioids

In 1974, Gorodetzky and Kullberg [20] analyzed saliva samples by radio-immunoassay after single intravenous doses of 5–10 mg heroin hydrochloride/70 kg. Using 60 ng morphine equivalents/ml as the criterion for a positive result saliva samples tested positive during 1–2 h after dosing and morphine equivalents were detectable in plasma for 2–4 h. During chronic administration, the detection window in saliva had increased to 3–4 h after the last dose. Heroin, 6-acetylmorphine and morphine were readily detected by GC/MS in saliva following parenteral heroin administration [19]. Jenkins et al. [31] described the excretion profiles of smoked and intravenous heroin which was detected in saliva 2 min after administration by both routes. After smoking, higher concentrations of the parent drug occurred, which were detectable 2–24 h after administration. Intravenously given heroin was detectable for approx. 1 h, and morphine for approx. 2–4 h using a detection limit of 1 ng/ml. Cone [6] reported the appearance of heroin and major metabolites in saliva samples and in plasma after 12 mg heroin hydrochloride was introduced nasally. Saliva concentrations of heroin were highly elevated over plasma concentrations for 1 h and were detectable for 4–8 h in saliva and plasma. Drug-induced effects had disappeared when drug levels in saliva and plasma were no longer detectable. Intramuscularly injected morphine (10, 20 mg) peaked in saliva after 0.5 h at about 10–37 ng/ml and was detectable for 24 h at a sensitivity limit of 0.6 ng/ml for the radio immunoassay used [7]. Peak concentrations (< 546 ng/ml) in saliva after oral administration of 60 mg codeine phosphate were observed at 1–3 h. Codeine could be detected with the Drugwipe

Table 1 Opioid saliva/plasma concentration ratio (S/P), concentration range (ng/ml) and detection time after dosing from experimental studies mentioned in the text, and findings from roadside testing (ng/ml) [52]. –: no data available

Drug	Experimental studies			Roadside testing nNg/ml
	S/P	ng/ml	time	
Heroin	0.02– 0.9	6 – 30	2 min – 4 h	–
6-Acetylmorphine	–	18 – 141	0.5 min – 8 h	
Morphine	0.1 – 3.5	0.6– 37	< 24 h	311
Codeine	2.0 – 6.6	0.6– 546	< 36 h	–
Dihydrocodeine	2.5 – 7.5	30 –1400	< 24 h	–
Methadone	0.5 –10	200	24 h	642

equipment, however, numerous false negative results were obtained [40]. The saliva/plasma concentration ratios found for codeine were highly variable ranging from 2.0 to 6.6 [55]. While Cone measured about the same concentrations of codeine in saliva and plasma [5], Sharp et al. [57] found a considerably higher concentration of codeine in saliva than in plasma. Variable saliva/plasma concentration ratios > 2.5 have recently been established in a pharmacokinetic study after orally administered dihydrocodeine by HPLC/fluorescence detection (unpublished). Methadone has been detected in mixed saliva after acute and chronic dosing [44]. The saliva/plasma concentration ratio was found to range from 3.0–10 [6], and was only 0.51 in a study where subjects rinsed their mouth with water prior to saliva collection [33]. The most important data on opioid findings in saliva are summarized in Table 1.

Cannabinoids

In 1984, Peel et al. [49] reported on cannabinoid detection in saliva sampled from impaired drivers by a modified immunoassay procedure with a limit of detection for cannabinoid compounds of 10 ng carboxytetrahydrocannabinol equivalents/ml. Positive findings were thought to be due to the presence of the acid metabolite as well as to tetrahydrocannabinol and its primary metabolite being sequestered in the oral cavity during smoking. In addition to tetrahydrocannabinol [32], cannabidiol and cannabinol have been detected in saliva after smoking by thin layer chromatography using dansyl chloride for visualization [22]. Although saliva concentrations of cannabinoids greatly varied and seemed to depend on various factors such as frequency of drug administration, smoking habits, the content of tetrahydrocannabinol of cannabis products and the time interval since last administration, a positive blood level was found to be accompanied by a positive saliva result [21]. After smoking a single cigarette containing 1.75% or 3.55% of tetrahydrocannabinol, detection times of cannabinoids in saliva by radio immunoassay were on average 6 or 10 h [27]. When GC/MS was used for the identification of cannabinoids from saliva samples, neither carboxytetrahydrocannabinol [27, 52] nor 11-hydroxytetrahydrocannabinol [27] was detected in any sample, in contrast to Schramm et al. [55], who reported the detection of small amounts of carboxytetrahydrocannabinol, 11-hydroxytetrahydrocannabinol and cannabidiol in a single saliva sample by LC/MS. Although the presence of

Table 2 Cannabinoid saliva/plasma concentration ratio (S/P), concentration range (ng/ml) and detection time after dosing from experimental studies mentioned in the text, and findings from roadside testing (ng/ml) [52]

