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Drugs of Abuse in Saliva: A Review

Willfried Schramm*, **Richard H. Smith**, and **Paul A. Craig**
BioQuant, Inc., 1919 Green Road, Ann Arbor, MI 48105

David A. Kidwell
Naval Research Laboratory, Chemistry Division, Code 6177, Washington, DC 20375-5000

Abstract

There has been substantial interest in the use of saliva as a diagnostic medium for drugs of abuse because it can be obtained noninvasively. Although drugs of abuse have been investigated in saliva for more than a decade, the role of saliva remains uncertain. A clear picture is difficult to obtain because of variations in (1) the analytical methods used; (2) the dose regimen of subjects, which was either unknown or differed between studies; and (3) the elapsed time between drug intake and sample collection. This communication summarizes the studies on the quantitative determination of different drugs of abuse in saliva to elucidate the current status in this area. Marijuana, cocaine, phencyclidine, opiates, barbiturates, amphetamines, and diazepines (or their metabolites) have all been detected in saliva by various analytical methods, including immunoassay, gas chromatography/mass spectrometry, and thin layer chromatography. Initial studies with cocaine and phencyclidine suggest a correlation between saliva and plasma concentrations of these drugs, indicating a dynamic equilibrium between saliva and blood. Tetrahydrocannabinol, the active component in marijuana, on the other hand, does not appear to be transferred from plasma to saliva. However, tetrahydrocannabinol is sequestered in the buccal cavity during smoking and can be detected in saliva. These findings point to the potential role of saliva in the analysis of many illicit drugs. To clearly identify the role of saliva as a diagnostic medium for drugs of abuse, research efforts should be directed towards (1) performing systematic studies on correlations between saliva, blood, and urine and (2) determining the concentrations of drugs and their metabolites in saliva as a function of dose and time after intake.

Introduction

The traditional media for the quantitative measurement of most physiologically active substances, drugs, and poisons are blood plasma and urine. Many substances and their metabolites are present in different concentrations in plasma and urine. While plasma can provide an estimate of the actual circulating concentration of the analyte of interest, urine permits measurement of the accumulated concentration of analytes since the last void of the bladder. Unfortunately, the concentration of substances in urine is also dependent on fluid intake, which can vary substantially.

Although more information is available on drugs of abuse and their metabolites in plasma, the single most frequently used source for the measurement today is urine. The reason is obvious: only a noninvasively obtained sample is typically acceptable for routine collection. Yet, even the acceptability of collecting a urine sample is being disputed in view of the potential invasion of privacy, especially if a directly observed collection is advisable to prevent adulteration or substitution of the sample. Unlike a urine sample, saliva can be obtained under supervision without direct observation of private functions.

Many drugs are highly bound to blood proteins, but it is only the free fraction that is physiologically active. Because saliva is an ultrafiltrate of interstitial fluid and contains the free component of drugs, it has the potential to better indicate a state of intoxication. Therefore, saliva has been increasingly used as the diagnostic medium for the measurement of therapeutic drugs and endogenous markers (1–5). The concentrations and clearance rates of salivary analytes are different from those in plasma and urine. Therefore, the diagnostic value of saliva for the determination of each drug needs to be investigated separately.

For the measurement of drugs of abuse, saliva was suggested as early as the seventies as an alternative medium (6). Particular interest in saliva has been expressed by law enforcement agencies for road-side testing of potentially intoxicated drivers (3,7–9).

In one of these studies, the authors came to the following conclusion: "The results of this investigation have shown that it is possible to obtain and analyze samples of saliva for drivers who have been suspected of impaired driving. This investigation indicates the potential versatility of using saliva as a non-invasive technique of determining the occurrence and frequency of drug use in impaired drivers" (7).

The purpose of this review is to examine the advantages and limitations of saliva in the measurement of drugs of abuse. Several classes of drugs have been investigated to a limited extent in saliva, including cannabinoids, cocaine, phencyclidine, opioids, barbiturates, diazepines, and amphetamines. Available information on each drug class will be summarized and the need for fur-

* Author to whom correspondence should be addressed.

ther studies will be discussed. Tables I–III are included to provide the reader with convenient access to the references.

