NISTIR 7139

Evaluation of Saliva/Oral Fluid as an Alternate Drug Testing Specimen - Final Report

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Coordination by
U.S. DEPARTMENT OF COMMERCE
Office of Law Enforcement Standards
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and Technology
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This report was conducted under the direction of Alim A. Fatah, Program Manager for Chemical Systems and Materials, and Kathleen M. Higgins, Director of OLES.

FOREWORD

The Office of Law Enforcement Standards (OLES) of the National Institute of Standards and Technology (NIST) furnishes technical support to the National Institute of Justice (NIJ) program to strengthen law enforcement and criminal justice in the United States. OLES's function is to develop standards and conduct research that will assist law enforcement and criminal justice agencies in the selection and procurement of quality equipment.

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This document covers research conducted by OLES under the sponsorship of the NIJ. Additional reports as well as other documents are being issued under the OLES program in the areas of protective clothing and equipment, communications systems, emergency equipment, investigative aids, security systems, vehicles, weapons, and analytical techniques and standard reference materials used by the forensic community.

Technical comments and suggestions concerning this report are invited from all interested parties. They may be addressed to the Office of Law Enforcement Standards, National Institute of Standards and Technology, 100 Bureau Drive, Stop 8102, Gaithersburg, MD 20899–8102.

Kathleen M. Higgins, Director
Office of Law Enforcement Standards

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COMMONLY USED SYMBOLS AND ABBREVIATIONS

A	ampere	Hf	high frequency	o.d.	outside diameter
ac	alternating current	Hz	hertz	Ω	ohm
AM	amplitude modulation	i.d.	inside diameter	p.	page
cd	candela	in	inch	Pa	pascal
cm	centimeter	IR	infrared	Pe	probable error
CP	chemically pure	J	joule	pp.	pages
c/s	cycle per second	L	lambert	Ppm	parts per million
d	day	L	liter	Qt	quart
dB	decibel	lb	pound	Rad	radian
dc	direct current	lbf	pound-force	Rf	radio frequency
°C	degree Celsius	lbf·in	pound-force inch	Rh	relative humidity
°F	degree Fahrenheit	Lm	lumen	S	second
dia	diameter	Ln	logarithm (base e)	SD	standard deviation
emf	electromotive force	Log	logarithm (base 10)	sec.	Section
eq	equation	M	molar	SWR	standing wave ratio
F	farad	m	meter	uhf	ultrahigh frequency
fc	footcandle	μ	micron	UV	ultraviolet
fig.	figure	min	minute	V	volt
FM	frequency modulation	mm	millimeter	vhf	very high frequency
ft	foot	mph	miles per hour	W	watt
ft/s	foot per second	M/s	meter per second	λ	wavelength
g	acceleration	Mo	month	wk	week
g	gram	N	newton	wt	weight
gr	grain	N·m	newton meter	yr	year
H	henry	Nm	nanometer		
h	hour	No.	number		

area=unit^2 (e.g., ft^2, in^2, etc.); volume=unit^3 (e.g., ft^3, m^3, etc.)

PREFIXES

d	deci (10^{-1})	Da	deka (10)
c	centi (10^{-2})	H	hecto (10^2)
m	milli (10^{-3})	K	kilo (10^3)
μ	micro (10^{-6})	M	mega (10^6)
n	nano (10^{-9})	G	giga (10^9)
p	pico (10^{-12})	T	tera (10^{12})

COMMON CONVERSIONS (See ASTM E380)

0.30480 m =1 ft	4.448222 N = 1 lbf
25.4 mm = 1 in	1.355818 J = 1 ft·lbf
0.4535924 kg = 1 lb	0.1129848 N m = 1 lbf·in
0.06479891 g = 1 gr	14.59390 N/m = 1 lbf/ft
0.9463529 L = 1 qt	6894.757 Pa = 1 lbf/in^2
3600000 J = 1 kW·hr	1.609344 km/h = 1 mph

psi = mm of Hg x (1.9339 x 10^{-2})
mm of Hg = psi x 51.71

Temperature: T °$_C$ = (T °$_F$ –32)×5/9
Temperature: T °$_F$ = (T °$_C$ ×9/5)+32

Evaluation of Saliva/Oral Fluid as an Alternate Drug Testing Specimen - Final Report

Dennis J. Crouch, Jayme Day, Jakub Baudys, University of Utah, Center for Human Toxicology, Salt Lake City, UT 84112–9457.

This report presents findings from a study to determine whether saliva/oral fluid is a suitable specimen for drug testing in the criminal justice system. The study involved a literature review; a report based on findings from the review; and a clinical study, using codeine as a model drug, to assess the practical problems of collecting and analyzing oral fluid samples.

Findings

- The study found that oral fluid is a promising specimen for drug testing and has several advantages over other testing specimens: (1) It may be collected simply, noninvasively, and under direct supervision; (2) because oral fluids are a filtrate of blood, the oral fluid-drug concentration should reflect blood-drug concentrations; (3) because oral fluid is relatively free of blood constituents, it can be easily processed for testing by conventional drug screening and confirmation methods.
- Researchers discovered that the technique used to collect oral fluid affected the drug concentration, and nonstimulated spitting was the most effective technique because it produced the highest levels of drug concentration.
- The current method of using a specified concentration level of the oral fluid immunoglobulin G (IgG) appeared to be ineffective in determining if an oral sample had been diluted. Thus, more research is needed to identify a chemical marker that will ensure the validity of oral fluid specimens.

Further Research

Using oral fluid to test for drugs requires further research to determine:

- How drug concentrations in oral fluid correlate with drug concentrations in other body fluids.
- How factors such as pKa, physical size, and the degree of protein-binding lipophilicity affect drug transfer into oral fluid.

The authors would like to acknowledge Wayne Barlow, President; Kent Thomas, Business Manager; Lewis Webster, Senior Scientist; and Dennis Briscoe, Chief Engineer from Wescor, Inc., for their assistance to modify the collector, and the technical assistance provided in this regard. The authors would also like to acknowledge the cooperation and support of Mr. Jerome Robinson and his staff from the PSA. Without the support and cooperation of Wescor and PSA, the pilot and field studies could not have been successfully completed.

1. INTRODUCTION

1.1 Drug Testing Methods

Studies have shown that more than two-thirds of people who are incarcerated admit that they used drugs while incarcerated, and one-third admit that they used drugs while committing their crimes (Bray and Crouch, 1997). In addition, studies reveal that alcohol or drugs are detected in about two-thirds of the drivers who are involved in fatal automobile accidents (Bray and Crouch, 1997). Workplace drug testing has become fairly routine due to concerns about employee safety, health, and productivity. Given the prevalence and impact of drug abuse, it is not surprising that drug testing has become an integral part of society and criminal and civil investigations.

Drug testing is conducted at all stages of the criminal justice system: at arrest, before a trial, and during incarceration, probation, and parole (Timrots, 1992). To date, testing has been done primarily on blood, plasma, and urine, but alternate biological specimens that can be collected easily and noninvasively and can complement or replace urine and blood are being evaluated, including hair, sweat, and saliva. Each has its own strengths and weaknesses. For example, hair may contain a history of a donor's drug use, but, if drugs are smoked in the vicinity of the donor, then the hair is subject to external contamination from touching with the hands and drug vapors. Sweat can be collected using sweat patches, but little is known about interpreting the concentrations of drugs detected in the patches.

1.2 Oral Fluid as a Drug-Testing Specimen

Oral fluid, sometimes called "mixed saliva," comes from three major and several minor salivary glands. Strictly speaking, oral fluid is the mixed saliva from the glands and other constituents present in the mouth. "Saliva" is the fluid collected from a specific salivary gland and is free from other materials. This report will use the term oral fluid because it best describes the specimen examined in the studies.

Oral fluid contains plasma electrolytes such as potassium, sodium, chloride, and bicarbonate and many other plasma constituents, such as enzymes, immunoglobulins, and DNA. The total volume of oral fluid produced by an adult may be in excess of 1000 mL/day with typical flows of 0.05 mL/min while sleeping, 0.5 mL/min while spitting, and 1 mL/min to 3 mL/min while chewing gum.

Many drugs of interest in the criminal justice setting have been detected in oral fluid, including ethanol, methamphetamine, amphetamine, barbiturates, benzodiazepines, heroin, cocaine, and cannabinoids. It has been reported (Cone, 1993) that oral fluid-amphetamine concentrations exceeded blood concentrations for 48 hours after use, PCP-oral fluid concentrations may exceed plasma concentrations, and detection times for morphine concentrations in oral fluid were longer than for those in plasma following a single dose of heroin. However, much remains to be learned about the potential to detect drugs in oral fluid and how drug concentrations in oral fluid correlate with drug concentrations in other body fluids.

Several factors may affect drug transfer into saliva, such as pKa, physical size, degree of protein binding, and lipophilicity of the drug. In addition, parent drugs and not metabolites are often found in oral fluid because they are more lipid soluble and therefore pass more easily through the capillary and acinar membranes into the oral fluid.

1.3 Oral Fluid: Pros and Cons

The presence of parent drugs in oral fluid can be advantageous and disadvantageous. The disadvantage is that antibodies in most commercially available immunoassay drug screen tests target metabolites. The advantage is that parent drugs are less polar, more easily extracted, and are less likely to require derivatization prior to confirmational analysis.

Another consideration for using oral fluid for drug testing also has pros and cons. Drugs that are ingested orally (like ethanol) as well as those that can be smoked (i.e., methamphetamine, PCP, marijuana, and cocaine) may be detected in high concentrations in oral fluid following recent use due to residual drugs remaining in the oral cavity. However, under these circumstances, results may not be accurate because the drug concentration found in the oral fluid may not reflect the blood-drug concentration. At the same time, these transiently elevated concentrations improve the likelihood of detecting the drug.

1.4 Objectives

The objectives of this research included the following:

1. To perform a thorough literature search to establish the current state of scientific knowledge about the use of oral fluid as a testing specimen and to prepare a comprehensive report on the scientific literature related to oral fluid drug testing.

2. To perform a controlled clinical study with the following objectives:
   - To assess the practical problems of obtaining oral fluid samples from donors.
   - To determine if there is a predictable relationship between codeine concentrations in plasma and oral fluid following controlled administration of the model opiate drug codeine.
   - To develop and refine methods for analyzing codeine and its metabolites in oral fluids.

3. To determine the optimum method of collecting oral fluids and the effect of collection devices on drug concentration.

4. To determine if the current standards that are used to assess whether an oral fluid sample is "valid" (i.e., has not been diluted to circumvent testing protocols) are appropriate.

2. LITERATURE SEARCH

2.1 Search Results

In year 1 of this study, researchers conducted a thorough literature search to determine the state of knowledge about the use of oral fluid/saliva as a drug-testing specimen. Information gathered in the search was used to prepare a report that summarized the scientific literature on oral fluid drug testing. Results of the literature search were then used to design a controlled clinical study in which subjects were dosed with codeine, and oral fluid and plasma samples were collected.

In this section, no differentiation was made between oral fluid and saliva because most of the literature did not make that distinction. Generally, the term "saliva" is used in the literature regardless of collection technique and actual fluid harvested.

The literature review resulted in the identification of 134 references from which reprints were obtained for 85 articles.

The reprints were separated into the following categories:

- Saliva collection and predicting saliva/plasma concentrations–20
- Amphetamines–8
- Cocaine and metabolites–22
- Marijuana and metabolites–11
- Opiates and codeine–10
- Review articles–11
- Therapeutic and other drugs–33
- Miscellaneous–11

Many reprints contained information about one or more drugs and their metabolites. Articles also often contained information about collecting, testing, and the pharmacokinetics of one or more drugs. Therefore, the number of references in the bibliography may not be equal to the total number of reprints listed in each category. Following are summaries of the information obtained in each category.

2.1.1 Saliva Collection and Predicting Saliva/Plasma Concentrations

As stated, several fluids combine to constitute what is commonly referred to as "saliva." These fluids are excreted by the major salivary glands, minor salivary glands, and gingival crevices. A mixture of fluids from the various glands is variously referred to as whole saliva, mixed saliva, oral fluid, or oral fluids (Malamud, 1993). Whole saliva may also contain other materials that are in the mouth such as shed mucosal cells or food residues (Schramm et al., 1993a). Salivary composition and flow can be affected by many factors, including oral diseases (Dawes, 1993; Mandel, 1990).

A variety of methods are available for collecting saliva. Some involve stimulating saliva production, while others target collection of unstimulated (also referred to as nonstimulated) saliva.

Unstimulated saliva can be collected by the draining method, which is performed by allowing saliva to drip from the mouth into a collection container (Navazesh, 1993). Several techniques may be used to collect stimulated saliva. The simplest involves tongue, cheek, or lip movements without the use of an external stimulus (Mucklow et al., 1978; Jones, 1995). Chewing paraffin wax, Parafilm®, teflon, rubber bands, gum base, or chewing gum are usually referred to as mechanical methods of stimulating saliva production (Chang, 1976; Mucklow et al., 1978; Dabbs, 1991; Navazesh, 1993; Hold et al., 1996). A lemon drop or citric acid can be placed in the mouth to provide a gustatory stimulus for saliva production (Mucklow et al., 1978; Dabbs, 1991; Navazesh, 1993).

Following stimulation by one or more of these methods, saliva can be spit, suctioned, or swabbed from the mouth (Navazesh, 1993). Some collection techniques combine stimulation and collection of the saliva using absorbent materials such as cotton balls or cotton rolls. After the absorbent material becomes saturated with saliva, it is removed from the mouth and the saliva is extracted by centrifugation or by applying pressure to the material (Chee et al., 1993; Lamey and Nolan, 1994).

There are several potential problems associated with stimulating saliva production. Parafilm has been shown to absorb some drugs and, therefore, give erroneous results when saliva is tested for drugs or drug metabolites (Chang, 1976). Also, paraffin contains compounds that may affect chromatographic analyses—again affecting drug testing accuracy (Chang, 1976). Some salivary stimulants may change the salivary composition and, therefore, affect the saliva-drug concentration. For example, citric acid may change saliva pH and consequently alter drug concentrations in the saliva. Citric acid and cotton have also been shown to alter immunoassay drug test results (Mucklow et al., 1978; Dabbs, 1991; Cheever, 1997).