Compound	Experimental studies			Roadside testing ng/ml
	S/P	ng/ml	time	
THC	0.1 –2	5–1000	4–14 h	< 30
11-OH-THC	0.06–0.1	trace	–	–
THCCOOH	–	–	–	not detected

Cannabinol and cannabidiol were occasionally detected
–: no data available. THC: tetrahydrocannabinol, 11-OH-THC: 11-hydroxy-THC, THCCOOH: carboxy THC

tetrahydrocannabinol metabolites in saliva is still unclear, drug metabolism of sequestered tetrahydrocannabinol may also occur in the oral cavity [74]. Saliva/plasma concentration ratios of tetrahydrocannabinol vary over a wide range (Table 2) [28, 55]. As a result of high individual variability, the correlation of saliva levels of tetrahydrocannabinol from a single test with behavioral and physiological effects was assumed not to be significant [27]. In contrast, Menkes et al. [46] reported that subjective intoxication and heart rate were significantly correlated with the log of salivary levels of tetrahydrocannabinol.

Amphetamines

The oral route of amphetamine administration enabled detection by thin layer chromatography [67]. When absorption was complete the saliva/plasma drug concentration ratio was relatively constant and averaged 2.8 [71]. Wan et al. [71] reported on saliva amphetamine concentrations exceeding plasma concentrations by a factor of 2–3, in contrast to Smith [60], who observed that the amount of amphetamine in saliva measured by radio immunoassay was similar to that in whole blood. Elimination was pH dependent and abuse of amphetamine was detectable up to 48 h following last abuse [71]. The same detection interval has been reported for methamphetamine [13]. Methylenedioxymethamphetamine, methylenedioxyethylamphetamine and methylenedioxyamphetamine were also detected in saliva in a forensic study [52]. The high number of false positives by immunoassay (42%) as evidenced by GC/MS reanalysis of saliva samples was assumed to be caused by food containing phenylalkylamine

Table 3 Amphetamine saliva/plasma concentration ratio (S/P), concentration range (ng/ml) and detection time after dosing from experimental studies mentioned in the text, and findings from roadside testing (ng/ml) [52]

Compound	Experimental studies			Roadside testing ng/ml
	S/P	ng/ml	time	
Amphetamine	2.8	20–40	< 50 h	< 30
Methamphetamine	–	trace	< 50 h	–
MDA, MDMA, MDEA	–	–	–	< 16000

–: no data available: methylenedioxyamphetamine, MDMA: methylenemedioxymethamphetamine, MDEA: methylene-dioxyethylamphetamine

compounds such as tyramine, which highly cross-reacted in the amphetamine assay [52] (Table 3).

Cocaine

Excretion of radiolabelled cocaine in saliva has been investigated by Inaba et al. [29] in 1978 and Peel et al. [49] reported on cocaine detection in a saliva sample from an impaired driver. The presence of cocaine in saliva was confirmed by GC/MS [9, 66], and non-stimulated saliva contained substantially more drug than stimulated saliva [34]. More detailed studies following different administration routes revealed benzoylecgonine, ecgonine methyl ester and, after crack use, anhydroecgonine methyl ester to be present in saliva samples [31, 42]. Nevertheless, cocaine was the predominant analyte identified in saliva samples after a single dose [34]. After intravenously administered cocaine, highly significant correlations of saliva cocaine to plasma cocaine concentrations were observed in stimulated, mixed saliva samples [66]. The high variability in saliva excretion during the initial phase after drug administration has been attributed to pH effects [8]. In this study, a close relationship was observed between cocaine saliva levels and cocaine-induced behavioral and physiological effects. After oral administration of cocaine, subjective rating of intoxication and euphoria correlated with salivary cocaine concentration, but stimulant effects still persisted when salivary levels had returned to pre-cocaine levels [63]. After a 40 mg dose of intravenous cocaine hydrochloride, cocaine levels in saliva peaked at 237–1843 ng/ml after 10 min and declined to an average of 29 ng/ml after 5 h [8]. When cocaine was smoked or administered intranasally, elevated saliva/plasma concentration ratios were found in the early period after drug administration, which was attributed to drug contamination of the oral cavity [31]. After smoking 40 mg cocaine base, saliva/plasma concentration ratios remained elevated for about 1 h and thereafter, were approximately equivalent to the particular ratios present after 44.8 mg intravenously administered cocaine hydrochloride. The average detection time of cocaine in saliva was 7.4 h after smoking compared with 8.5 h after intravenous application, while cocaine was detected in plasma for about 4.0 h after smoking compared to 6.3 h after intravenous injection (Table 4).

Table 4 Saliva/plasma concentration ratios (S/P) of cocaine and cocaine metabolites, concentration range (ng/ml) and detection time after dosing from experimental studies mentioned in the text, and findings from roadside testing (ng/ml) [52]

Compound	Experimental studies			Roadside testing ng/ml
	S/P	ng/ml	time	
Cocaine	0.36–9.74	< 1927 10 d, chronic use	2 min – 24 h	ca. 4000
Benzoylecgonine	ca. 0.4	< 122	< 24 h	–
Ecgoninemethyl ester	–	< 135	< 24 h	–

–: no data available

Prolonged occurrence of cocaine in saliva has been detected by immunoassay up to 5–10 days following abstinence, possibly due to the longer half-life of cocaine metabolites and the storage of the parent drug in body compartments [34]. In chronic abusers, unmetabolized cocaine could be measured in saliva by GC/MS assay during the first 24 h after drug intake [7]. During this time, subject scores for cocaine craving and depression significantly correlated with the cocaine saliva concentration.