Cannabinoids

Marijuana is the most frequently used illicit drug in the United States (10). Therefore, the incidence of recent marijuana use in motorists who exhibit erratic driving has attracted the attention of law enforcement agencies. Elevated levels of Δ^9-tetrahydrocannabinol (THC), the active component in marijuana, have been found in a relatively high percentage of this population in both plasma (14.4%) (11,12) and saliva (9%) (7).

Attempts to measure THC in saliva date back to the early seventies (13–15). Even with the relatively insensitive method of thin layer chromatography, cannabinoid derivatives can be detected. Several studies report on the determination of THC by other analytical methods as well [HPLC (16), GC (17), Immunoassay (18,19); See Table I].

A controlled study of 352 samples collected from 25 male and 10 female volunteers was undertaken to investigate the detection of THC in saliva (18). The data presented by the authors document that salivary THC reliably reflects positive testing in blood samples even though the concentrations in the two body fluids do not correlate precisely.

Plasma vs. saliva. The calculated saliva/plasma ratios of THC and the metabolite 11-hydroxy-Δ^9-tetrahydrocannabinol, based on the dissociation constants of the substances and the Henderson-Hasselbalch equation, are approximately 0.1 (3). This is in disagreement with the actual measured levels which are likely to be equal to or greater in saliva than in serum (16) (Table III). The high concentration is not a result of transfer from blood because radiolabeled THC administered by intravenous injection cannot be detected in saliva (20). Therefore, it seems that THC or its metabolites do not pass into the saliva or lungs from the blood but rather are sequestered in the buccal cavity during smoking. In some cases cannabinoids may be detected in saliva for a longer time than in plasma (16) because it is sequestered in the mouth.

Urine vs. saliva. Cannabinoids can be detected longer in urine

Table I. Drugs of Abuse Measured in Saliva and Methods of Detection*

Drugs	Methods of detection	References
Cannabinoids	HPLC (EC)	16
	GC (ECD)	17
	IA	18, 19
	TLC; MS	14
	TLC	13, 15
Cocaine	IA	7, 28
	GC (NPD)	26
	GC/MS (CI); GC (NPD)	27
Phencyclidine	IA	35
	HPLC (RD)	89
Opioids	HPLC (FD)	46, 47
	IA	6, 44, 48, 49
	GC (NPD)	43
	GC/MS (SID)	45
Barbiturates	HPLC (UV)	90
	GC (NPD)	43, 51, 63
	GC (ECD)	52
	GC (FID)	50
	IA	58, 61, 91, 92
Diazepines	GC (NPD)	93
		43, 66, 65, 73
	GC (EC)	7, 68
	RRA	70, 72, 76
	RRA; GC (EC)	64, 71
Amphetamines	TLC	82
	GC/MS (SID)	77

* Abbreviations CI: chemical ionization; EC: electrochemical detection; ECD: electron capture detection; FD: fluorescence detection; FID: flame ionization detection; GC: gas chromatography; HPLC: high peformance liquid chromatography; IA: immunoassay; MS: mass spectrometry; NPD: nitrogen-phosphorus detector; RD: radiochemical detection; RRA: radioreceptor assay; SID: single ion detection; TLC: thin layer chromatography; UV: ultraviolet.

Table II. Concentrations of Drugs of Abuse in Saliva*

Drug class	Analyte	Conc. (ng/mL)	Time of detection**	Reference
Cannabinoids	Δ^9-THC	5–200	~ 7 h	16
	Δ^9-THC	30–250	< 4 h	17
	Δ^9-THC	6–330	4–6 h	18
	Δ^9-THC	5–100	< 8 h	20
	Δ^9-THC	qual.	14 h	14
	Δ^9-THC	qual.	< 6 h	15
Cocaine	Cocaine	0.5	5–10 d	28
	Cocaine	1–10	12–24 h	28
Phencyclidine	Phencyclidine	2–600	n.a.	35
Opioids	Morphine	qual.	3–4 h	6
		0.6	24 h	42
	Codeine	120	3 h	43
		0.6	36 h	42
	Pholcodine	20	~ 4 d	47
	Hydromorphone	0.3–1	4–10 h	48
	Methadone	200	24 h	45
Barbiturates	Amobarbital	200	50 h	52
	Amobarbital	100	50 h	50
	Hexobarbital	100	12 h	51
	Phenobarbital†	~ 8000	24 h	54
Methaqualone	Methaqualone	20	> 24 h	63
		300	3 h	43
Diazepines	Diazepam	2	50 h	64
	Diazepam	700	< 5 h	71
	Diazepam	20	24 h	66
	Diazepam	2	8 h	70
	Nordiazepam	692	24 h	66
	N-Desmethyld.	1100	< 5 h	71
Amphetamines	Amphetamine	20–40	50 h	77