Several devices are commercially available for collecting saliva.[1] Some devices are based on the collection techniques just discussed. They carry names such as Oral Diffusion Sink® (Shipley et al., 1992; Hold et al., 1996), Proflow Sialometer™ (Jones, 1995), Orasure® (Gomez et al., 1994), and Salivette™ (Shipley et al., 1992). They have been advocated for saliva collection when testing for ethanol, steroids, and many other drugs.

Mathematical models have been developed for predicting saliva to plasma (S/P) drug concentration ratios for acidic and basic drugs (Matin et al., 1974). The equations are:

$$\text{Acidic Drugs - S/P} = \frac{1 + 10^{(\text{pHs-pKa})} \times \text{fp}}{1 + 10^{(\text{pHp-pKa})} \times \text{fs}}$$

[1] Certain products or materials are identified in this report to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology (NIST), nor does it imply that products are necessarily the best available for the purpose.

$$\text{Basic Drugs - S/P} = \frac{1 + 10^{(pKa-pHs)} \times fp}{1 + 10^{(pKa-pHp)} \times fs}$$

where S = concentration of drug in saliva
P = concentration of drug in plasma
pKa = pKa of drug
pHs = pH of saliva
pHp = pH of plasma
fp = free (unbound) fraction of drug in plasma
fs = free (unbound) fraction of drug in saliva

When using these equations, plasma pH is assumed to be constant at 7.4 and drug protein binding is assumed to be negligible in the saliva. Therefore, a value of 1 is used for fs (Hold, 1996). The binding of drugs to plasma proteins varies from drug to drug. However, it remains fairly consistent between individuals. Normally, saliva pH may vary from 6 to 8. If the pH is measured at the time of sample collection, one can modify this variable in the equation and, theoretically, predict the plasma concentration of a drug given its saliva drug concentration (Mucklow et al., 1978). Much of the pharmacokinetic and pharmacodynamic research reported on therapeutic and abused drugs has been based on plasma drug concentrations. Therefore, if S/P ratios can be shown to be predictable using mathematical models, then the databases on plasma pharmacokinetic, physiological, and behavioral data could be used to support interpretation of saliva drug concentrations. This would greatly enhance the value of saliva as a matrix for drug testing.

2.1.2 Amphetamines

In the context of drug abuse, the term "amphetamines" usually refers to amphetamine and *d*-methamphetamine (methamphetamine). However, there are several structurally related sympathomimetic amines such as phentermine, *l*-methamphetamine, ephedrine, pseudoephedrine, phenylephrine, methylenedioxyamphetamine (MDA), and methylenedioxymethamphetamine (MDMA/Ecstacy) that are sometimes referred to as amphetamines (Baylor and Crouch, 1993). This section focuses on amphetamine and methamphetamine. These drugs may be used therapeutically in the treatment of obesity, narcolepsy, and attention deficit disorder (Basalt and Cravey, 1995). However, they are commonly abused for their central nervous system stimulant properties. They may be taken by oral ingestion, IV injection, or smoked (Cone, 1993). In the body, methamphetamine is metabolized by n-demethylation to amphetamine.

A limited number of published reports on amphetamine or methamphetamine in saliva indicate that they both have been detected in saliva. Wan et al. (1978) reported a study involving oral administration of 10 mg of amphetamine (as free base) to four subjects. Plasma and saliva amphetamine concentrations were determined and the S/P concentration ratio was calculated. During the absorption phase, the S/P ratio was generally higher than theoretically predicted. The authors hypothesized that this was due to contamination of the oral cavity during dosing. Following the absorption phase, the S/P ratio was 2.76. Using the model for calculating

the S/P ratio of basic drugs, the authors determined that the S/P ratio of amphetamine, theoretically, should have been 2.21. They suggested their data confirmed that mathematical models could be used to estimate plasma amphetamine concentrations when the saliva amphetamine concentration was known.

Amphetamine was also detected in the saliva of a subject undergoing amphetamine therapy (Wan et al., 1978). The authors reported that the salivary concentration of amphetamine was similar to that in the subject's whole blood.

There were two reports of detecting methamphetamine in saliva (Kajutani et al., 1989; Suzuki et al., 1989). Suzuki et al. (1989) detected methamphetamine in saliva from drug users for up to 2 d after their last use. Of the 19 saliva samples collected, 3 had detectable concentrations of methamphetamine. The amount of drug recovered following extraction of the saliva samples was 0.3 μg, 0.5 μg, and 2.1 μg. Amphetamine was not detected in any of the samples.

Vapaatalo et al. (1984) reported that the low concentrations of amphetamines found in saliva made thin-layer chromatography (TLC) methods unsuitable for use in the detection of these drugs in saliva.

2.1.3 Cocaine

Cocaine is a potent central nervous system stimulant that occurs naturally in the leaves of the *Erythroxylon coca* plant. In solution, it is used as a local anesthetic during ophthalmological and otorhinolaryngeal procedures. More often, though, cocaine is abused for its stimulant and euphoric effects. Cocaine may be self-administered by IV injection and nasal insufflation or smoked as a free base (Clauwaert et al., 1995). Cocaine is metabolized primarily to benzoylecgonine (BZE) and ecgonine methyl ester (EME) and to a lesser extent to norcocaine and ecgonine.

A number of articles reported detecting cocaine and its metabolites in saliva. Inaba et al. (1978) demonstrated that radioactivity was detected in the saliva of subjects who had ingested radiolabeled cocaine. Peel et al. (1984) reported detecting cocaine in the saliva of impaired drivers. Thompson et al. (1987) questioned whether the cocaine reported in these two earlier studies was actually absorbed and distributed into the saliva, or if it was the result of salivary contamination from oral use or insufflation. He designed a study in which cocaine was administered by IV injection. Sour candy containing citric acid was used to stimulate saliva production. The stimulated saliva and corresponding plasma samples were collected and analyzed by gas chromatography/mass spectrometry (GC/MS) for cocaine. Saliva cocaine concentrations paralleled those detected in the plasma. S/P ratios were calculated and showed an overall mean of 1.25, with a range from 0.5 to 2.96.

Two investigators reported significant correlations between saliva cocaine concentrations and physiological effects. In 1988, Cone et al. reported on a study in which cocaine was administered by IV to five subjects. Saliva production was stimulated with sour candy containing citric acid. Plasma and saliva samples were collected and analyzed by GC with nitrogen-phosphorus detection (GC-NPD). Plasma cocaine concentrations reached 273 ng/mL 10 min after a 15 mg

dose, and they declined below the assay sensitivity by 5 h post dose. Following the same dose, saliva cocaine concentrations ranged from 100 ng/mL to 520 ng/mL at 10 min. The saliva concentration declined to an average of 8 ng/mL by 5 h. At 10 min after a 40 mg IV dose, plasma cocaine concentrations ranged from 204 ng/mL to 523 ng/mL and declined to an average of 6 ng/mL by 5 h. The corresponding saliva concentrations following the 40 mg dose ranged from 237 ng/mL to 1843 ng/mL at 10 min and averaged 29 ng/mL at 5 h. The saliva and plasma cocaine concentrations were compared with several subjective and physiological measures and were found to have a significant correlation ($p \leq 0.05$). The subjective measures included self-rating scales such as feelings of "good, bad, restless, rush, and anxious." The physiological measures included respiration, pulse, and blood pressure.

Stillman et al. (1993) also reported on a study that compared saliva cocaine concentrations with subjective and physiological measures. Thirteen subjects were administered oral gelatin capsules containing cocaine hydrochloride (2 mg/Kg). Dental cotton rolls were placed in each subject's mouth to collect the saliva, pressure was applied to the rolls to remove the absorbed saliva for analysis. Physiological measures such as blood pressure, temperature, pupil size, and subjective measures including intoxication (orientation, subjective rating of high, and mood) paralleled saliva cocaine concentrations. Saliva cocaine concentrations peaked at 75 min after capsule ingestion, with a peak concentration of about 826 ng/mL. No cocaine was detected at 3 h post dose.

Several authors reported detecting metabolites of cocaine in saliva. Schramm et al. (1993b) was the first to report BZE in saliva. Saliva was collected from 69 volunteers who self-reported using cocaine within the previous 24 h. The saliva was assayed for cocaine and BZE by radioimmunoassay (RIA). A selected number of the samples were also analyzed by GC/MS. Cocaine concentrations ranged from 3 ng/mL to 1990 ng/mL, and BZE concentrations ranged from 9 ng/mL to 1960 ng/mL. Correlating these concentrations with drug dose was difficult because the route and time of administration were not controlled. Cone et al. (1994) developed a GC/MS method to simultaneously detect cocaine, BZE, EME, and five other metabolites in biological samples. They successfully detected cocaine, BZE, and EME in saliva and plasma, but the concentrations of the other metabolites were below the limit of detection (LOD) of the assay. The peak saliva cocaine concentration was about 3 times that found in plasma. BZE concentrations were lower in saliva than plasma, however, saliva EME concentrations were higher in saliva than in plasma.

The route of administration can affect the distribution of cocaine and its metabolites into biological specimens. In a 1995 study by Jenkins et al., male subjects were administered cocaine intravenously or as free base by smoking. After smoking 40 mg of free base cocaine, cocaine, BZE, and anhydroecgonine methyl ester were detected in saliva. Cocaine was the major analyte detected. Concentrations of cocaine ranged from 15852 ng/mL to 504880 ng/mL, declined to 100 ng/mL by 4 h, and were less than 1 ng/mL in all subjects by 24 h. Cocaine was also detected in saliva after IV administration. After administration of 44.8 mg of cocaine-HCl by IV, peak drug concentrations were reached within 30 min of the dose and were lower than those found after smoking (428 ng/mL to 1927 ng/mL). Anhydroecgonine methyl ester was only detected in saliva and only after smoking. Therefore, the authors concluded that anhydroecgonine methyl ester (a thermal degradation product of cocaine) could be used as a marker of smoked-cocaine.

Cone et al. (1997) reported a study comparing saliva cocaine concentrations following intravenous, intranasal, and smoked administration. Saliva cocaine concentrations peaked 5 min after IV administration and ranged from 258 ng/mL to 1303 ng/mL. S/P ratios ranged from 1.3 to 10.1. S/P ratios following intranasal and smoked administration were generally higher than those after IV administration. Regardless of route of administration, cocaine usually remained detectable for about 12 h. Peak BZE and EME concentrations occurred at 10 min to 4 h and were consistently lower than peak cocaine concentrations. The authors suggested that the elevated S/P ratios observed after smoked and intranasal administration may have resulted from oral contamination during dosing. The elevated S/P ratios persisted for about 15 min after smoking and for about 2 h after intranasal administration. The authors also determined that the duration of cocaine detection in saliva paralleled its pharmacological effects.

Saliva cocaine concentrations may differ between stimulated and unstimulated collections. Kato et al. (1993) reported that cocaine concentrations in unstimulated saliva were higher than those detected in saliva collected using citric acid candy stimulation. In six subjects, the mean ratio of unstimulated to stimulated saliva cocaine concentrations was 5.2 and ranged from 3.0 to 9.5.

As demonstrated in the previous discussion, several methods have been used to detect cocaine and its metabolites in saliva. Kidwell (1990) reported the detection of cocaine, BZE, and ecgonine in saliva using liquid chromatography/mass spectrometry (LC/MS). He reported that saliva could be analyzed by direct injection using LC/MS. This technique eliminated the need to extract the analytes from the saliva and to derivatize the cocaine metabolites prior to instrumental analysis. This streamlined method resulted in a savings of time and, potentially, costs when compared to GC/MS techniques.

Based on the literature discussed above, there appears to be a reasonable correlation between saliva and plasma cocaine concentrations. There also appears to be a relationship between cocaine concentrations in saliva and physiological and pharmacological effects.

2.1.4 Marijuana

Tetrahydrocannabinol (THC) is the major psychoactive compound found in marijuana plants. Marijuana may contain up to 15% of THC by weight. It can be administered either by oral ingestion or by smoking (Cone, 1993). THC may produce sedation, euphoria, hallucinations, and temporal distortion. It does not usually cause physical dependence or withdrawal symptoms, but it can cause psychological dependence.

THC has a pKa of approximately 9.5 (Idowu and Caddy, 1982). It is metabolized to 11-hydroxy-THC and 8-beta-hydroxy-THC and is further metabolized to its major urinary metabolite, 11-carboxy-THC (THC-COOH). About 70% of a dose of THC is excreted within 72 h.

A number of articles were identified that discussed the detection of THC and other cannabinoids in saliva. Using an enzyme immunoassay and GC/MS, Peel et al. in 1984 reported detecting cannabinoids in saliva samples collected from 6 of 56 impaired drivers. Gross et al. (1985) developed an RIA method for detecting THC in saliva. This method was subsequently used to test saliva samples collected from 25 male and 10 female marijuana smokers. Ten of the male and

5 of the female subjects were chronic smokers; the remaining 15 male and 5 female subjects reported occasional use of marijuana. All subjects smoked from one-half to two cigarettes containing 27 mg of THC. One-half hour after smoking, peak saliva THC concentrations were attained. They averaged 324 ng/mL in the chronic smokers and 172 ng/mL in the occasional smokers. THC could be detected in the saliva samples for up to 5 h after smoking. In another study performed by Maseda et al. in 1986, gas chromatography with electron capture detection (GC-ECD) was used to quantitate THC in saliva collected from eight subjects. Four of the subjects drank 200 mL of beer after smoking marijuana. The remaining four subjects abstained from eating or drinking after smoking. One hour after smoking, saliva THC concentrations from all subjects tested ranged from 73.5 ng/mL to 250.0 ng/mL and had decreased to 34.1 ng/mL to 74.1 ng/mL by 4 h. The subjects who drank the beer after smoking had lower saliva THC concentrations than the nondrinkers. The authors concluded that this resulted from a washing effect of the oral cavity by the beer. However, two other authors suggested that eating and drinking have little effect on THC concentrations in saliva (Thompson and Cone, 1987; Menkes et al., 1991).

Thompson and Cone (1987) used a liquid chromatography with electrochemical detection method to analyze saliva for THC. They found that concentrations of THC in saliva were initially higher than those in plasma. They suggested that this resulted from oral contamination during smoking.