Aspects of collection, processing and analysis of saliva samples

Saliva can be collected by expectoration, a method which may certainly lead to unpleasant situations in roadside testing and therefore cannot be recommended. An overview on commercially available saliva collection devices with absorbing materials was given by Häckel and Hänecke [25]. Most advantageous for on-site testing seems the use of the Salivette (Sarstedt, Nümbrecht, Germany) which consists of a stoppered centrifuge tube and an insert containing a cotton wool roll. The absorbing material is left in the buccal cavity where it absorbs a sufficient amount (1–2 ml) of saliva within 30–60 s and the fluid is usually collected by centrifugation. To simplify this procedure for roadside testing, the roll is transferred to a 5 ml disposable syringe, soaked with saline (0.9%, 0.5–1.0 ml) and fluid is collected upon squeezing.

At present, a very limited number of one-step dip-and-read, nonisotopic assays are available for on-site drug testing. All of those tests have been developed for urine screening and are less suitable for the detection of the parent drug for the antibodies are designed to drug metabolites and only poorly cross react. Additionally, the cut-off levels of the assays meet the requirements for urine concentrations resulting in poor sensitivity for saliva testing. Bogusz et al. reported on findings of urine and saliva samples collected from cocaine body packers [3]. Urine samples were clearly immunochemically positive using 300

ng benzoylecgonine equivalents/ml as the cut-off, whereas examination of saliva revealed negative results. At present, an immunological screening for opiate or cocaine abuse may be performed using tests with considerable cross reactivity to the parent drug such as Frontline dipsticks (Boehringer, Mannheim, Germany) or a multi-drug panel such as Triage (Merck, Darmstadt, Germany). Using the Triage panel, the membrane in the detection cup is moistened by 1–2 drops of 1% Triton X 100 (Sigma, Deisenhofen, Germany) to aid chromatography without interfering with antibody binding. For later confirmation, an aliquot of the remaining eluate or an additionally collected sample may be processed and analyzed by GC/MS according to routine procedures for blood or plasma specimens [18, 47, 72]. Identity can be established by several PCR systems if questioned [51]. The omission of mouth rinsing prior to sampling and an additional wiping of the buccal area will enhance the probability to detect drug consumption, especially when drugs of abuse have recently been smoked or administered by the oral or nasal route.

Biophysical data on transdermal drug excretion

The initial observations on outward transcutaneous drug delivery date back to 1844 when Valentin [68] verified quinine in perspiration fluid. The presence of various drugs on the skin surface has been reported, including amphetamines [16, 30, 36, 64, 69], cocaine [1, 4, 10, 58, 59, 61, 62], opioids [10, 26, 37, 38, 40, 48, 51, 58] and phencyclidine [12]. Although it is poorly understood how nonvolatile chemicals exit the body through the skin, potential pathways for drug molecules to reach the skin surface are via (Fig. 2):

- perspiration and / or sebum
- intercellular diffusion along the cell membrane complex
- transcellular diffusion and/or transport by the keratinocytes.

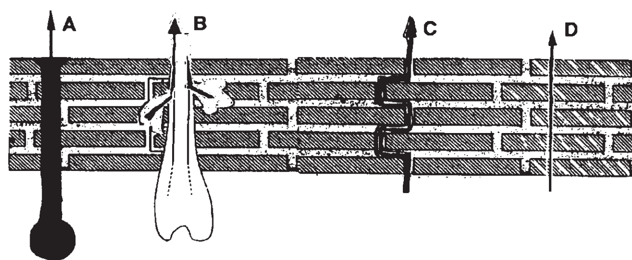
The passage of drug molecules from the skin capillaries into perspiration can be considered a passive diffusion process which is governed by the same factors as the secretion into saliva. The elimination of a substance via sebum is delayed for many days as is the transcellular diffusion and transport by the keratinocytes. The rating of the importance of these different routes still remains controversial. Additionally, drug binding to various skin fractions [70] and reabsorption of drugs from the skin have been observed [15]. Therefore, a continued presence of drugs on the skin surface results in the time period when blood or urine levels are already undetectable [4]. From these observations and on the basis of the current knowledge the following conclusions can be drawn:

- The material collected on the skin surface, i.e. perspiration, consists of various constituents and originates from various sources.
- The main analyte found on the skin surface is predominantly the parent drug.
- In single drug consumption a time lag exists. The time interval between drug consumption and detection on the skin surface depends on the nature of the particular drug and on the sensitivity of the analytical method used.
- In chronic abusers, drug molecules are permanently present on the skin due to the temporary reservoir of the stratum corneum.