* Abbreviations: n.a.: not available; qual: qualitative.
** This is the time after use when the sample was collected and does not necessarily represent the maximal time for reliable detection of the drug.
† Phenobarbital concentration in saliva during treatment for epileptic seizure control, 6 to 10 µg/mL.

(21–23) than in saliva (14–18,20), i.e., between 5 to 20 days (Table IV) vs. 14 h (Table II), depending on the dose and frequency of use. Therefore, for the determination of "past use," urine is more suitable than saliva. However, for the indication of current intoxication or specific time of use, urine is inadequate and saliva has been recommended as the body fluid of choice (20).

Determination in saliva. Concentrations of THC between 5 and 330 ng/mL (16–18,20) (Table II) have been reported for up to 8 h after smoking, although qualitative tests could detect the metabolite for up to 14 h (15). These concentrations can be analyzed with existing analytical methods (immunoassays, GC/MS).

Very little is known about the composition of cannabinoid metabolites in saliva. As a result of enzymatic metabolism primarily in the liver, THC is converted via several intermediates into 11-nor-Δ^9-THC-9-carboxylic acid (THC-COOH) which circulates in blood and is the most prevalent metabolite found in urine (20). Therefore, this metabolite has been recommended for confirmatory testing of samples identified as positive in screening procedures (24). If THC-COOH is not transferred from blood to saliva (20), confirmatory tests for THC and other metabolites may be needed with saliva.

Preliminary evidence suggests that saliva contains a number of cannabinoid metabolites that have been found only in minute amounts, if at all, in urine. We have investigated a urine and a saliva sample (as an ultrafiltrate), collected simultaneously from an individual who had smoked marijuana. The urine sample contained only the stable metabolite, THC-COOH (Figure 1A). In the saliva sample, THC-COOH and three metabolites could be detected (Figure 1B): Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH). Because it appears that cannabinoid metabolites do not cross from the blood system into the salivary gland (20), the THC metabolites detected in saliva may have come directly from the marijuana smoke or from metabolism in the mouth.

Based on the evidence available today, the detection of marijuana metabolites in saliva is a good indicator of recent use. Concern has been expressed about the possibility of intentionally removing traces of the drug from the mouth (8). Surprisingly, normal food and liquid intake does not interfere substantially with the detection of cannabinoids in saliva (16,18,20). Whether

Table III. Saliva-to-Plasma Ratios (S/P) of Drugs of Abuse

Drug	S/P	References
Marijuana	1 to 2	16
Cocaine	1 to 2	26, 27
Phencyclidine	1.5 to 3.0	89
Opiates		
morphine	~ 0.2	42
codeine	1	42
	3	43
methadone	0.5	45
pholcodine	4	46
hydromorphone	~ 1	48
Barbiturates		
amobarbital	0.3–0.4	50
secobarbital	0.3	43
hexobarbital	0.3	51
phenobarbital	0.3–0.5	55, 59, 61
methaqualone	0.1	43, 63
Diazepines		
diazepam	0.03	70, 76
	0.02	66
nordiazepam	0.03	66
Amphetamine	2.8	77

Table IV. Reported Time During Which Drugs of Abuse Can Be Measured in Urine

Drug	Comment	Time	References
Cannabinoids	Moderate smoker	5 d	21
	Heavy smoker	10 d	22
	Chronic use	20 d	23
Cocaine	Metabolite BE	1–4 d	28–31
Phencyclidine	Moderate use	8 d	94
	Chronic use	8–30 d	95
Opiates	–	2 d	29
Barbiturates	Short acting	1 d	96
	Long acting	2–3 wk	97–99
Diazepines	Therapeutic dose	3 d	100
Amphetamines	–	2 d	29, 101