In an early study, seven subjects were asked to smoke a moderate strength (11 mg of THC) marijuana cigarette (Maseda et al., 1986). Data from this study suggested that the effects of THC were associated with salivary THC concentrations. However, Huestis et al. (1992) concluded that a single THC saliva test could not reliably predict performance effects. It has been suggested by other authors that the presence of THC in saliva is due solely to oral contamination during drug administration and that no cannabinoids pass from the blood into saliva (Cone, 1993). If this is true, any correlation of saliva THC concentrations with blood THC concentrations and physiological or behavioral effects would be coincidental. However, saliva THC detection may still be a useful predictor of recent cannabinoid exposure. Clearly, more research is needed to relate saliva THC concentrations to administered dose and effects.

2.1.5 Opiates (Codeine)

The review of the literature for opiate drugs focused on codeine because this drug was used as the "model" opiate drug in the clinical studies. Codeine is a narcotic analgesic with antitussive properties. It is derived from opium and was first isolated in the early 1800s.

GC-NPD analytical methods have been shown to be sufficiently sensitive to detect codeine in saliva following 30 mg oral doses of codeine phosphate (Sharp et al., 1983). The codeine was given to 11 male and 3 female subjects. Codeine S/P ratios ranged from 2.0 to 6.6 and averaged 3.3 following drug administration.

In a study reported by Cone (1990), one subject was administered morphine and a second subject received codeine. Saliva and plasma were collected and tested by RIA. Following intramuscular (IM) doses of 10 mg and 20 mg of morphine sulfate, plasma morphine concentrations in

subject one peaked within 30 min at 66.1 ng/mL and 150.2 ng/mL, respectively. Saliva morphine concentrations peaked at 30 min postdose and were 10.8 ng/mL after the 10 mg dose and 37.8 ng/mL after the 20 mg dose. The morphine concentrations were less than the analytical sensitivity of 0.6 ng/mL by 24 h. Following IM doses of 60 mg and 120 mg of codeine, peak plasma concentrations in subject 2 of 212.4 ng/mL and 272.4 ng/mL, respectively, were reached in less than 30 min. Corresponding peak saliva concentrations of 183.9 ng/mL and 307.6 ng/mL were reached in 30 min to 45 min after the dose. All concentrations were estimates based on immunoreactivity.

Cone also demonstrated that saliva concentrations of heroin and its metabolites, 6-monoacetyl morphine (6-MAM) and morphine, were "highly elevated" over plasma concentrations for the first hour after intranasal heroin administration (Cone, 1993). The concentrations remained higher than those found in plasma for up to 6 h after the dose was administered.

It has been demonstrated that opiates can be detected in saliva by immunoassay techniques such as RIA and enzyme immunoassay (EIA) (Gorodetzky and Kullberg, 1974; Cone, 1990). Gorodetzky showed that following single 5 mg/70 Kg or 10 mg/70 Kg IV doses of heroin, opiates could be detected in saliva by EIA for 1 h to 2 h (Gorodetzky and Kullberg, 1974). Opiates were not consistently detected in saliva following a dose of 2.5 mg/70 kg. Gorodetzky and Kullberg also reported that morphine could be detected in saliva by EIA for 3 h to 4 h following chronic subcutaneous administration of 30 mg of morphine.

Wang et al. (1994) reported detecting opiates in the saliva of a single subject who received a 12 mg intranasal dose of heroin. In this subject, saliva heroin and 6-MAM concentrations peaked at 10 min following dosing at concentrations of 307.8 ng/mL and 58.7 ng/mL, respectively. One hour after administration, the morphine concentrations peaked at 25.4 ng/mL. By 3 h postdose, the concentrations of all three drugs were less than the LOD of the assay. The saliva samples were analyzed by GC/MS.

Jenkins et al. (1995) reported a study designed to compare heroin concentrations in saliva and blood after administering the drug intravenously and by smoking. Two subjects were administered heroin through smoking (2.6 mg, 5.2 mg, and 10.5 mg doses) and also by IV administration (5 mg, 10 mg, and 20 mg doses). Saliva heroin concentrations peaked 2 min after smoking and 2 min to 5 min after IV administration. After smoking, peak heroin concentrations ranged from 3,534 ng/mL to 20,580 ng/mL. Peak heroin concentrations from 6 ng/mL to 30 ng/mL were measured following the IV dose. The 6-MAM and morphine were also detected in blood and saliva after both routes of administration. Saliva to blood (S/B) concentration ratios were greater than 5 at all time points following smoked administration. Following IV administration, S/B ratios were always less than 2. The authors attributed the differences in S/B ratios to contamination of the saliva following smoking.

Kidwell (1990) reported detecting morphine and 6-MAM in the saliva of a heroin user by LC/MS. The use of LC/MS for the analysis of morphine and 6-MAM in saliva is of particular interest because the saliva was analyzed directly. This technique offered significant advantages such as reduced analysis time and costs compared to GC/MS methods primarily because LC/MS did not require preextraction of the saliva or derivatization of the opiate drugs and metabolites.

Several other opioid drugs (opiates or synthetic drugs with opiate-like actions) and their metabolites have been reported in saliva. Examples are propoxyphene, pholcodine, hydromorphone, buprenorphine, meperidine, and methadone (Mucklow et al., 1978; Idowu and Caddy, 1982; Schramm et al., 1992; Cone, 1993; Sharp et al., 1983; Jenkins et al., 1995).

2.1.6 Review Articles

A number of articles were identified that provided reviews of the drugs-in-saliva literature. Articles were obtained from as early as 1977 (Horning et al., 1977). Early articles focused primarily on the detection of therapeutic drugs in saliva and the use of saliva as a diagnostic specimen for clinical purposes (Horning et al., 1977; Mucklow et al., 1978). They explored the S/P ratios of these drugs and attempted scientific explanations for variations in these ratios, such as protein binding, pKa of the drug, and salivary pH. The reviews warned of factors that can affect salivary flow and ultimately the disposition of drugs into saliva (Mandel, 1990). Later reviews provided summaries of the saliva literature on many drugs of abuse such as opiates, barbiturates, methaqualone, and marijuana (Schramm et al., 1992; Cone, 1993). In addition, they provided information on therapeutic drug classes such as anticonvulsants, antidepressants, and antiarrhythmics (Drobitch and Svensson, 1992). Each review article and the major topics discussed are listed below:

1. Use of Saliva in Therapeutic Drug Monitoring (Horning et al., 1977). Major drugs/drug classes discussed: antipyrine, caffeine, ethosuximide, phenobarbital, phenytoin, primidone, and theophylline.

2. Drug Concentration in Saliva (Mucklow et al., 1978). Major drugs/drug classes discussed: antipyrine, chlorpropamide, meperidine, phenobarbital, phenytoin, propranolol, and tolbutamide.

3. A Review of the Use of Saliva in the Forensic Detection of Drugs and Other Chemicals (Idowu and Caddy, 1982). Major drugs/drug classes discussed: barbiturates, benzodiazepines, cannabinoids, ethanol, lithium, heavy metals, and methaqualone. Major topics discussed: secretion of drugs into saliva and advantages and disadvantages of saliva testing. This article also contains a table outlining the pKa, percent plasma protein binding, calculated S/P ratios, experimental S/P ratios, and journal references for 106 common drugs of abuse, therapeutic drugs, and other chemicals.

4. Drugs of Abuse in Saliva: A Review (Schramm et al., 1992). Major drugs/drug classes discussed: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methaqualone, opioids, and phencyclidine. Major topics discussed: advantages and disadvantages of saliva testing.

5. The Diagnostic Uses of Saliva (Mandel, 1990). Major topics discussed: saliva collection, detecting oral diseases, detecting systemic diseases, clinical analyses, hormone monitoring, drug monitoring, antiviral antibody screening, and viral antigen screening.

6. Therapeutic Drug Monitoring in Saliva (Drobitch and Svensson, 1992). Major drugs/drug classes discussed: antidepressants, carbamazepine, cyclosporin, digoxin, disopyramide, ethosuximide, lidocaine, lithium, methotrexate, phenobarbital, phenytoin, primidone, procainamide, quinidine, and theophylline. Major topics discussed: composition, anatomy and physiology of saliva and salivary glands, factors affecting salivary drug concentrations, and saliva collection.

7. Laboratory Tests for Rapid Screening of Drugs of Abuse in the Workplace: A Review (Schwartz et al., 1993). Topics discussed: salivary ethanol tests.

8. Saliva Testing for Drugs of Abuse (Cone, 1993). Major drugs/drug classes discussed: amphetamines, barbiturates, benzodiazepines, caffeine, cocaine, ethanol, inhalants, lysergic acid diethylamide, marijuana, opioids, phencyclidine, and nicotine.

9. Methods for Collecting Saliva (Navazesh, 1993). Major topics discussed: methods for collecting whole saliva, and methods for collecting saliva from individual glands.

10. Forensic Science (Brettell and Saferstein, 1995). Major drugs/drug classes discussed: cocaine, ethanol, and opioids.

11. Saliva as an Analytical Tool in Toxicology (Hold et al., 1996). Major topics discussed: composition, anatomy and physiology of saliva and salivary glands, saliva collection, analytical methods, and the secretion of drugs into saliva.

2.1.7 Therapeutic and Other Drugs and Miscellaneous Topics

This section encompassed a large number of articles. They were collected primarily because they provide useful background information and present a historical perspective of drugs in saliva. The articles discuss saliva collection protocols, pharmacokinetics, and therapeutic value. The following drugs or endogenous compounds were discussed.

1. Acetaminophen (Drehsen and Rohdewald, 1981)
2. Antidepressants (Drobitch and Svensson, 1992)
3. Antipyrine/capillary electrophoresis (Horning et al., 1977; Mucklow et al.,1978; Perrett and Ross, 1995)
4. Barbiturates (Horning et al., 1977; Mucklow et al., 1978; Idowu and Caddy, 1982; Sharp et al., 1983; Drobitch and Svensson, 1992; Schramm et al., 1992; Cone, 1993)
5. Basic drugs/capillary electrophoresis (Stalberg et al., 1995)
6. Benzodiazepines (Tjaden et al., 1980; Idowu and Caddy, 1982; Valentine et al., 1982; Sharp et al., 1983; Hart't and Wilting, 1988; Schramm et al., 1992; Cone, 1993)
7. Caffeine (Horning et al., 1977; Moncrieff, 1991; Cone, 1993)
8. Carbamazepine (Drobitch and Svensson, 1992; Chee et al., 1993)
9. Chlorpropamide (Mucklow et al., 1978)
10. Cyclosporin (Drobitch and Svensson, 1992)
11. Desipramine (Pi et al., 1991)
12. Digoxin (Drobitch and Svensson, 1992)

13. Diphenhydramine (Sharp et al., 1983)
14. Disopyramide (Drobitch and Svensson, 1992)
15. Ephedrine/capillary electrophoresis (Chicharro et al., 1995; Jones, 1995)
16. Ethanol (Jones, 1979; Idowu and Caddy, 1982; Bates et al., 1993; Cone, 1993; Jones, 1993; Kiesow et al., 1993; Schwartz et al., 1993; Jones, 1995)
17. Ethosuximide (Horning et al., 1977; Drehsen and Rohdewald, 1981; Paton and Logan, 1986; Drobitch and Svensson, 1992)
18. Heavy metals (Idowu and Caddy, 1982)
19. Human immunodeficiency virus antibodies (Gomez et al., 1994)
20. Ibuprofen (Steijger et al., 1993)
21. Inhalants (Cone, 1993)
22. Lidocaine (Drobitch and Svensson, 1992)
23. Lysergic acid diethylamide (Cone, 1993)
24. Lithium (Idowu and Caddy, 1982; Drobitch and Svensson, 1992)
25. Methadone (Wolff and Hay, 1991)
26. Meperidine (Mucklow et al., 1978)
27. Methaqualone (Peat and Finkle, 1980; Idowu and Caddy, 1982; Sharp et al., 1983; Schramm et al., 1992)
28. Methotrexate (Drobitch and Svensson, 1992)
29. Nicotine/cotinine (Benkirane et al., 1991; Cone, 1993)
30. Phencyclidine (Bailey and Guba, 1980)
31. Phenytoin (Horning et al., 1977; Mucklow et al., 1978; Paton and Logan, 1986; Drobitch and Svensson, 1992)
32. Primidone (Horning et al., 1977; Drobitch and Svensson, 1992)
33. Procainamide (Drobitch and Svensson, 1992)
34. Propranolol (Mucklow et al., 1978)
35. Quinidine (Drobitch and Svensson, 1992)
36. Salicylates (Drehsen and Rohdewald, 1981)
37. Steroids (Dabbs, 1991; Schramm et al., 1990; Shipley et al., 1992; Quissell, 1993)
38. Theophylline (Horning et al., 1977; Moncrieff, 1991; Drobitch and Svensson, 1992)
39. Tolbutamide (Mucklow et al., 1978)

Miscellaneous:

1. Capillary electrophoresis (Northrop et al., 1994; Perrett and Ross, 1995; Caslavska et al., 1995)
2. Acetylator phenotyping (Hutchings and Routledge, 1996)
3. Frontline test sticks (Iwersen and Schmoldt, 1996)

2.1.8 Discussion and Conclusions

The literature search demonstrated that a substantial amount of scientific information is known about drugs in saliva. This is particularly true of therapeutic drugs where 33 reprints were obtained. Less is known about drugs of abuse in saliva. Only eight articles were identified that reported amphetamines in saliva. These were limited in scope and did not provide current scientific data. Twenty-two reprints were obtained that discussed cocaine and its metabolites in saliva.

They provided substantial information about sample collection, testing, S/P ratios, and interpretation. Testing procedures for cocaine and its metabolites in saliva included RIA, GC-NPD, GC/MS, and LC/MS methods. Several authors calculated S/P ratios. Significant correlations were demonstrated between saliva cocaine concentrations and physiological and behavioral effects. Eleven articles reported detecting THC in saliva. Testing methods for THC and its metabolites included RIA, EIA, GC-ECD, GC/MS, and LC with electrochemical detection. The articles clearly demonstrated that cannabinoids can be detected in saliva. They also showed that a controversy exists about whether cannabinoids detected in saliva are the result of oral contamination from the route of administration, or actually reflect circulating blood concentrations of the drug. Ten articles were identified that discussed opiates in saliva. The opiates included codeine, morphine, 6-MAM, and heroin. Testing methods included RIA, EIA, GC-NPD, GC/MS, and LC/MS. The articles presented saliva pharmacokinetic profiles of these drugs and metabolites following oral, intranasal, IV, and smoked administration. Several articles presented S/P or S/B drug concentration ratios.