Findings on forensically relevant drugs in perspiration

Opioids

In 1942, Oberst detected morphine in skin excretion [48] which was later confirmed by Ishiyama et al. [30] and Balabanova and Schneider [1] and Balabanova et al [2]. After heroin abuse, heroin and 6-acetylmorphine but little morphine was found in perspiration samples as evidenced by GC/MS analysis [17]. Further opiod-type substances including codeine [37, 40], dihydrocodeine [59] and methadone [26] have been detected in skin excretions. After opioid administration, perspiration contained preferably the parent opioid and lipophilic metabolites. Except for methadone, virtually all data on drug species and their concentrations in perspiration were obtained from experimental studies on healthy persons at single doses and low dosage. Recently, a solid phase enzyme immunoassay on microtiter plates for drug testing perspiration has been developed with a cut-off concentration of 10 ng morphine equivalents/ml [17]. Fogerson et al. [17] reported that single doses of less than 20 mg intravenously administered



Drugs reach the skin surface **via**:

A	sweat
B	sebum
C	intercellular diffusion along the cell membrane complex
D	transcellular diffusion and transportation by the keratinocytes

Fig. 2 Potential pathways for drug molecules to reach the skin surface

Table 5 Opiate concentrations (GC/MS) in skin swabs (skin area: 25 cm^2) from opiate-related fatalities ($n = 8$) [59], and in perspiration patches from patients treated with constant, but individually different doses of methadone (0.4 – 1.2 mg/kg, $n = 8$) [58]

Drug substance	Concentration (ng) per skin swab (25 cm^2) or perspiration patch (14 cm^2)
6-Acetylmorphine	62–14090
Morphine	3–20325
Codeine	13– 2050
Dihydrocodeine	90– 2725
Methadone	47– 1431
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	3– 57

heroin hydrochloride produced no positive result within 24 to 48 h using a perspiration patch for collection and the particular immunoassay for qualitative detection.

In opiate-related deaths, a wide range in drug concentrations was observed from skin swabs, and a relationship to acute, subacute or chronic drug abuse could not be established [59] (Table 5).

Cocaine

Cocaine findings on skin have been reported by Smith and Liu [61], Cone et al. [10], and Schneider and Balabanova [53]. After intranasal administration of single doses of 50 or 126 mg cocaine hydrochloride using a band-aid collection device, Burns and Baselt [4] concluded that even a single dose of cocaine can be detected in perspiration stains for up to 7 days, but the concentrations found showed no correlation either to dose nor to time of use. In a controlled study, Cone et al. [10] detected cocaine in skin excretions 1-2 h after dosing by GC/MS and peak concentrations within 24 h after administration of the drug. Cocaine appeared in an apparent dose-dependent manner and intrasubject variability was suggested to be low, in contrast to intersubject variability. When chronically used, cocaine was found in stimulated perspiration by radio immunoassay up to 6 days after abstaining from the drug [2]. Benzoylecgonine and ecgonine methyl ester were always identified in small concentrations in cases where relatively high concentrations of cocaine could be measured in the particular samples, the concentrations of ecgonine methyl ester exceeding those of benzoylecgonine [59].

Evidence of crack abuse was possible by GC/MS identification of anhydroecgonine methyl ester [39]. Kidwell et al. [35] screened cocaine in skin swabs from a university population. Controlled experiments showed that cocaine could remain on the skin for about 3 days after external exposure. From these results they concluded that cocaine concentrations > 15 ng per skin swab would appear to indicate either recent use or exposure (Table 6).

Recently, Spiehler et al. [62] presented a modified enzyme immuno assay involving microtiter plates for analysis of cocaine in perspiration with a cross-reactivity for cocaine of 102% relative to 100% for the benzoylecgonine calibrators and a cut-off concentration of 10 ng/ml cocaine or benzoylecgonine equivalents.

Table 6 Cocaine and cocaine metabolite concentrations (GC/MS) in skin swabs (skin area: 25 cm^2) from fatal cases of drug abuse ($n = 4$) [59]

Drug substance	Concentration (ng) per skin swab (25 cm^2)
Cocaine	500–2925
Ecgonine methyl ester	45– 750
Benzoylecgonine	1– 725

Cannabinoids

Reports on cannabinoid findings from skin swabs or perspiration patches are rare. In pilocarpine-stimulated perspiration samples, cannabinoids were detected by radio immunoassay ranging from 19–456 carboxytetrahydrocannabinol equivalents/ml [2], while Ehorn [14] used a highly sensitive GC/MS/MS assay for identification of tetrahydrocannabinol in perspiration stains. In addition to tetrahydrocannabinol, the 11-hydroxy-metabolite was present in skin swabs [59], while other cannabinoids or metabolites were not detected by GC/MS. Similiar findings have been published by Kintz et al. [38] who observed concentrations of 4–38 ng of tetrahydrocannabinol/patch from perspiration patches applied to drug abusers (Table 7).