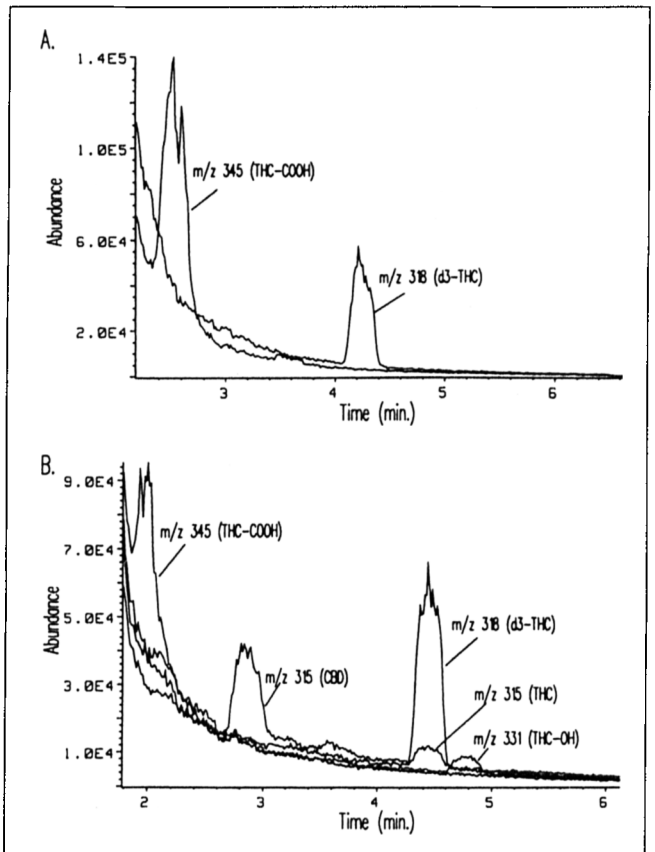

Figure 1. Cannabinoid metabolites in (A) a urine sample and (B) a simultaneously collected saliva sample. The samples were analyzed by direct injection of 200 µL fluid onto an HPLC column. The eluent was applied via a Vestec thermospray unit to a mass spectrometer (88). Saliva was collected as an ultrafiltrate with an osmotic device (75). We used as an internal standard deuterated THC (d_3-THC).

the concentration of cannabinoid metabolites can be substantially lowered by a mouth rinse as suggested (19), for example with alcohol, needs to be further investigated.

Cocaine

Plasma vs. saliva. In an early study, the metabolism of orally administered radiolabeled cocaine was followed in saliva and plasma (25). Recent investigations have established that cocaine can be detected in saliva after intravenous administration (16,26), demonstrating that cocaine enters the salivary glands from the blood circulation and is not just a residue from oral or nasal self-administration. The concentration of cocaine in saliva is usually higher than in plasma (26,27) (Table III), although a saliva/plasma ratio between 3 and 0.5 has been reported for one subject over time (27). Using immunoassay technology, cocaine can be detected in saliva as much as 10 days after cessation of intake (28) (Table II).

Because very little is known about the concentration of benzoylecgonine in saliva, the following considerations might serve as a working hypothesis for the expected distribution between plasma and saliva. The effects of pH on the predicted drug distribution between saliva and plasma for cocaine and benzoylecgonine are shown in Figure 2. Cocaine is a basic drug (pKa = 8.6), while benzoylecgonine is a zwitterion containing both acidic and basic functional groups (pKa = 2.25 and 11.2). For comparison, we have also shown the expected distribution of the acidic antiepileptic drug valproic acid (pKa = 4.6). While the saliva/plasma ratio of cocaine is high at low salivary pH and decreases at higher salivary pH, the concentration of benzoylecgonine is not significantly affected by the acidity of saliva. It should, however, be noted that the prediction of distribution on the basis of this passive diffusion model is not always in agreement with experimentally measured results (3), and solubility in the lipid bilayer of the membranes of the acinar cells in the salivary gland and even potential active transfer mechanisms can contribute to the saliva/plasma ratio.