The literature demonstrated that testing for drugs of abuse in saliva can be readily performed. However, more scientific data are needed to fully assess the utility of saliva as a testing specimen for amphetamines, cannabinoids, and opiates.

A number of methods for collecting saliva were presented in the literature. Collections may produce either stimulated or unstimulated saliva. Procedures used to stimulate saliva production may affect the pH of the saliva, which may affect both deposition of the drug into saliva and immunoassay drug tests. A related and controversial topic is the potential for contamination of the saliva with the administered drug. This phenomenon is a potential problem when drugs are administered orally (by mouth), intranasally (through the nose), or are smoked. It was reported as a problem when testing for cocaine, THC, and opiates in saliva. It may also be a potential problem when testing for amphetamine in saliva, since methamphetamine is now commonly abused by smoking. Relating a saliva drug concentration to a dose may be difficult due to the potential for oral contamination and the elevated saliva concentrations seen in many of the studies immediately following drug administration. Relating saliva drug concentrations to blood concentrations, accurately predicting S/P ratios, and relating saliva drug concentrations to physiological and behavior effects is also problematic when the potential for oral contamination exists.

Several issues about saliva collection need to be clarified. First, for each drug of interest, the kinetics of the drug in stimulated and unstimulated saliva need to be described. Second, collection procedures need to be optimized to reduce the potential for erroneous results from items that inadvertently stimulate saliva production during collection. In addition, collection procedures need to address the potential for oral contamination from the route of drug administration. Perhaps oral contamination can be eliminated by simply "rinsing" the oral cavity with a suitable liquid prior to saliva collection.

In most of the studies reviewed above, the authors neglected to address the effect of the saliva collection procedure on their results. All future studies performed on saliva as a matrix for drug testing need to be preceded by a thorough investigation of the potential effects of collection procedures on the data. They should include rationale for the collection procedure chosen and a

discussion of the potential effects of the procedure on the saliva drug concentrations, results, and conclusions.

A variety of analytical methods have been used to test for drugs of abuse in saliva. The thin-layer chromatography (TLC), RIA, EIA, GC-NPD, GC-ECD, GC/MS, and LC/MS were reported. Only TLC procedures lacked the sensitivity to detect drugs in saliva. One topic that was not thoroughly discussed by the authors was the efficacy of commercial immunoassy kits for saliva drug testing. Most commercially available immunoassay kits have primary reactivity to urinary drug metabolites. For cocaine and THC, the major urinary metabolites are BZE and THC-COOH, respectively. However, cocaine and THC were found in greater concentrations in saliva than their metabolites. In addition, saliva concentrations of drugs of abuse are similar to blood concentrations and much lower than urinary concentrations. Therefore, the kits may not have adequate sensitivity to reliably detect drugs of abuse in saliva. An additional potential problem when testing saliva with immunoassay kits is that salivary pH can vary considerably (and be affected by the collection technique). Some analysis kits, such as those for cannabinoids, are pH sensitive. Therefore, some commercial immunoassay test kits may have limited utility for the analysis of saliva samples. The use of LC/MS for the detection of cocaine, BZE, ecgonine, morphine, and 6-MAM in saliva is of particular interest because the saliva can be analyzed directly. This technique offers additional advantages. It has reduced analysis time and costs compared to conventional methods because it does not require preextraction of aqueous samples or derivatization of the polar drug metabolites. Also, glucuronide metabolites may be analyzed without hydrolysis procedures.

Although drugs of abuse are readily detected in saliva, care must be taken in selecting an immunoassay screening method to ensure that the method is not pH dependent, is sufficiently sensitive, and is specific for the drug or metabolite found in saliva. LC/MS shows promise as a technique to analyze saliva samples for drugs of abuse and their metabolites.

Do saliva concentrations of abused drugs reflect blood concentrations? Much of the pharmacokinetic literature focuses on serum, plasma, or blood drug concentrations. The literature relating physiological and behavioral effects to drug concentrations also focuses on these specimens. Urinary drug and drug metabolite concentrations do not correlate with physiological or behavioral effects. Therefore, if saliva drug concentrations correlate with blood drug concentrations, then saliva would be an extremely valuable specimen for interpretative purposes in the criminal justice system, impaired-driving cases, and post-accident testing, and for therapeutic drug monitoring.

Mathematical models have been developed to predict the saliva to plasma ratio for acidic and basic drugs. For amphetamine, the experimentally determined S/P ratios supported the ratios predicted from the model. Cocaine may be ingested orally and is commonly abused by insufflation or by smoking. When ingested by these routes, elevated S/P ratios are observed immediately after consumption. These elevated ratios are attributed to oral contamination of the saliva with cocaine. With the exception of this problem, there appears to be a reasonable correlation between saliva and plasma cocaine concentrations. THC and its metabolites have been detected in saliva. Some reports indicate that there is a relationship among dose, saliva, and blood drug concentration; however, more research is needed to define this relationship. Opiates may be ingested orally

and are commonly abused intravenously or by smoking. When opiates are smoked, S/P ratios are elevated immediately after consumption. These elevated ratios are attributed to oral contamination of the saliva with the drug. Despite this problem, there appears to be a dose-response relationship for opiates in saliva, and S/P ratios may be useful in predicting blood concentrations from saliva data.

There is a dose-response relationship for most drugs of abuse found in saliva. Therefore, saliva concentrations may be used to predict plasma concentrations. However, this should be done with extreme caution since many factors—most notably route of administration—may confound predictions.

2.2 Controlled Clinical Study 1

The second phase of the research was designed to determine through in vivo human studies whether saliva was a suitable specimen for drug testing in the criminal justice system. The study was designed to assess the utility of saliva as a specimen for drugs of abuse testing and for estimating the circulation of blood drug concentrations. Codeine was used as a model drug for this work. It was chosen because it is representative of drugs commonly abused in the criminal justice system and, unlike marijuana, PCP, and cocaine (which are illegal, highly addictive, or dangerous drugs), there were no ethical concerns to consider when administering codeine to human subjects. Codeine is commonly prescribed, very safe to administer, and (like heroin) it is metabolized to morphine. In addition, both codeine and morphine are readily detected by immunoassay screening techniques and by GC/MS. The protocol described below allowed for the collection of saliva and plasma in a controlled clinical environment. The data obtained were invaluable in assessing the usefulness of saliva as a testing specimen, for relating saliva concentrations to dose, and for comparing saliva drug concentrations to those in other specimens. In this section, no distinction is made between oral fluid and saliva.

2.2.1 Human Subjects and Study Protocol

Seventeen human subjects were recruited at the University of Utah Health Sciences Center to participate in the Institutional Review Board (IRB) approved study. Subjects were required to sign informed consent forms and be drug free to enter the study. To ensure that the subjects were drug free, urinalysis drug tests were performed for the following drugs: amphetamines, opiates, BZE, cocaine, 9-carboxy-Δ^9-tetrahydrocannabinol, benzodiazepines, and phencyclidine using EMIT® (Syva Corp., Palo Alto, CA). Subjects were excluded if they had taken any medications containing opiates during the preceding 6 months, or if they had a history of acute or chronic illnesses. Subjects were admitted to the Clinical Research Center at the University of Utah Health Sciences Center on the evening before the study. The following morning, the subjects were given a single 30 mg dose of liquid codeine phosphate. The codeine dose was administered under direct supervision. The subjects brushed their teeth with toothpaste and/or vigorously rinsed their mouths following drug administration and prior to saliva collection. Blood (10 mL) was collected in heparinized tubes at the following times: predose, 15 min, 30 min, and 60 min, and 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h. Plasma was separated from the blood by centrifugation and stored at -20 °C until analysis. Saliva was collected at the same time points by having the sub-

jects spit into 5 mL inert polyethylene tubes. The pH of the saliva was recorded at the time of collection and the samples were then stored at -20 °C until analysis.

2.2.2 Analysis

Reference solutions of codeine and morphine were combined and diluted with methanol to obtain stock solutions containing 10 ng/μL and 1.0 ng/μL. These stock solutions were used to prepare calibrators. Saliva calibration curves contained the following concentrations of codeine and morphine: 0.0 ng/mL, 5.0 ng/mL, 10.0 ng/mL, 25.0 ng/mL, 50.0 ng/mL, 100.0 ng/mL, 200.0 ng/mL, 400.0 ng/mL, and 500.0 ng/mL. A similar procedure was used to make separate stock solutions of codeine and morphine for preparation of quality control (QC) samples. Batches of QC samples were prepared in saliva at 5.0 ng/mL and 250.0 ng/mL, aliquoted into silanized glass tubes in 0.5 mL volumes, and stored at -20 °C until analysis. For the plasma analysis, QC samples were prepared in plasma at 50.0 ng/mL and 100.0 ng/mL, aliquoted in 2.0 mL volumes, and stored at -20 °C until analysis. Concentrated internal standard solutions were combined and diluted in methanol to achieve a final concentration of 1.0 ng/μL of codeine-d_3 and morphine-d_3.

Drug-free saliva was collected from healthy volunteers and stored at -4 °C until use. In 0.5 mL aliquots of calibrators, controls, and samples were transferred to labeled and silanized glass tubes. Codeine-d_3 (25 μL) and morphine-d_3 (25 ng) were added to each tube. Distilled water (4 mL) was added followed by 2 mL of 0.1 M phosphate buffer (pH 6.0). The specimens were mixed and then centrifuged at 2000 G for 10 min. Clean Screen® ZSDAU020 (United Chemical Technologies, Horsham, PA) solid phase extraction (SPE) columns were conditioned with methanol (3 mL), distilled water (3 mL), and phosphate buffer (1 mL). Supernatants of the specimens were added to the appropriately labeled SPE column. The columns were washed with distilled water (2 mL), 0.1 M acetate buffer, pH 4.5 (2 mL), and methanol (3 mL).

Codeine and morphine were eluted with 3 mL methylene chloride:isopropanol (80:20) containing 3% ammonium hydroxide. The eluates were evaporated to dryness at <40 °C under a stream of nitrogen. The dried extracts were reconstituted in 100 μL chloroform, derivatized with trifluoroacetic anhydride (TFAA) (100 μL) for 30 min at 70 °C and evaporated to dryness at <40 °C under a stream of nitrogen. Derivatized extracts were reconstituted in 50 μL of ethyl acetate and analyzed on a Finnigan-Mat 4500 GC/MS (Finnigan-Mat, San Jose, CA) using positive-ion chemical ionization.

Reconstituted residue (1 μL) was injected onto the column and the instrument was operated in the splitless mode. Methane-ammonia was used as the reagent gas, helium as the carrier gas, and the column was a DB1 (15 m x 0.32 mm x 0.25 μm) capillary column (J&W Scientific, Folsom, CA). The initial column temperature of 135 °C was held for 0.5 min and then programmed to 300 °C at the rate of 18 °C/min. The final temperature was held for 1 min. Temperatures of the injection port, interface, and ionizer were 250 °C, 250 °C, and 130 °C, respectively. The MH^+ ion for each analyte was monitored with masses at *m/z* 396, 399, 478, and 481 for trifluoroacetyl derivatives of codeine, codeine-d_3, morphine, and morphine-d_3, respectively.

Peak height ratios of codeine and morphine to their respective internal standards were calculated and the concentration of each analyte in the subject samples was determined by comparing analyte response ratio to the least-squares equations generated from peak height ratios of the calibrators. The standard curves were linear from 5 ng/mL to 500 ng/mL ($r \geq 0.98$) for codeine and morphine. The level of detection (LOD) and level of quantitation (LOQ) were determined using serial dilutions of the lowest calibrator. The LOD for each analyte was the lowest concentration with a signal to noise (S/N) ratio of 3 or greater for each injection (n=5). The LOQ was the lowest concentration yielding a result within ± 20% of the target concentration and a coefficient of variation (CV) less than 10% (n=5). The S/N ratio for the LOQ was 10 or greater. Two QC samples (low and high concentration) were run with every 30 specimens.

The 1 mL to 2 mL of plasma were added to internal standards, 2 mL distilled water and 2 mL (10%) trichloroacetic acid. After mixing, the plasma mixture was centrifuged for 5 min. After separating and adjusting the pH to 9.0 with 10N NaOH, the supernatants were transferred to Bond Elut Certify™ (Varian, Harbor City, CA) extraction columns. The columns were prewashed with methanol and distilled water. The columns were rinsed with distilled water, 0.1 M acetate buffer (pH 4.0), and methanol. Codeine and morphine were eluted with methylene chloride:isopropanol with 2% ammonium hydroxide. The elution step was repeated twice, and the final combined eluates containing drugs were evaporated to dryness at <40 °C under nitrogen. The dried extracts were derivatized in TFAA (200 µL) with 200 µL chloroform for 30 min at 70 °C and dried under nitrogen at <40 °C. Derivatized extracts were reconstituted in 50 µL of chloroform and analyzed on a Finnigan Magnum ion trap mass spectrometer (Finnigan-Mat, San Jose, CA) in the positive-ion chemical ionization mode using acetone as the reagent gas, helium as the carrier gas, and a DB5MS (30 m x 0.25 mm x 0.25 µm) capillary column (J&W Scientific, Folsom, CA). Reconstituted extracts (1 µL) were injected in the splitless mode. The column temperature was held at 175 °C for 1 min, increased to 300 °C at 15 °C/min and held at 300 °C for 0.5 min. The injector and transfer line temperatures were maintained at 250 °C, and the manifold was maintained at 225 °C. Masses at *m/z* 396, 399, 478, and 481 were monitored for the trifluoroacetyl derivatives of codeine, codeine-d_3, morphine, and morphine-d_3, respectively. The standard curves were linear from 1 ng/mL to 1500 ng/mL for codeine and morphine.