Table 7 Cannabinoid concentrations (GC/MS) in skin swabs (skin area: 25 cm^2) from fatal cases of drug abuse ($n = 6$) [59]

Drug substance	Concentration (ng) per skin swab (25 cm^2)
Tetrahydrocannabinol	0.5–15
11-Hydroxy-tetrahydrocannabinol	trace (< 1.0)
Carboxy tetrahydrocannabinol, cannabinol, cannabidiol	not detected (< 0.5)

Amphetamines

In 1972 the detection of amphetamines in human perspiration as well as in stains on clothes worn next to the skin after administration of 20–25 mg L-dimethylamphetamine hydrochloride was reported by Vree et al. [69]. Methamphetamine and its major metabolite amphetamine were found in perspiration samples, whereas methamphetamine only was detected in saliva by GC/MS [64]. N-Methyl-1-(3,4-methylenedioxylphenyl)-2-butanamine (MBDB) and 3,4-methylenedioxyphenyl-2-butanamine (BDB) were excreted into perspiration with an increase in concentration during the first 36 h following a single dose of 100 mg MBDB in one subject [36]. In skin patches an-

alyzed by GC/MS the peak concentrations were 44 ng MBDB/patch and 23 ng BDB/patch. Recently, an enzyme immunoassay was modified for analysis of methamphetamine from perspiration with an optimum cut-off concentration of 10 ng amphetamine equivalents/ml [16].

Aspects of collection, processing and analysis of perspiration samples

Recently, an immunological test kit was investigated for perspiration samples and road side testing [59]. Drugwipe is a pen-sized test strip based on an immunological technique. It is simply to apply and results are available within 2–3 min. For the detection of opiates and cocaine, cross-reactivities of the antibodies satisfy the demands for skin testing, however sensitivity seems poor [59]. The antibody of the Drugwipe cannabis system preferably reacted with carboxytetrahydrocannabinol and its glucuronide (≥ 25 or 10 ng of the pure substance), but the test kit failed to detect tetrahydrocannabinol [59].

Although, recently modified enzyme immunoassays for detection of cocaine, heroin and methamphetamine abuse in perspiration samples [16, 17, 62] seem to be both specific and sensitive, handling of microtiter plates is not considered to be suitable for roadside testing. The use of ion mobility spectrometry (IMS, Barringer, Canada) is thought to be more sucessfull due to increased sensitivity and the possibility to detect the parent drug. In a preliminary study, abuse of heroin and cocaine could always be detected in cases tested positive for 6-acetylmorphine and morphine or cocaine by GC/MS (unpublished). Sample collection can be simply and quickly managed by wiping an appropriate skin area with a cotton wool roll from a Salivette system moistened with 70% ethanol, preferably on the sternal or axillary regions to avoid environmental contamination as far as possible. The skin swabs can be directly blotted onto the filter pads. Before in situ analysis, it is recommended to dry the pads with a hair-drier. For later confirmation, the cotton wool rolls can be stored frozen until GC/MS analysis by routine procedures. A skin swab collected by a cotton wool roll always contains superficial dermal cells allowing identification of the particular person [51].

Concluding remarks

Perspiration and saliva samples can be easily collected and processed to be measured in situ. However, in contrast to urine testing the assay used for both perspiration and saliva must be based on the detection of the parent drug. A large concentration range in perspiration and saliva samples has already been observed for all drugs of abuse [10, 52, 59]. Therefore, the sensitivity of the test system seems to be most crucial. The use of ion mobility spectrometry covers both sensitivity and specificity but requires advanced equipment and special handling. Currently, enzyme immunoassays on microtiter plates are available, exhibiting an adequate cross-reactivity to the parent drug. For on-site testing however, a dip-and-read test for rapid and easy screening should be favored without the need of an automatic analyzer and special storage or handling conditions. At present, a simple and easy assay serving all the purposes mentioned above is not commercially available.

Besides the technical demands, saliva and perspiration show completely different drug excretion patterns. The skin acts a drug reservoir and even in single dose application, there is a considerable time delay until the drug molecules will appear on the skin. Provided that environmental exposure could be excluded, skin testing may identify a substance abuser, but seems less appropriate to detect recent or actual drug administration.

Saliva can be regarded as an in vitro model for transmembrane transport and may therefore give information on the particular drug at the location where it exerts its pharmacological action. The theory and the results of a limited number of studies involving heroin and cocaine suggest that saliva concentrations are highly correlated to physiological and behavioral effects [6, 8, 31, 63], which favors saliva for estimation of recent or actual drug administration. Besides Röhrich et al. [52], detailed experiences of saliva testing from a roadside survey including 2235 specimens have been reported by Krüger [41]. Without extensive cleaning of the oral cavity prior to sampling, the collected saliva is in equilibrium with the oral mucosa, which has high local drug concentrations when drugs have been orally or nasally administered or inhaled, such as amphetamines, cocaine or cannabis. Oral drug sequestration in the early stage of drug abuse will even enhance its detection provided that specific and sensitive tests are used. In future, efforts have to be made to develop simple and appropriate test kits for roadside drug testing and to evaluate their applications under controlled conditions as well as under real life situations. Meanwhile, any indication of a possible actual impairment due to drug consumption should result in a reliable analysis of the blood sample taken from the particular driver.