Urine vs. saliva. A good correlation between cocaine concentration in urine and saliva has been reported (28), but the concentration is 20- to 50-fold higher in urine than in saliva. However, with GC/MS as the detection method, cocaine could be reliably measured in saliva for 12 to 36 h after administration. Immunoassays, which are more sensitive than GC/MS, permitted detection of cocaine in saliva for 5 to 10 days after withdrawal (28) (Table II). As a comparison, the cutoff level for cocaine and its metabolites recommended by the National Institute on Drug Abuse (NIDA) for urine screening at 300 ng/mL (24) can be reached in saliva 1 to 4 days after cessation of cocaine use (28–31) (Table IV).

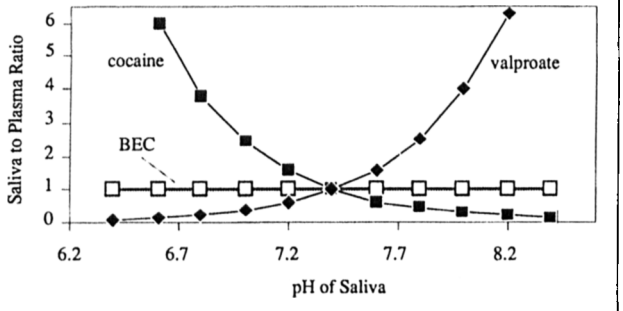

Figure 2. The effect of salivary pH on the saliva-to-plasma ratio of a drug. The distribution ratios were determined based on the net charge on the molecules at the indicated salivary pH, using the Henderson-Hasselbalch equation. Plasma pH was assumed to be 7.4.

To our knowledge, the concentrations of the major metabolites of cocaine, benzoylecgonine (BE), ecgonine methylester, and ecgonine have not been reported for saliva. Because the half-life of cocaine in blood is about 40 min (26,32,33) but that of BE is 7.5 h (28,34), BE is used for confirmatory measurement by GC/MS. It seems reasonable to expect that BE can be found in saliva in correlation with the concentration in plasma. In fact, in preliminary investigations, we found cocaine as well as benzoylecgonine and ecgonine in the saliva of a cocaine user (Figure 3). Other samples contained ecgonine methyl ester as well. However, the relative amount of each species varied widely, possibly depending on the length of time since the last use of cocaine.

Phencyclidine

Plasma vs. saliva. Only two studies have been published on the concentration of phencyclidine (PCP) in saliva (35,36)(Table I). Exchange of PCP between the blood system and the salivary gland has been shown by the detection of radioactively labeled derivatives in saliva after intravenous infusion. Concentrations were actually higher in saliva than in plasma with the ratio of saliva/plasma between 1.5 and 3.0 (36) (Table III). From these investigations, saliva seems to be a suitable medium for the determination of this drug. Paired serum and saliva samples obtained from 100 emergency department patients showed a good correlation between the two media for positive and negative confirmation of PCP (35).

Urine vs. saliva. Saliva is a good alternative to analysis of urine because the excretion of PCP and its metabolites in urine varies substantially with the pH of the urine (36,37) and can therefore lead to misinterpretation. The pH of urine normally ranges from 4.5 to 8.0 (38), whereas the pH of saliva, although variable, is usually in a more narrow range, between 6.5 and 7.2 (39,40). Stimulation of saliva flow with citric acid does increase

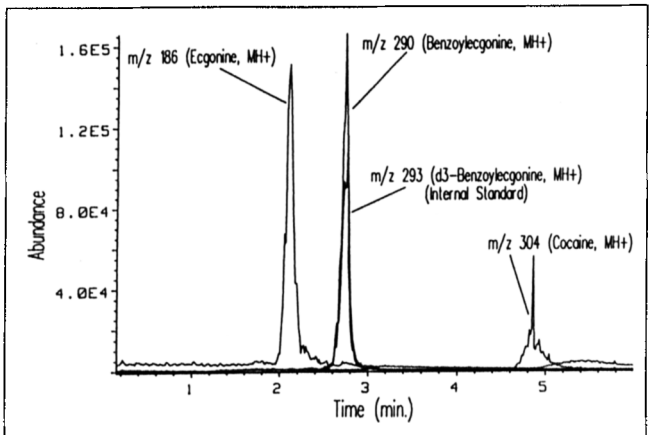

Figure 3. Cocaine and metabolites in a saliva sample of a cocaine user. The samples were analyzed as described in Figure 1. Saliva was collected as an ultrafiltrate with an osmotic device (75). Deuterated BE was used as an internal standard.

the pH (41), but even with stimulation the pH remains reasonably constant. The reported concentration of PCP in saliva ranges between 2 and 600 ng/mL (35) (Table II) and is comparable to that in urine. No data are available describing how long the drug is detectable in saliva.