The areas under the pharmacokinetic curves (AUC) were computed for the interval 0 h to 24 h by the trapezoidal rule. The elimination rate constant (k) was estimated by linear regression of the plasma or saliva concentration data points after 2 h. The terminal half-life ($t_{1/2}$) was estimated from $0.693/k$.

2.2.3 Results and Discussion

Only 0.5 mL of saliva was needed for the saliva GC/MS assay, and a LOQ of 5 ng/mL and a LOD of 1 ng/mL were routinely achieved. This method was fully validated for LOD, LOQ, precision, and accuracy. Figure 1 shows a GC/MS chromatogram of a saliva specimen containing 5 ng/mL of codeine.

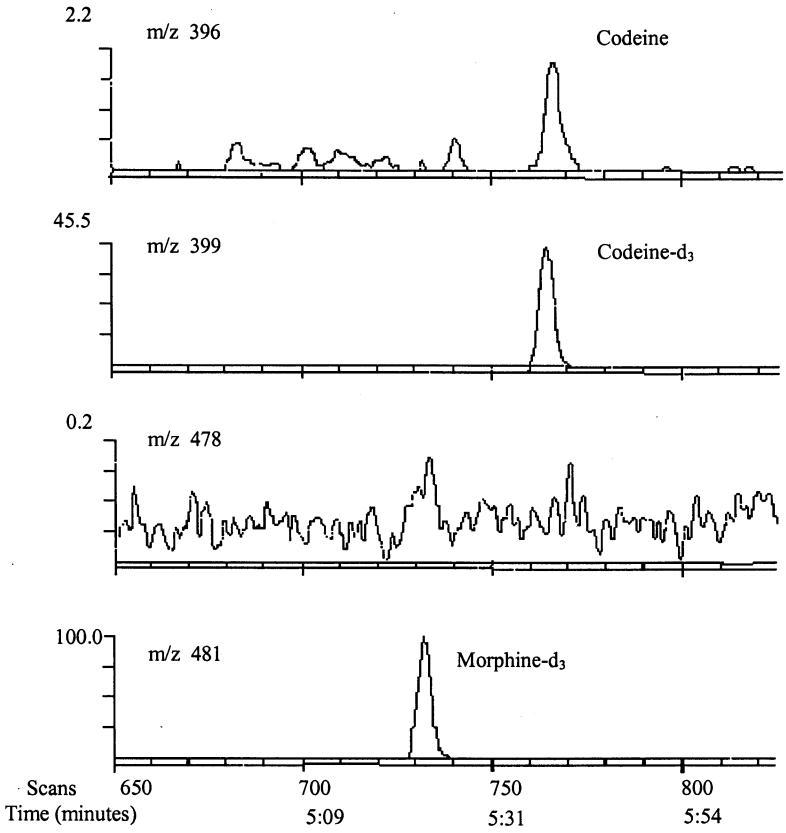

Figure 1. Chromatogram of 5 ng/mL codeine

One objective of this study was to determine if codeine and morphine could be detected in saliva following a single oral dose. The mean time course for the appearance and disappearance of codeine in plasma and saliva are shown in figure 2. The figure shows the mean codeine concentration for the 17 subjects at each time point. No codeine or morphine was detected in the predose plasma or saliva specimens for any of the subjects. Morphine was not detected in any of the plasma specimens. After the oral administration, the plasma codeine concentrations peaked between 30 min and 2 h (mean = 30 min) at concentrations ranging from 19 ng/mL to 74 ng/mL, with a mean of 46 ng/mL. Plasma concentrations declined rapidly, approaching the LOD for the assay within 12 h. No codeine was detected in any of the plasma specimens collected 24 h after drug administration. Cone (1993; see sec. 2.1.6) reported that heroin and its metabolites, 6-acetylmorphine (6-AM) and morphine were "highly elevated" compared to plasma concentrations for the first hour after intranasal heroin administration. Figure 2 demonstrates that the same phenomenon was observed in this study. Elevated saliva codeine concentrations were detected at the early collection times. Contamination of the oral cavity with codeine from the oral administration was evident in the saliva specimens collected for at least the first hour after administration. Codeine concentrations in the 15 min saliva specimens ranged from 690 ng/mL to over 15,000 ng/mL. The rapid decline in the mean saliva codeine concentration observed in the first 2 h following administration was the result of elimination of codeine from the oral cavity by

"natural cleansing" and does not reflect metabolism or redistribution. After an initial 2 h period, the mean saliva codeine concentration appeared to decline at a rate similar to that observed in the plasma (fig. 2). However, the mean saliva codeine concentrations remained 3 to 4 times greater than the plasma codeine concentrations throughout the 24 h interval.

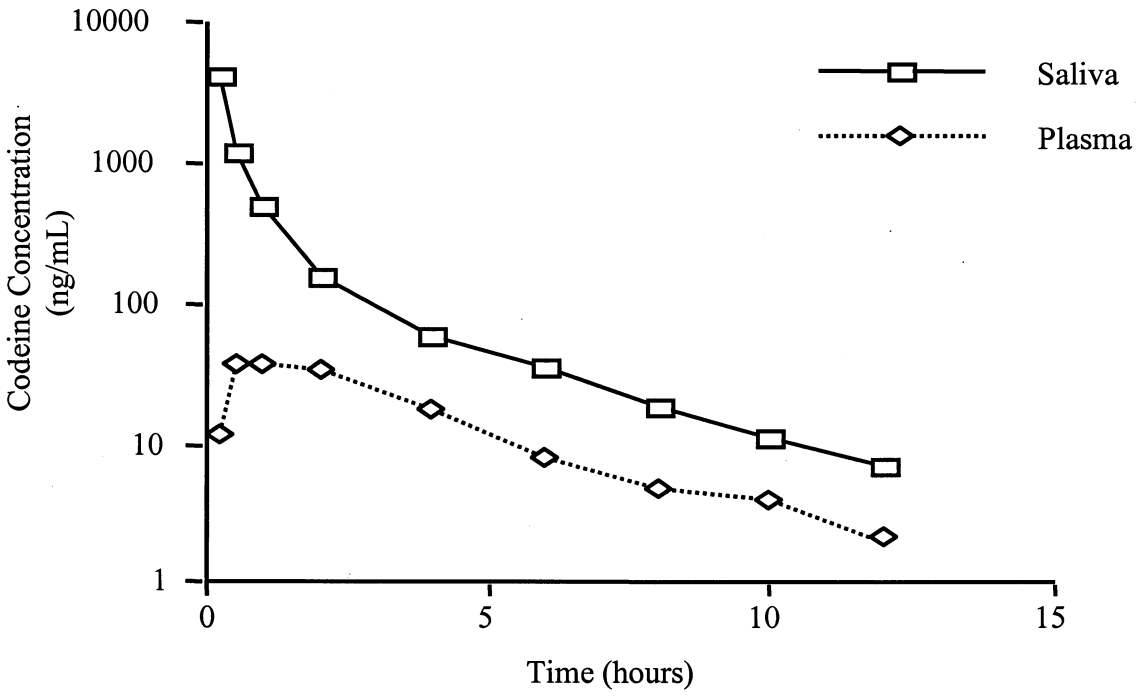

Figure 2. Mean saliva and plasma codeine concentrations versus time

Even though the slope of the decay curves for the plasma and saliva codeine concentrations were similar, significant differences were observed in the detection times for codeine in the two specimens due to the greater saliva concentrations. Codeine was detected in the 24 h saliva specimens from 11 of the 17 subjects. The concentrations were below the LOQ for the assay. The saliva from one subject contained 5 ng/mL of codeine at 24 h. No codeine was detected at 24 h in any plasma samples. Morphine (<5 ng/mL) was detected in three saliva specimens collected 15 min or 30 min post administration, but was not detected in any plasma samples.

Estimates of the elimination rate constant, $t_{1/2}$, and AUC for codeine in plasma and saliva are listed in table 1. Half-life estimates for codeine in plasma and saliva appear to be equivalent, 2.6 h and 2.9 h, respectively. These estimates were consistent with those reported by other researchers. Studies involving administration of other drugs of abuse have reported variations in half-life estimates between saliva and plasma. Cone et al. (1993; see sec. 2.1.6) reported similar saliva and plasma $t_{1/2}$ estimates for cocaine administered intravenously (34.7 min and 34.9 min, respectively). In contrast, Jenkins et al. (1995; see sec. 2.1.6) observed differing pharmacokinetic results with cocaine and heroin in saliva versus plasma. The saliva $t_{1/2}$ of heroin after smoking was reported to be approximately 14 to 60 times longer than that for blood and 2 to 208 times

longer after intravenous administration, but only two subjects were included in the study. For cocaine (n=7), Jenkins reported that the $t_{1/2}$ was shorter in saliva than in plasma after smoking (52 min versus 113 min).

Table 1. Mean PK summary

Parameter	Plasma (SEM)*	Saliva (SEM)*
k (1/h)	0.33 (.043)	0.28 (.021)
$t_{½}$ (h)	2.6 (.32)	2.9 (.280)
AUC (ng-h/mL)	183 (17)	2,365 (202)
*Standard error of mean.		

Although the $t_{1/2}$ estimates for codeine were similar in saliva and plasma, the AUC estimate for codeine in saliva was approximately 13 times greater than for plasma AUC. This was partially due to contamination of the oral cavity during the first 2 h after drug administration. In addition, saliva codeine concentrations exceeded plasma codeine concentrations throughout the 24 h period. However, this is an advantage in using saliva as a specimen for drug testing because increased saliva concentrations resulted in longer detection times.

An objective of the study was to determine if there was a predictable relationship between the saliva and plasma codeine concentrations. As shown in figure 3, saliva codeine concentrations correlated with plasma codeine concentrations for time ≥2 h ($r=0.809$, $p<0.05$). This correlation indicates that saliva codeine concentrations may be predictors of plasma codeine concentrations. These data are consistent with those of other investigators who have reported a significant correlation between saliva and plasma drug concentrations. Thompson reported that plasma and saliva cocaine concentrations correlated significantly ($p<0.001$) in a study in which three doses of cocaine were administered intravenously. Cone (1988) also reported a significant correlation between cocaine concentrations in saliva and plasma ($r=0.89$, $p<0.01$) after IV administration. However, due to the oral contamination, a poor correlation was observed for saliva and plasma codeine concentrations if the specimens collected in the first hour after drug administration were included in the evaluation ($r=0.036$). The average time for elimination of oral contamination in the 17 subjects was approximately 2 h. As shown in figure 3, higher codeine concentrations did not correlate as well due to oral contamination.

To further assess whether saliva codeine concentrations could be used to estimate plasma codeine concentrations, S/P ratios were calculated for the 17 subjects. Table 2 shows the mean plasma and saliva codeine concentrations with the corresponding S/P ratios. Contamination of the saliva from the liquid codeine produced elevated S/P ratios for at least the first hour after codeine administration. S/P ratios in specimens collected at 15 min and 30 min ranged from 75 to 2,580. At 1 h, the S/P ratios were still elevated, but after 2 h, contamination was not a factor in most subjects and the S/P remained constant with a mean ratio of 3.7 (fig. 4). These data are consistent with those of Sharp et al. (1983), who administered 30 mg oral doses of codeine phosphate to study subjects. These investigators reported codeine S/P ratios ranged from 2.0 to 6.6 with a mean of 3.3.

Table 2. Mean S/P ratios

Time	Plasma	Saliva	S/P Ratios
Hours	(ng/mL)	(ng/mL)	
0	0	0	0
.25	12	4129	344
.5	38	1172	337
1	37	480	13
2	34	154	4.5
4	18	60	3.3
Mean (2 h to12 h)			3.7

A variety of methods are used to collect saliva and collection protocols may have an effect on saliva drug concentrations. For example, oral contamination was observed even when subjects were instructed to cleanse their oral cavities by brushing their teeth or vigorous rinsing of their mouths. Some collection methods stimulate saliva production, while others are designed to collect nonstimulated saliva. Nonstimulated saliva can be collected by spitting into containers or by the draining method. Techniques to collect stimulated saliva include chewing paraffin wax, Teflon®, rubber bands, or gum. A lemon drop or citric acid crystals can also be placed in the mouth to provide a stimulus for saliva production. Saliva collected by stimulation may differ in composition from saliva collected by spitting due to changes in saliva flow rate. As saliva flow rate increases, the concentration of bicarbonate in the saliva increases. Therefore, the saliva pH increases and this affects saliva drug concentrations in a pH dependent fashion. Salivary pH in normal individuals is usually between 6.2 and 7.4. The pH of stimulated saliva is reported to fall within a narrow range around 7.4. The pH of the specimens in this study ranged from 6.0 to 8.0, but only 8 specimens had a pH above 7.0.

As discussed, the pH of saliva may be an important factor in saliva drug concentrations. For basic drugs, as the pH decreases, a greater concentration of drug will be ionized and the salivary concentration will increase. The theoretical S/P ratio for a particular drug can be estimated using the following mathematical model based on the Henderson-Hasselbach equation:

$$S/P = \frac{1 + 10^{(pKa-pHs)} \times fp}{1 + 10^{(pKa-pHp)} \times fs}$$

Therefore, small changes in saliva pH should result in profound changes in the S/P. For codeine (pKa 8.2), the theoretical S/P at pH 6.0 is 20. However, at pH 7.0, it is only 2.1. In this study, the observed S/P ratios at pH 6.0 were not as high as predicted by the equation (except within the first hour of drug administration when oral contamination was still present). The observed mean S/P was 4.7 at pH 6.0, 3.4 at pH 7.0, and 1.8 at pH 8.0. Although there was a large inter- and intrasubject variability observed for the S/P ratios (fig. 5), the mean S/P ratio decreased with increasing pH as predicted. However, the decrease was not as dramatic as predicted by the model.