References

1. Balabanova S, Schneider E (1990) Detection of drugs in sweat. Beitr Gerichtl Med 48:45–49
2. Balabanova S, Schneider W, Wepler R, Hermann B, Boschek HJ, Scheitler H (1992) Significance of drug determination in pilocarpine sweat for detection of drug abuse. Beitr Gerichtl Med 50:111–115
3. Bogusz MJ, Althoff H, Erkens M, Maier RD, Hofmann R (1995) Internally concealed cocaine: analytical and diagnostic aspects. J Forensic Sci 40:811–815
4. Burns M, Baselt RC (1995) Monitoring drug use with a sweat patch: an experiment with cocaine. J Anal Toxicol 19:41–48
5. Cone EJ (1990) Testing hair for drugs of abuse. I. Individual dose, time and profiles of morphine and codeine in plasma, saliva, urine and beard compared to drug-induced effects on pupils and behavior. J Anal Toxicol 14:1–7
6. Cone EJ (1993) Saliva testing for drugs of abuse. Ann N Y Acad Sci 694:91–127

7. Cone EJ, Weddington WW (1989) Prolonged occurrence of cocaine in human saliva and urine after chronic use. J Anal Toxicol 13 : 65–68
8. Cone EJ, Kumor K, Thompson LK, Sherer M (1988) Correlation of saliva cocaine levels with plasma levels and with pharmacologic effects after intravenous cocaine administration in human subjects. J Anal Toxicol 12 : 200–206
9. Cone EJ, Hillsgrove M, Darwin WD (1994) Simultaneous measurement of cocaine, cocaethylene, their metabolites, and "crack" pyrolysis products by gas chromatography-mass spectrometry. Clin Chem 40 : 1299–1305
10. Cone EJ, Hillsgrove MJ, Jenkins AJ, Keenan RM, Darwin WD (1994) Sweat testing for heroin, cocaine and metabolites. J Anal Toxicol 18 : 298–305
11. Cone EJ, Oyler J, Darwin WD (1997) Cocaine disposition in saliva following intravenous, intranasal, and smoked administration. J Anal Toxicol 21 : 465–475
12. Cook CE, Brine RD, Jeffcoat AR, Hill JM, Wall ME, Prez-Reyes M, DiGuiseppi SR (1982) Phencyclidine disposition after intravenous and oral doses. Clin Pharmacol Exp Ther 31 : 625–634
13. Cook CE, Jeffcoat AR, Hill JM, Pugh DE, Patetta PK, Sadler BM, White WR, Perez-Reyes M (1993) Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. Drug Metab Dispos 21 : 717–723
14. Ehorn C (1994) Ion trap GC/MS/MS for analysis of THC from sweat. Abstracts of the TIAFT-SOFT joint meeting, Tampa, Florida, Abstract 11
15. Faergemann J, Zehender H, Denouel J, Millerioux L (1993) Levels of terbinafine in plasma, stratum corneum, dermis-epidermis (without stratum corneum), sebum, hair and nails during and after 250 mg terbinafine orally once per day for four weeks. Acta Derm Venereol 73 : 305–309
16. Fay J, Fogerson R, Schoendorfer D, Niedbala RS, Spiehler V (1996) Detection of methamphetamine in sweat by EIA and GC-MS. J Anal Toxicol 20 : 398–403
17. Fogerson R, Schoendorfer D, Fay J, Spiehler V (1997) Qualitative detection of opiates in sweat by EIA and GC-MS. J Anal Toxicol 21 : 451–458
18. Geier A, Bergemann D, v Meyer L (1996) Evaluation of a solid-phase extraction procedure for the simultaneous determination of morphine, 6-monoacetylmorphine, codeine and dihydrocodeine in plasma and whole blood by GC/MS. Int J Legal Med 109 : 80–83
19. Goldberger BA, Darwin WD, Grant DM, Allen AC, Caplan YH, Cone EJ (1993) Measurement of heroin and its metabolites by isotope dilution electron-impact mass spectrometry. Clin Chem 39 : 670–675
20. Gorodetzky CW, Kullberg MP (1974) Validity of screening methods for drugs of abuse in biological fluids. II. Heroin in plasma and saliva. Clin Pharmacol Ther 15 : 579–587
21. Gross SJ, Worthy TE, Nerder L, Zimmermann EG, Soares JR, Lomax P (1985) Detection of recent cannabis use by saliva delta-9THC radioimmunoassay. J Anal Toxicol 9 : 1–5
22. Hackel R (1972) Detection of cannabinoids in the saliva following use of hashish. Arch Toxicol 29 : 341–344
23. Häckel R (1990) Relationship between intraindividual variation in the saliva/plasma- and of the arteriovenous concentration ratio as demonstrated by the administration of caffeine. J Clin Chem Clin Biochem 28 : 279–284
24. Häckel R, Hänecke P (1993) The application of saliva, perspiration and tear fluid for diagnostic purposes. Ann Biol Clin (Paris) 50 : 903–910