Opioids

Plasma vs. saliva. According to an early study, morphine, the major metabolite of heroin, is detectable for a shorter time in saliva than in urine (6) (Table V). More recently, simultaneous measurement of morphine in three body fluids showed increasing concentrations in saliva, plasma, and urine, in that order (42) (Table VI). In saliva and plasma, the concentrations approached the sensitivity limit of the immunoassay after about 24 h, and in urine after 3 to 6 days. The 300-ng/mL cutoff recommended by NIDA for screening procedures in urine is much higher than the detection limit of the immunoassay. The NIDA cutoff level was reached within 24 to 36 h in urine samples.

The saliva/plasma ratio of codeine has not been determined with certainty. Following the oral administration of 30 mg of codeine phosphate per subject, 120 ng/mL could be measured in saliva after 3 h (43) (Table II). A higher concentration was found in saliva than in plasma with much variation between individuals, the saliva/plasma ratio ranging from 2 to 6.6 with an average ratio of 3 (Table III). On the other hand, Cone (42) measured about the same concentration of codeine in plasma and saliva (Table VI). While the first group used gas chromatography as a detection method, the second group measured the levels by RIA with an antibody that may show cross-reactivity with a metabolite. Codeine is a weak base with a pKa of 8.21 and the salivary pH would affect the saliva/plasma ratio, providing another possible explanation for differences in the studies.

Urine vs. saliva. Salivary morphine concentrations may attain about 10% the level of urinary morphine. This ratio, however, depends largely on the accumulated volume of urine after the last void (42) (Table VI). The concentration of "morphine equivalents," i.e., opiate derivatives recognized by the antibody used, typically does not exceed 200 ng/mL, although a value of 20 µg/mL was reported for a single saliva sample after recent use (injection of an unspecified amount of heroin 20 min before sample collection) (44). In comparison, the urinary concentrations usually do not exceed 6 µg/mL.

Synthetic opioids. Methadone has been measured in saliva. The saliva/plasma ratio is about 0.5 (Table III) and the correlation between saliva and plasma is excellent (45). After taking a dose of 90 mg, about 200 ng/mL of methadone is detectable in saliva 24 h later (45) (Table II). Likewise, pholcodine, a synthetic opioid widely used as an antitussive drug has been determined in saliva and compared with plasma and urine levels (46,47). Good correlation between the three media was reported ($r > 0.99$) with concentrations in saliva about four times greater than in plasma (46) (Table III). The drug could still be detected four days after administration of 60 mg per individual (47) (Table II). Saliva also has been suggested as an alternative medium for the measurement of hydromorphone, an analgesic alternative to morphine (48). In the elimination phase (more than1 h after administration), the drug is found in saliva at the same concentration as in plasma (48) (Table III) and can be detected up to 10 h after intake (48) (Table II). Enkephalin, a naturally occurring opioid-like peptide, has also been detected in saliva (49).

Table V. Time of Detection of Morphine in Plasma and Saliva after Administration of Different Amounts of Heroin (6).

Heroin (mg/70 kg)	Saliva (h)	Plasma (h)
2.5	n.d.	n.d.*
5 and 10	1–2	2–4
30 over 6 h**	3–4	6

* n.d.: not reliably detectable.
** 30 mg administered over 6 h. The measurements were taken after the last dose was given.

Barbiturates and Methaqualone

Analytical methods suitable for the detection of barbiturates in saliva have been described for amobarbital, secobarbital, phenobarbital, hexobarbital, and pentobarbital (Table I). For amobarbital (50), secobarbital (43), and hexobarbital (51) about 30 to 40% of the total concentration in plasma is found in saliva (Table III). Because the pKa of the barbituric acids falls in the range 7.4–8.3, the transfer into the salivary gland is pH dependent. However, correlations between matched plasma and saliva samples are good [amobarbital (50), secobarbital (43), hexobarbital (51)] if the pH is taken into account.