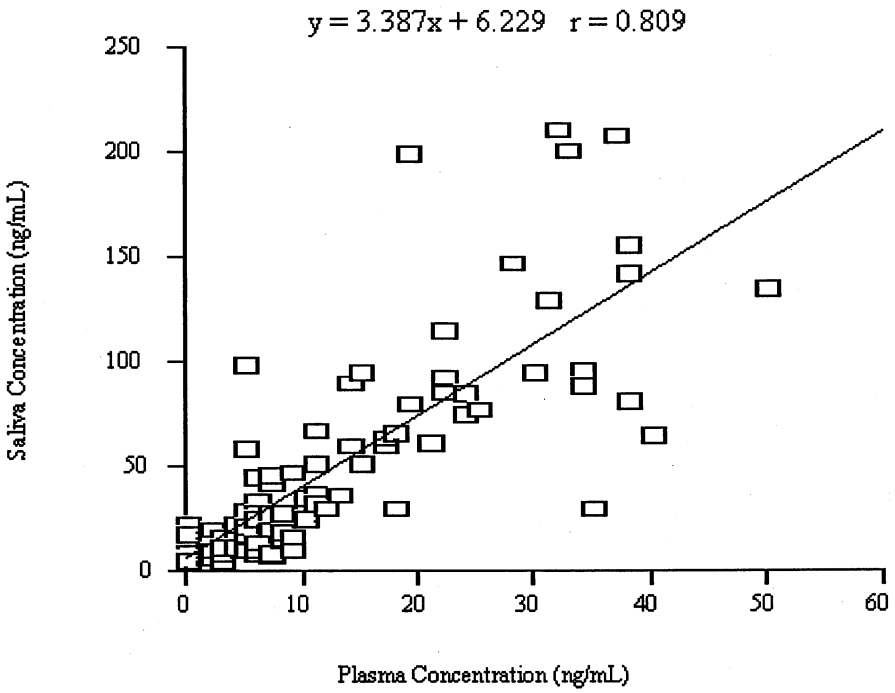

Figure 3. Saliva concentration versus plasma codeine concentrations

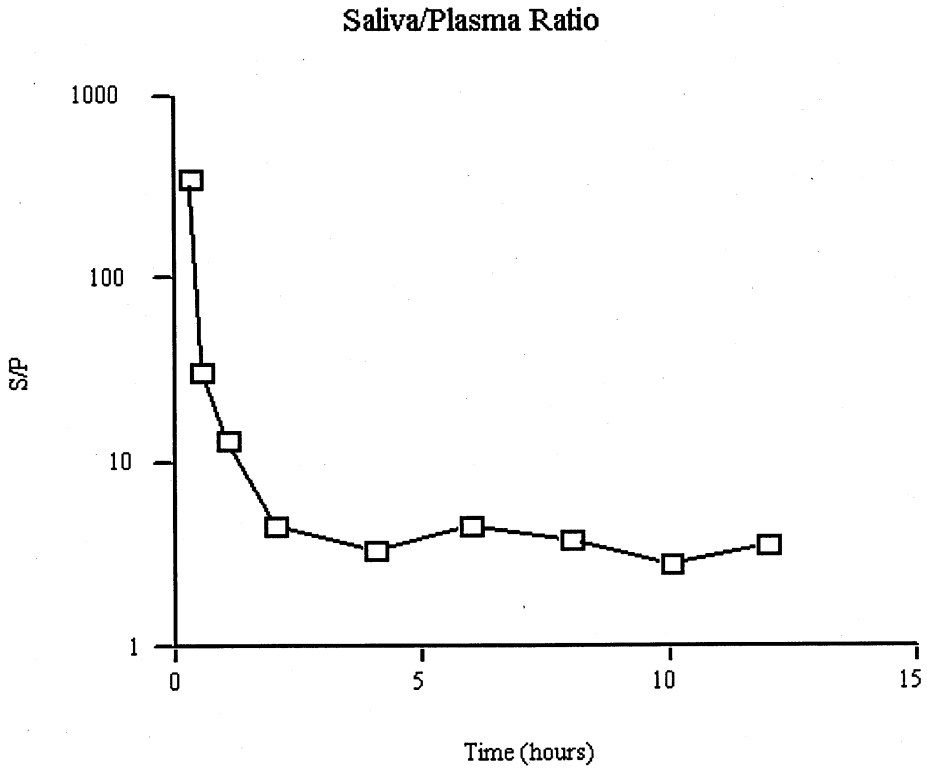

Figure 4. Mean S/P ratio over time

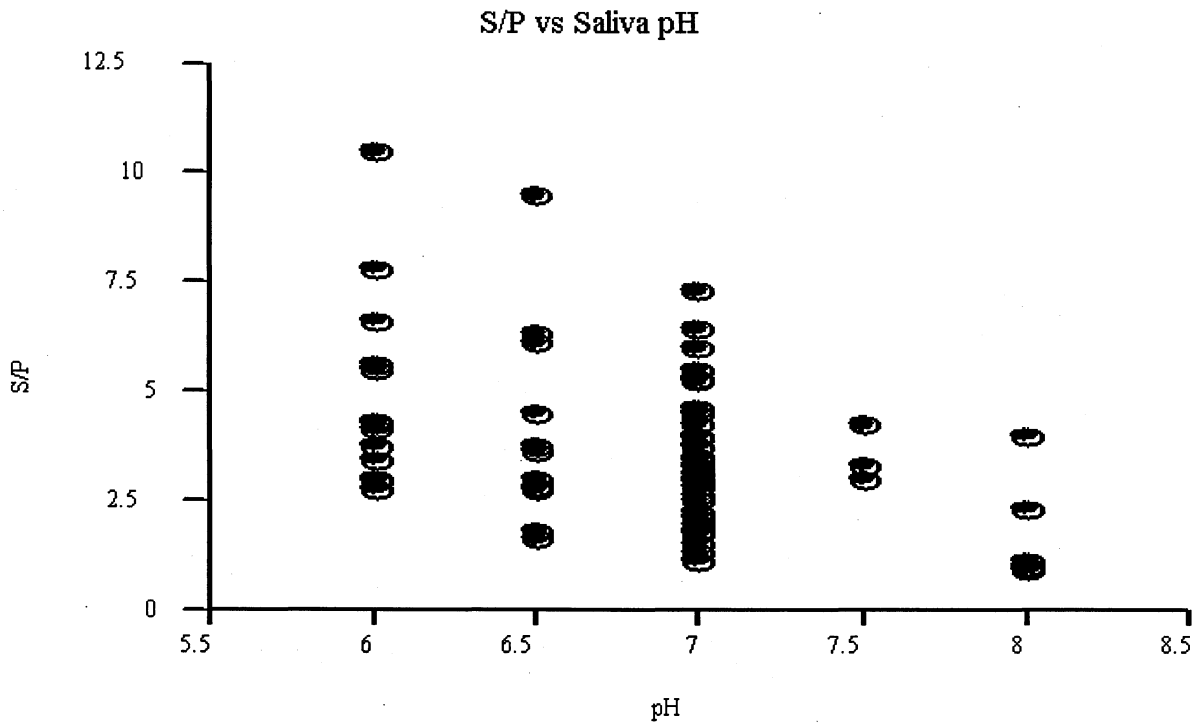

Figure 5. pH versus codeine concentration

2.2.4 Conclusion

1. Saliva can be easily and noninvasively collected under direct observation. This eliminates the concerns about specimen integrity and identity often associated with urine collections. From 1 to 2 mL of saliva can be collected in 3 min to 5 min and small specimen volumes can be utilized for GC/MS analysis.

2. The detection time for codeine in saliva is at least 12 h longer than in plasma. Therefore, saliva provides a much longer detection window for codeine use than does plasma.

3. A disadvantage of saliva drug testing is the potential for contamination of the oral cavity when the drug is administered orally, intranasally, or by smoking.

4. Elevated S/P ratios were observed for 1 h to 2 h after drug administration. However, after the absorption phase, a significant correlation (r=0.809) between saliva and plasma concentrations was observed in this study, and the mean S/P ratio remained constant. Saliva codeine concentrations could be used to estimate plasma concentrations through use of the S/P ratio once the oral contamination has been eliminated. However, these estimates should be made cautiously. One must ensure that oral contamination is not a factor. Simple rinsing procedures did not remove the contamination to the oral dose.

5. Although small changes in saliva pH were predicted to result in profound changes in S/P ratios for codeine, this was not observed.

2.3 Clinical Study 2

Clinical Study 1 was published by O'Neal et al. (1999). As with most research projects, although this study answered many questions about the potential for use of saliva/oral fluid for drug testing in criminal justice settings, additional questions were raised. The most critical question involved the effect of collection technique on the drug concentration detected in the oral fluid. To address this question, a second clinical study using codeine was performed. By that time, distinctions were being made between saliva and oral fluid, and the term oral fluid is used below.

2.3.1 Human Subjects and Study Protocol

Human subjects were recruited as described in the first clinical study. The study was approved by an Independent Research Board (IRB) at the University of Utah and at NIST. Subjects signed informed consent forms and were required to be drug free to enter the study. Subjects were excluded if they had taken any medications containing opiates during the preceding 6 months, or if they had a history of acute or chronic illnesses. They were admitted to the Clinical Research Center at the University of Utah Health Sciences Center on the evening prior to the study and the following morning were given a single 30 mg dose of liquid codeine phosphate. After administration, the subjects brushed their teeth with toothpaste before any oral fluid was collected. Subjects were placed into experimental groups depending on the method of oral fluid collection. For the control group (nonstimulated oral fluid), oral fluid was collected by having the subjects spit into 5 mL inert polyethylene tubes (n=22, this includes the 17 subjects from study 1). Stimulated oral fluid was collected by having the subjects place either a lemon drop (n=5 subjects) or sugarless gum (n=5 subjects) in their mouths 1 min to 2 min prior to their spitting into inert tubes. For the next five subjects, two devices were used simultaneously to collect stimulated oral fluid specimens, the Salivette and the Finger Collector. The devices were placed between the cheek and gum, one on each side of the subjects' mouths, for 5 min, removed, and then the oral fluid was harvested from the device. The Salivette devices were centrifuged in the conical storage tubes to remove the oral fluid from the cotton roll. Oral fluid was removed from the Finger Collectors by vigorously "milking" the foam applicator. All specimens were refrigerated during the 24 h collection period. Control and acidic and nonacidic stimulated oral fluid specimens and the oral fluid specimens collected with the Salivette and the Finger Collector were then stored at –20 °C until analysis.

Oral fluid from an additional group of four subjects was collected "simultaneously" by both the control and the Salivette methods. At each time point, control specimens were collected by having the subjects spit into the inert tube. When 1 mL of oral fluid had been collected (approximately 1 min to 2 min), the subject then placed a Salivette between the cheek and gum for 5 min. All specimens were then processed and stored as described above.

A similar procedure was followed for two additional subjects, except an Orasure device instead of the Salivette was placed between each subject's cheek and gum for 5 min. The oral fluid was

removed from the Orasure device by centrifugation into the storage tube containing buffer and stored at -20 °C until analysis.

In in vitro experiments, morphine and codeine were added to drug-free oral fluid in the following concentrations: 0.0 ng/mL, 10.0 ng/mL, 25.0 ng/mL, 100.0 ng/mL, and 200.0 ng/mL. Salivette and Finger Collector devices were placed in 2 mL aliquots of the fortified whole oral fluid (n=5) at each concentration. The oral fluid was allowed to completely saturate the device. The oral fluid was then removed from each device by centrifugation or milking and analyzed for codeine and morphine as described above. The recovery of codeine and morphine from each device was determined by comparison to oral fluid specimens of the same concentration.

2.3.2 Analysis

The analysis procedures paralleled those of the initial clinical study. Drug-free oral fluid was collected from healthy volunteers and stored at -4 °C until use. The 0.5 mL aliquots of calibrators, controls, and samples were extracted by SPE, derivatized with TFAA, and analyzed on a Finnigan Mat 4500 GC/MS using positive-ion chemical ionization.

The AUCs were computed for the interval 0 h to 24 h by the trapezoidal rule. The elimination rate constant (k) was estimated by linear regression of the plasma or oral fluid concentration data points after 2 h. The terminal half-life ($t_{1/2}$) was estimated from $0.693/k$.

2.3.3 Results and Discussion

The time course of codeine elimination from oral fluid for subjects using each of the five collection methods is shown in figure 6. Because the first clinical study demonstrated that contamination from the oral codeine produced elevated concentrations in the first 1 h to 2 h after administration, only mean concentrations from 2 h to 12 h are shown. Except for the 8 h time point, codeine concentrations in specimens collected by the control method (spitting) were consistently higher than those detected in specimens collected by the other methods. The control concentrations were, on average, 3.6 times higher than concentrations in specimens collected by acidic stimulation and 1.3 to 2.0 times higher than concentrations in specimens collected by the other three methods (fig. 6a and table 3). Because oral contamination appeared to be a significant problem for the 15 min to 30 min controlled collections, the 0.25 h time point was excluded when the average concentration ratio (alternate method/control method) was also calculated.

Differences in the duration of the detection time were also observed for the five oral fluid collection methods (table 4). In the control group, codeine was detected in all of the 12 h specimens and only 3 of 22 specimens had concentrations <5 ng/mL. Codeine was detected in 15 of 22 specimens (68%) at 24 h after drug administration. Using the Salivette and Finger Collector devices, all specimens collected at 12 h contained codeine. However, 60% of the samples contained codeine concentrations <5 ng/mL. At 24 h after administration, only two (40%) and one (20%) of the Salivette and Finger Collector samples, respectively, contained detectable codeine. Nonacidic oral fluid stimulation yielded only three (60%) positive specimens at 12 h (two of the subjects had codeine concentrations >5 ng/mL at 12 h and two (40%) positive specimens at 24 h). Using the acidic oral fluid stimulation method, no specimens collected at 24 h contained codeine and four of five specimens collected at 12 h were also negative.