25. Häckel R, Hänecke P (1996) Application of saliva for drug monitoring. An in vivo model for transmembrane transport. Eur J Clin Chem Clin Biochem 34 : 171–191
26. Henderson GL, Wilson BK (1973) Excretion of methadone and metabolites in human perspiration. Res Comm Chem Pathol Pharmacol 5 : 1–8
27. Huestis MA, Dickerson S, Cone EJ (1992) Can saliva THC levels be correlated to behavior? American Academy of Forensic Science publication. Fittje Brothers Printing, Colorado Abstract 92–2, p 190
28. Idowu OR, Caddy B (1982) A review of the use of saliva in the forensic detection of drugs and other chemicals. J Forensic Sci Soc 22 : 123–135
29. Inaba T, Stewart DJ, Kalow W (1978) Metabolism of cocaine in man. Clin Pharmacol Ther 23 : 251–256
30. Ishiyama I, Nagai T, Komuro E, Momose T, Akimori N (1979) The significance of drug analysis of perspiration in respect to rapid screening for drug abuse. Z Rechtsmed 82 : 251–256
31. Jenkins AJ, Oyler JM, Cone EJ (1995) Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. J Anal Toxicol 19 : 359–374
32. Just WW, Filapovic N, Werner G (1974) Detection of delta-9-tetrahydrocannabinol in saliva of men by means of thin layer chromatography and mass spectrometry. J Chromatogr 96 : 189–194
33. Kang GL, Abbotts FS (1982) Analysis of methadone and metabolites in biological fluids with gas chromatography-mass spectrometry. J Chromatogr 23 : 311–319
34. Kato K, Hillsgrove M, Weinhold L, Gorelick DA, Darwin WD, Cone EJ (1993) Cocaine and metabolite excretion in saliva under stimulated and nonstimulated conditions. J Anal Toxicol 17 : 338–341
35. Kidwell DA, Blanco MA, Smith FP (1997) Cocaine detection in a university population by hair analysis and skin swab testing. Forensic Sci Int 84 : 75–86
36. Kintz P (1997) Excretion of MBDB and BDB in urine, saliva, and sweat following single oral administration. J Anal Toxicol 21 : 570–575
37. Kintz P, Tracqui A, Jamey C, Mangin P (1996) Detection of codeine and phenobarbital in sweat collected with a sweat patch. J Anal Toxicol 20 : 197–201
38. Kintz P, Tracqui A, Mangin P, Edel Y (1996) Sweat testing in opioid users with a sweat patch. J Anal Toxicol 20 : 393–397
39. Kintz P, Sengler C, Cirimele V, Mangin P (1997) Evidence of crack use by anhydroecgonine methylester identification. Hum Exp Toxicol 16 : 123–127
40. Kintz P, Cirimele V, Ludes B (1998) Codeine testing in sweat and saliva with the Drugwipe. Int J Legal Med 11 : 82–84
41. Krüger HP (1995) Medikamente im Strassenverkehr. Epidemiologische Ergebnisse zu Auftreten und Risiken. In: Krüger HP, Kohnen R, Schöch H (eds) Medikamente im Strassenverkehr. Gustav Fischer, Stuttgart Jena New York, pp 3–34
42. Leonard J, Doot M, Martin C, Airth-Kindree W (1994) Correlation of buccal mucosa transudate collected with a buccal swab and urine levels of cocaine. J Addict Dis 13 : 27–33
43. Lillsunde P, Korte T, Michelson L, Portman M, Pikkarainen J, Seppälä T (1996) Drugs usage of drivers suspected of driving under the influence of alcohol and/or drugs. A study of one week's samples in 1979 and 1993 in Finland. Forensic Sci Int 77 : 119–129
44. Lynn RK, Olsen GD, Leger RM, Gordon WP, Smith RG, Gerber N (1976) The secretion of methadone and its major metabolites in the gastric juice of humans: comparison with blood and salivary concentrations. Drug Metab Dipos 4 : 504–509
45. Matin SB, Wan SH, Knight JB (1977) Quantitative determination of enantiomeric compounds. I. Simultaneous measurement of the optical isomers of amphetamine in human plasma and saliva using chemical ionization mass spectrometry. Biomed Mass Spectrom 4 : 118–121
46. Menkes DB, Howard RC, Spears GF, Cairns ER (1991) Salivary THC following cannabis smoking correlates with subjective intoxication and heart rate. Psychopharmacology (Berl) 103 : 277–270
47. Musshoff F, Daldrup T (1993) Evaluation of a method for simultaneous quantification of codeine, dihydrocodeine, morphine and 6-acetylmorphine in serum, blood, and postmortem blood. Int J Legal Med 106 : 107–109