The half-lives for the moderate-acting barbiturates amobarbital and pentobarbital in saliva are about 24 h and 18 h, respectively (52), and for the short-acting hexobarbital 3.3 h (51). The concentration in saliva 50 h after an oral dose of 120 mg amobarbital has been measured between 100 ng/mL (50) and 200 ng/mL (52).

Phenobarbital has been extensively measured in saliva, especially for the management of seizure control in epileptic patients (53–61). The reported correlation coefficients between the concentrations in plasma and saliva usually exceed 0.9. With a pKa of 7.2, the ratio of distribution between saliva and plasma is pH dependent. By taking the pH of saliva into consideration, the level of the free drug in plasma can be calculated (62). However, some authors did not find a significant effect of the pH in saliva on the saliva/plasma ratio (58,61).

The sedative-hypnotic drug methaqualone can be detected in

Table VI. Concentrations of Morphine and Codeine in Three Body Fluids after Intramuscular Injection of Different Doses (42)*

Drug mg/subject	Saliva (ng/mL)	Plasma (ng/mL)	Urine (ng/mL)
morphine, 10	11	66	1100
morphine, 20	38	150	2400
codeine, 60	184	212	1797**
codeine, 120	308	272	3251†

* The concentrations reflect the free, non-glucuronated opiates in all body fluids 0.25 to 0.5 h after intramuscular injection.
** Concentration after the first void (1 h after injection). The concentration in the second void (3.8 h after drug) was 4384 ng/mL.
† Concentration after the first void (1 h after injection).

saliva in good correlation with plasma (43,63); the saliva/plasma ratio is 0.1 (Table III). After administration of 250 mg, saliva still contains about 20 ng/mL of the drug 24 h later (63) (Table II).

Diazepines

The diazepines are tranquilizers prescribed as sedative-hypnotics, muscle relaxants, anticonvulsants, and for relief of anxiety and psychiatric disorders. Diazepam (Valium is the most widely known trademark) is the most popular among the benzodiazepines. In a number of investigations, the utility of saliva for the determination of several diazepines in diagnostic evaluation has been studied (Table I) with excellent (64–66), moderate (68), and poor (67) correlations to plasma being reported. Saliva has also been used for the detection of benzodiazepines in the screening of impaired drivers (7).

Several issues still must be considered for the appropriate evaluation of diazepines in saliva. About 97% of diazepam in plasma is bound to proteins (69). Therefore, saliva as an ultrafiltrate of blood should contain 3% of the total concentration in plasma. While some investigators have found this to be the case (64,70), others measured either higher (65) or lower concentrations (66,71). The binding of diazepines to saliva proteins has been suggested as one possible reason for this discrepancy (72). Another source of variation may be the observed nitro-reduction of at least some diazepines (73), which is known to occur with nitrazepam and clonazepam (67,74). A solution to these problems might be the collection of an ultrafiltrate directly in the mouth to exclude proteins, including enzymes, as has been reported for other free molecules (75).

Low concentrations of diazepines can be very effectively measured by a radioreceptor assay with homogenized cells from the cerebral cortex of rats (64,70,72,76) (Table I). Results obtained with this assay are in good agreement with gas chromatography as the analytical method (64).

Amphetamines

Amphetamine and methamphetamine are sold widely on the black market. The half-life of amphetamine in plasma is highly dependent on the acidity of the urine because renal excretion is the major elimination route (77). Because of the high amphetamine levels found in saliva and the strong dependency of urinary pH on the excretion of the drug (78,79), saliva has been recommended as the medium of choice for diagnostic evaluation (77). The concentration of amphetamine is about three times higher in saliva than in plasma (77) (Table III). After a dose of 10 mg per subject, 20 to 50 ng/mL can be detected in saliva 50 h later (77) (Table II).

After administration of methamphetamine, according to a recent report, only this substance, not its major metabolite, amphetamine, was detected over time in saliva (80). However, it seems unlikely that amphetamine does not cross from the blood system to the salivary gland because other investigators found a very good correlation in plasma and saliva over extended periods of time (77). The secretion of methamphetamine from blood into saliva has also been reported by a second group (81).