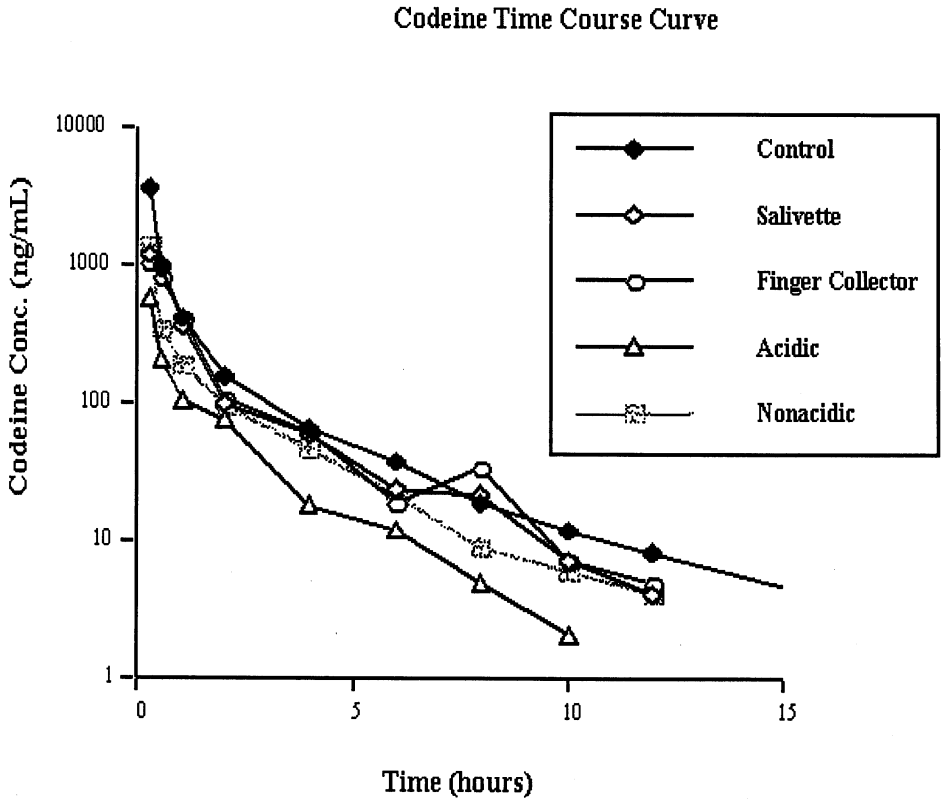

Figure 6. Codeine time course by device

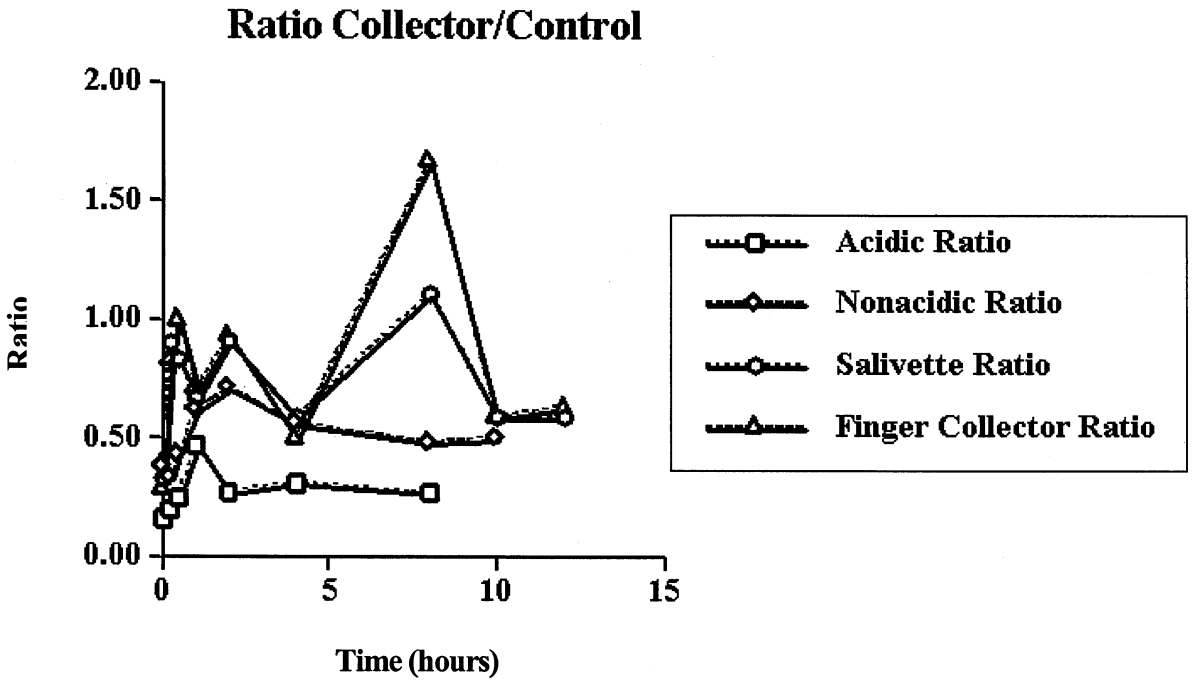

Figure 6a. Comparison of oral fluid collection methods

Table 3. Concentration comparisons by device used to collect oral fluid

Time (hours)	Control	SEM*	Acidic	SEM*	Ratio*	Nonacidic	SEM*	Ratio*	Salivette	SEM*	Ratio**	Finger Collector	SEM	Ratio*
0.25	3,542	727	556	301	6.4	1,365	441	2.6	1,171	389	3.0	1,013	337	3.5
0.5	1,000	180	203	58	4.9	338	106	3.0	916	309	1.1	825	475	1.2
1	413	53	103	32	4.0	183	56	2.3	356	69	1.2	412	86	1.0
2	152	19	74	22	2.1	94	15	1.6	99	17	1.5	106	43	1.4
4	66	10	18	3.8	3.7	47	6.3	1.4	60	6	1.1	62	14	1.1
6	38	5	12	2.6	3.2	21	7.2	1.8	23	4	1.7	19	2.8	2.0
8	19	2	5	1.3	3.8	9	1.7	2.1	21	7	0.9	34	12.2	0.6
10	12	1.5	<5			6	1.5	2.0	7	1	1.7	7	1.4	1.7
12	8	1.3	ND***			<5			<5			5	2	1.6
24	<5		ND***			<5			<5			ND***		
Average Ratio					3.6			2.0			1.3			1.3

*Standard error of mean.
**Control concentration/method or device concentration.
***Not determined.

Pharmacokinetic parameters, including estimates for the elimination rate constant, $t_{1/2}$, and AUC, were calculated from the codeine concentrations detected using each collection method. These data are shown in table 5, which illustrates that substantially different pharmacokinetic parameters were obtained using the codeine concentrations from the different collection methods. As illustrated in figure 6, the slope of the elimination curve for codeine using the acidic collections was steeper than that of the other four methods. Therefore, the calculated $t_{1/2}$ for the acidic method was significantly less than the $t_{1/2}$ for the control method, 1.8 h and 3.1 h, respectively. The $t_{1/2}$ for the acidic method was also less than the $t_{1/2}$ for the other methods, but the difference was less dramatic. Due to the higher codeine concentrations observed for the control method, especially within the first 30 min after drug administration, the control method AUC was significantly greater than the calculated AUC for the acidic and nonacidic collection methods. However, there was considerable interindividual variation in peak codeine concentrations observed among the collection methods and within each collection method group. For example, a concentration range of 82 ng/mL to 1,690 ng/mL was observed for acidic stimulation collection and 298 ng/mL to 16,500 ng/mL for the control collection method.

Table 4. Duration of positive codeine detection by device

Collection Method	≥ 1 ng/12 h	≥ 5 ng/12 h	≥ 1 ng/24 h
Control	100%	86%	68%
Salivette	100	40	40
Finger Collector	100	40	20
Nonacidic	60	40	40
Acidic	20	20	0

Table 5. Mean PK estimates

Collection Method	k(1/h)	$t_{½}$	AUC
Plasma #1	0.33	2.6	183
Control #1	0.28	2.9	2,365
Control	0.26	3.0	2,167
Salivette	0.30	2.7	1,307
Finger Collector	0.26	3.3	1,011
Acidic	0.40	1.8	480
Nonacidic	0.34	2.5	940

The in vitro recovery studies suggested that there might be a difference in codeine absorption or recovery between the Salivette and the Finger Collector. The percent recovery of codeine and morphine was 8.3% and 6.8% less than the control, respectively, for the Salivette and 46.7% and 39.1% less than the control, respectively, for the Finger Collector at concentrations from 10 ng/mL to 200 ng/mL (fig. 7). An additional disadvantage of the Finger Collector was identified during the in vitro study. Typically, 75% to 90% of the oral fluid was recovered from the Salivette, but only approximately 50% of the oral fluid was recovered from the Finger Collector.

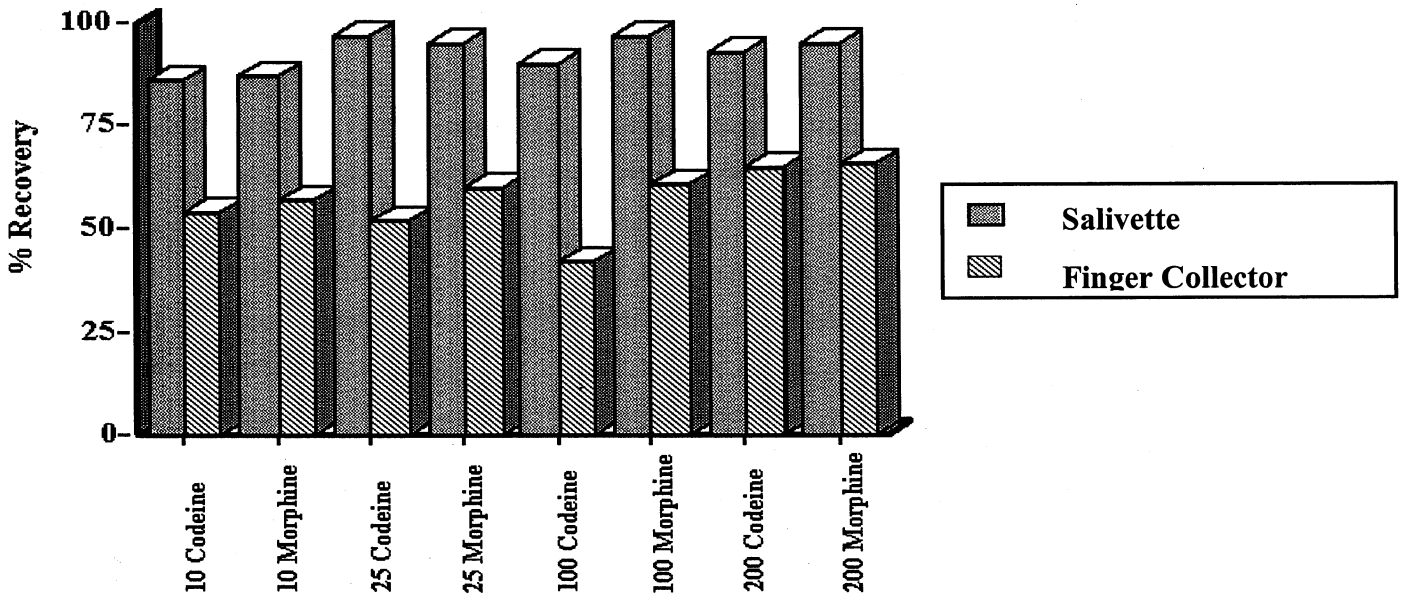

Figure 7. In vitro recovery of morphine and codeine

Because oral fluid specimens were collected from the same subjects at the same time with the two devices, a direct comparison of the in vivo collection with devices was possible. As illustrated in figure 8, the mean codeine concentrations using these two collection devices were similar throughout the 24 h collection interval. With the exception of the 8 h specimen, the time course of codeine elimination following collection with the devices followed a similar curve. However, during the clinical study, the authors were able to recover ≥500 µL of oral fluid from only 14 of 55 of the Finger Collector collections (25.5%) compared to 45 of 55 collections (81.8%) using the Salivette. Thirty-five of 55 specimens collected using the Finger Collector during the clinical study had a recovered volume of ≤300 µL. The authors were unable to analyze 9 of the 55 clinical specimens collected with the Finger Collector (all from the same 3 subjects) because no oral fluid could be milked from the foam applicator.

In a separate group of four subjects, oral fluid was collected almost simultaneously using the control method and the Salivette. The time course of codeine elimination from oral fluid for the subjects in this experiment is shown in figure 9. Codeine concentrations in oral fluid collected by the control method were consistently higher than concentrations in oral fluid collected with the Salivette. The codeine concentrations for the control group were approximately 2.3 times higher than concentrations in the oral fluid collected with the Salivette. A decreasing trend of 4.0 to 1.6 was observed through the experimental session. The first time point (0.25 h) was excluded when the average ratio was calculated for each object group. Even though the control codeine concentrations were typically greater than twice the Salivette concentrations, the detection time for codeine in these subjects was similar. Codeine concentrations were <5 ng/mL in oral fluid collected by both methods at 24 h after drug administration. The calculated $t_{1/2}$ for each group was similar (2.3 h and 2.5 h), but the AUC for the control group was more than three times that from the Salivette subject group.