48. Oberst FW (1942) Morphin im Schweiss. J Pharmacol Exp Ther 74 : 37
49. Peel HW, Perrigo BJ, Mikhael NZ (1984) Detection of drugs in saliva of impaired drives. J Forensic Sci 29 : 185–189
50. Pötsch L, Skopp G (1997) Zu Möglichkeiten und Grenzen chemisch-toxikologischer Untersuchungen an nicht konventionellen Asservaten. In: Pragst F (ed) Moderne Messverfahren im Rahmen der toxikologisch-forensischen Begutachtung. Helm, Heppenheim, pp 70–88
51. Pötsch L, Schneider PM, Lummer J, Skopp G (1997) Identity testing of alternative samples used in forensic toxicology. Proceeding of the TIAFT XXXV Annual Meeting TIAFT, Cleup Editrice, Padore, pp 544–547
52. Röhrich J, Schmidt K, Kauert G (1997) Detection of drugs in saliva using the immunoassay Triage. Blutalkohol 34 : 102–114
53. Schneider E, Balabanova S (1991) Detection of drugs in clothing. Arch Kriminol 188 : 97–105
54. Schoch H (1997) Alcohol, street drugs and therapeutic drugs in street traffic. Fortschr Med 115 : 39–42
55. Schramm W, Smith RH, Craig PA, Kidwell DA (1992) Drugs of abuse in saliva: a review. J Anal Toxicol 16 : 1–9
56. Schramm W, Craig PA, Smith RH, Berger GE (1993) Cocaine and benzoylecgonine in saliva, serum, and urine. Clin Chem 39 : 481–487
57. Sharp ME, Wallace SM, Hindmarsh KW, Peel HW (1983) Monitoring saliva concentrations of methaqualone, codeine, secobarbital, diphenhydramine, and diazepam after single oral doses. J Anal Toxicol 7 : 11–14
58. Skopp G, Pötsch L, Eser HP, Möller MR (1996) Preliminary practical findings on drug monitoring by a transcutaneous collection device. J Forensic Sci 31 : 933–937
59. Skopp G, Pötsch L, Zimmer G, Mattern R (1997) Zur Interpretation von Drogenbefunden auf der Haut. Blutalkohol 34 : 427–434
60. Smith FP (1981) Detection of amphetamine in bloodstains, semen, seminal stains, saliva, and saliva stains. Forensic Sci Int 17 : 225–228
61. Smith FP, Liu RH (1986) Detection of cocaine-metabolites in perspiration stain, menstrual blood stain, and hair. J Forensic Sci 31 : 1269–1273
62. Spiehler V, Fay J, Fogerson R, Schoendorfer D, Niedbala RS (1996) Enzyme immunoassay for qualitative detection of cocaine in sweat. Clin Chem 42 : 34–38
63. Stillman R, Jones RT, Moore D, Walker J, Welm S (1993) Improved performance 4 hours after cocaine. Psychopharmacology (Berl) 110 : 415–420
64. Suzuki SI, Inoue T, Hori H, Inayama S (1989) Analysis of methamphetamine in hair, nail, perspiration and saliva by mass fragmentography. J Anal Toxicol 13 : 176–178
65. Takatori T, Tomii S, Terazawa K, Nagao M, Kanamori M, Tomaru Y (1991) A comparative study of diazepam levels in bone marrow versus serum, saliva and brain tissue. Int J Legal Med 104 : 185–188
66. Thompson LK, Yousefnejad D, Kumor K, Sherer M, Cone EJ (1987) Confirmation of cocaine in human saliva after intravenous use. J Anal Toxicol 11 : 36–38
67. Vapaatalo H, Karkkainen S, Senius KE (1984) Comparison of saliva and urine samples in thin-layer chromatographic detection of central nervous stimulants. Int J Clin Pharmacol Res 4 : 5–8
68. Valentin (1844) Lehrbuch der Physiologie des Menschen, Braunschweig, cited from : Balabanova S, Schneider E (1991) Nachweis von Drogen in Wäschestücken. Arch Krim 188 : 97–105
69. Vree TB, Muskens ATJM, Van Rossum JM (1972) Excretion of amphetamines in human sweat. Arch Int Pharmacodyn Ther 199 : 311–317
70. Walter K, Kurz H (1988) Binding of drugs to human skin: influencing factors and the role of tissue lipids. J Pharm Pharmacol 40 : 689–693
71. Wan SH, Matin SB, Azarnoff DL (1978) Kinetics, salivary excretion of amphetamine isomers, and effect of urinary pH. Clin Pharmacol Ther 23 : 585–590
72. Wang WL, Darwin WD, Cone EJ (1994) Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography-mass spectrometry. J Chromatogr B Biomed Appl 660 : 279–290
73. Widmann M, Agurell S, Ehrnebo M, Jones G (1974) Binding of (+)-Δ1-tetrahydrocannabinols and (–)-7-hydroxy- and (–)-Δ1-tetrahydrocannabinol to blood cells and plasma proteins in man. J Pharm Pharmacol 26 : 914–916
74. Yamahara H, Lee VHL (1993) Drug metabolism in the oral cavity. Adv Drug Delivery Rev 12 : 25–40