While immunoassay methodologies may effectively detect amphetamines in saliva, the concentrations observed with a typical dose make thin layer chromatography unsuitable for the detection of amphetamines in saliva (82).

Advantages of Saliva

Noninvasive collection. As with urine, saliva can be obtained without the potential danger of infecting the subject. The sample can be collected without excessive inconvenience and the specimen can be easily handled without endangering personnel. The term "invasive," while traditionally used to indicate collecting a sample specimen by puncturing the skin, has assumed a different connotation in the area of drugs of abuse, that of invasion of privacy.

Protection of privacy. The collection of saliva, if properly performed, does not require special facilities and close supervision of private functions of the subject. Especially with the device for the collection of an ultrafiltrate in the mouth (75,83), aesthetic collection without spitting and the simultaneous collection from many subjects in the same room by one supervisor is possible.

Adulteration. Although the possibility of tampering with a sample by the donor can never be completely excluded, it is unlikely that any of the commonly used methods for adulteration of urine samples (84,85) can be easily applied to saliva. With the incidence of adulteration substantially reduced, the analysis becomes much less expensive.

Circulating concentrations. Unlike urine, saliva can be used to estimate the actual, protein-unbound, circulating concentration of some drugs or their metabolites at the time of collection. This becomes important for the evaluation of the impairment of individuals (once generally acceptable criteria for impairment have been established). Also, because the half-lives of metabolites are different, future investigations may reveal that the ratio of different metabolites in saliva can be used for the estimation of the time of ingestion.

Limitations of Saliva

One of the limitations in using saliva for drug evaluation will be overcome in the future by further investigations: our incomplete knowledge about the detectability over time with common analytical methods and the metabolism of many drugs. Although the body of information is rapidly increasing, many studies will still be required until generally accepted cutoff levels can be established (24). A general understanding of the diagnostic value of saliva for the major classes of abused drugs exists, but much of the published information needs to be confirmed by independent groups before saliva testing can be widely employed.

The concentrations of drugs are usually lower in saliva than in urine. Therefore, the methods established for urine as sample medium cannot be indiscriminately applied to saliva. At the same time, there do not seem to be technical limitations in measuring drugs and their metabolites in saliva by generally available analytical methods (Table I). Immunoassays are sufficiently sensitive to detect the substances in non-extracted samples. Fur-

thermore, GC/MS can be modified for the quantitative determination of analytes at concentrations in the pg/mL range (86,87).

Drugs of abuse can be measured in saliva for shorter periods than in urine (Tables II and IV) because the concentrations are higher in urine. However, it should be noted that many drugs have not been measured over extended periods in saliva and, therefore, the values shown in Table II may not represent the maximal time when the drug can be measured in saliva. Future studies for the determination of drug levels in saliva would also have to discriminate between chronic and occasional users of drugs because the measured concentration post administration may be considerably higher in chronic than in sporadic or occasional users (Table IV).

Conclusions

For the measurement of drugs of abuse, urine is the most widely used medium, primarily because it can be readily obtained. However, saliva may have potential as an alternative sample medium. Most of the commonly used drugs of abuse have been measured in saliva. Saliva eliminates the issue of protection of privacy and, to a large degree, of adulteration during sample collection. In contrast to urine as medium, measurement of drug concentrations in saliva provides an estimate of the actual circulating amount, and the results can therefore be used for the determination of current intoxication.

The major disadvantage of saliva is that many drugs are retained for a shorter period of time than in urine. Because the concentrations of drugs are lower in saliva than in urine, automated methods adjusted for urine cannot be indiscriminately used for saliva. Considering the number of classes of abused illicit drugs, the different metabolites within these classes, the number of metabolites for each substance, variation in drug metabolism by individuals, and potential differences caused by sporadic vs. chronic use, much information still needs to be accumulated before scientific and technical guidelines can be recommended for saliva as a medium for the measurement of drugs of abuse. However, projecting the pace of development of the last few years in this area into the future strongly indicates that saliva may soon have its place as the biological medium for many applications in the area of drugs-of-abuse monitoring.

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