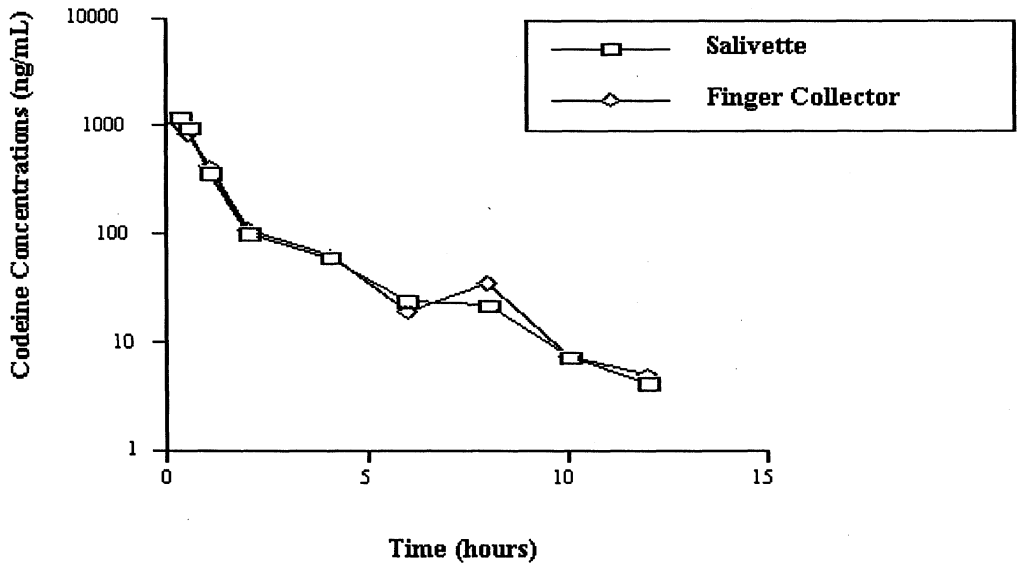

Figure 8. Salivette versus Finger Collector codeine concentrations

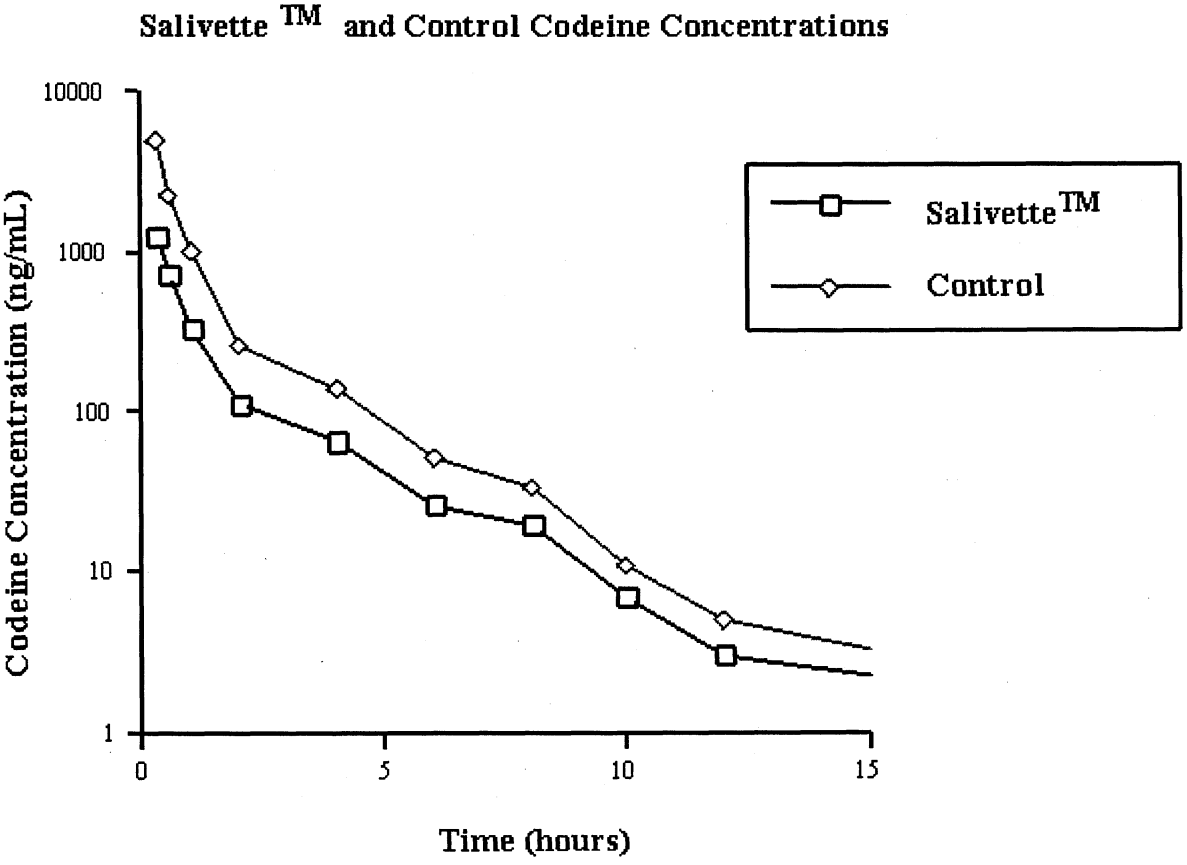

Figure 9. Control versus Salivette codeine concentrations

Results from the experiment in which oral fluid was collected by the control method and almost simultaneously with the Orasure device are shown in table 6. Differences in codeine concentrations for the two collection methods were also observed in this experiment. Data for each subject are shown rather than mean values. These data should be considered preliminary because only two subjects were tested, the control codeine concentration was considerably higher than in the previous experiment, and the ratios of the control to device oral fluid concentrations were quite variable (table 6). An additional variable was encountered in the Orasure collection procedure. After collection, the device was placed in a storage tube containing approximately 700 µL of buffer. This diluted the oral fluid. However, the dilution factor was dependent on the amount of oral fluid collected that was not known.

Table 6. Codeine concentrations in saliva collected by the control method and the Orasure device with corresponding ratios of control/Orasure concentrations, n=2 subjects

	Subject 1			Subject 2		
Time (hours)	**Orasure**	**Control**	**Ratio***	**Orasure**	**Control**	**Ratio***
0.25	668	8,410	12.6	342	5,440	15.9
0.5	295	3,070	10.4	223	2,970	13.3
1	256	607	2.4	127	493	3.9
2	107	202	1.9	105	229	2.2
4	36	132	3.7	26	82	3.2
6	9	96	10.7	11	25	2.3
8	8	21	2.6	8	19	2.4
10	5	9	1.8	<5	13	
12	6	9	1.5	<5	10	
24	<5	<5		0	0	

*Control concentration/Orasure concentration.

A thorough understanding of the effects of collection methods and devices on oral fluid collection and subsequent drug concentration is critical to interpreting testing results. For example, Kato et al. (1993) reported oral fluid concentrations of cocaine, BZE, and EME were substantially higher in nonstimulated than in stimulated oral fluid. In that study, saliva was stimulated by citric-acid-type sour candy and the ratio of the cocaine concentrations in nonstimulated versus stimulated saliva was 5.2 (3.0–9.5). The ratios for the metabolites, BZE and EME, were 6.0 and 5.5, respectively. The authors concluded that cocaine and metabolite concentrations in saliva are highly dependent on pH and the manner in which the oral fluid was collected. In the first clinical study, it was reported that a predictable relationship existed between oral fluid and plasma codeine concentrations for 2 h to 12 h after oral administration. However, in that study, nonstimulated oral fluid was collected by having the subjects spit into inert polyethylene tubes and highly elevated oral fluid codeine concentrations were observed in the first 1 h to 2 h after drug administration.

The collection methods evaluated in this study included acidic and nonacidic (mechanical) stimulation of oral fluid production and nonstimulated oral fluid collection by spitting. Although

the term "nonstimulated oral fluid" was used, the act of spitting is usually sufficient to produce some stimulation. A mechanical stimulus, such as chewing sugarless gum, stimulates a flow of approximately 1 mL/min to 3 mL/min. Citric acid stimulation may produce flows of from 5 mL/min to 10 mL/min. The Salivette and the Finger Collector devices collect oral fluid through use of an absorbent material. These methods also produce some stimulation of oral fluid production. It is important to note that subjects in this study were instructed not to chew either device to avoid stimulation. The codeine concentrations in nonstimulated oral fluid were greater than concentrations in oral fluid specimens collected by the other methods. The observed differences may be a function of the oral fluid flow rate in specimens collected under passively or actively stimulated conditions. The Salivette and Finger Collector devices collect oral fluid by the swab method, which introduces some stimulation of oral fluid flow, and concentrations found after these collections were, on average, about 77% of the control concentrations. The codeine concentrations in specimens actively stimulated by chewing gum were approximately one-half of the control codeine concentrations. Acidic stimulation (which stimulates oral fluid production at a faster rate than the above methods) appeared to have the greatest effect on the codeine concentrations. Codeine concentrations were, on average, less than 30% of those observed in the control specimens.

The simultaneous collection of control specimens and Salivette specimens from four subjects allowed for a direct comparison of these two collection methods (fig. 9). The concentration differences observed in these subjects were in general agreement with the results shown in table 3. However, the mean control concentrations and the average ratio of control/Salivette concentrations were higher for this smaller group of subjects than in the initial collection experiments. This is partially attributable to one control subject having considerably higher codeine concentrations than predicted. Codeine concentrations in the first two specimens collected from this subject were 14,300 ng/mL and 5,290 ng/mL. Due to the small number of subjects in the control group, results from this subject had an appreciable effect on the mean concentrations, the concentration ratio, and the AUC.

The Salivette and Finger Collector devices were compared in in vitro and in vivo experiments (fig. 8 and 9). In vitro codeine recovery from the Finger Collector device was 46.7% less than control compared to 8.3% less than control for the Salivette. In addition, 75% to 90% of the oral fluid was recovered from the Salivette, but only approximately 50% of the oral fluid was recovered from the Finger Collector. Approximately 75% of the specimens collected with the Finger Collector in the clinical study had less than the minimum volume desired for the GC/MS assay. The authors were able to recover $\geq$500 µL of oral fluid from 81.8% of the Salivette collection. The majority of the specimens collected using the Finger Collector during the clinical study had a recovered volume of $\leq$300 µL. The accuracy of the codeine determinations for the Finger Collector device was not compromised by the low volume of sample in this study, but may present a problem for routine testing using less sensitive screening and confirmation methods.

The Orasure device consists of a cotton pad on the end of a plastic applicator. The pad is impregnated with buffer salts and the device is designed to collect up to 1.0 mL of oral fluid. The manufacturer reports that when placed between the cheek and gum, this device collects gingival crevicular fluid (also called oral mucosal transudate (OMT)) instead of saliva. However, if it is

left in the mouth for the required 2 min to 5 min, the device likely absorbs saliva as well. After collection of the OMT, the cotton pad is placed in a storage tube containing approximately 700 μL of buffer. This results in the OMT specimen being diluted 1:2 or 1:3 with the buffer. Because there is no way to determine the amount of OMT collected, the dilution factor varies with each collection. This may have attributed to the variability observed in the ratios of control codeine concentrations to OMT codeine concentrations (1.5 to 15.9).

2.3.4 Conclusions

1. Of the collection methods and devices investigated, the subjects preferred the swab-type devices to spitting. The subjects found spitting into a tube unpleasant, especially if observed, but they considered this discomfort minor when compared to that of an observed urine specimen collection.

2. From a laboratory perspective, sample preparation was easier and less time-consuming for specimens collected by spitting because the additional steps required to isolate the oral fluid from a device were not required. Removal of oral fluid from the Finger Collector was often difficult and not readily performed by simply milking the device between the thumb and forefinger as suggested by the manufacturer. This difficulty may have contributed to the low sample of volume recoveries observed in this study.

3. Stimulation reduced saliva codeine concentrations. This was likely due to the dilution effect of stimulation because the previous study demonstrated that pH did not have a pronounced effect.

4. Spitting was the most effective collection technique. Saliva codeine concentrations were 3.6 times acidic stimulation, 2 times nonacidic stimulation, and 2.3 times to 1.3 times those that were found with the Salivette and the Finger Collector.

5. Differences in the duration of the detection time of codeine were also observed for the collection methods. In the control group, codeine was detected in all of the 12 h specimens and over more than two-thirds of the 24 h specimens. With the Salivette and Finger Collector devices, all specimens collected at 12 h contained codeine; however, at 24 h after administration, 40% and 20% of the Salivette and Finger Collector samples, respectively, contained detectable codeine. Nonacidic oral fluid stimulation yielded only 60% positive specimens at 12 h and 40% at 24 h. Using the acidic oral fluid stimulation method, only 20% of the 12 h and none of the 24 h specimens contained codeine.

3. REPORT ON THE EFFECTS OF COLLECTION DEVICES ON DRUG CONCENTRATION

3.1 Introduction

The potential effects of collection techniques on the resultant oral fluid drug concentrations have not been thoroughly evaluated or adequately recognized in the literature. For example, although repeated collections of 2 mL or more of oral fluid by spitting was readily accomplished in the authors' clinical studies, they were unable to recover ≥500 μL of fluid from 19.2% of the clinical samples collected with the Salivette and 74.5% of the samples collected with the Finger Collector. In the clinical study discussed above, codeine concentrations in specimens collected by spitting were consistently higher than concentrations in specimens collected by the other methods. The control collection method concentrations averaged 3.6 times higher than concentrations in specimens collected by acidic stimulation of oral fluid and 1.3 to 2.0 times higher than concentrations in specimens collected by nonacid stimulation or collection using the Salivette or Finger Collector devices. Pharmacokinetic AUC calculations showed the same trend: control collection > nonacidic stimulation > Salivette > Finger Collector > acidic stimulation. Recovery for codeine and morphine was less than the control with the Salivette and the Finger Collector. The potential effects of different collection techniques and devices on the collection volume and concentration of drugs in oral fluid were of concern and provided the impetus for the following studies.

3.2 Study Design and Results of In Vitro Oral Fluid Volume Absorption (Part 1)

Although several oral fluid collection devices are advertised as being commercially available (Accu-Sorb®, Oral-Diffusion Sink®, Saliva Sampler®, Orasure/Epitope®, Finger Collector, Hooded Collector, Intercept®, and the Salivette), some manufacturers (such as Saliva Diagnostic Systems, Inc. (Saliva Sampler)) did not return phone calls, e-mails, and other attempted communications. To date, the authors have received product information for and been able to procure the following devices for evaluation: Salivette, Intercept, Hooded Collector, ORALscreen, and Finger Collector. A series of experiments were designed to determine (1) the collection time required by each device, (2) the volume of oral fluid collected by each device, and (3) the volume of oral fluid that could be recovered from each device. Culture tubes were placed on a laboratory balance and a known weight of oral fluid (2 gm) was added to each tube. A device was then placed in each culture tube and allowed to remain for the time recommended by the manufacturer. If no collection time was specified by the manufacturer, devices were left for up to 5 min. This time exceeded that generally recommended by the manufacturers. The devices were then removed and the final weight of each culture tube and its remaining contents were recorded and used to calculate the amount of oral fluid absorbed by each device. In addition, the time for absorption and the volume of oral fluid that was recovered ("harvested") from each device were recorded. In the harvesting portion of the experiments, the manufacturer's recommended procedures were not always followed. Rather, the authors attempted to recover as much oral fluid as possible and, therefore, they usually exceeded the manufacturer's recommendations. For example, instead of milking the Hooded Collector and the ORALscreen, each device was placed in a plastic pipette and centrifuged to increase the volume of oral fluid harvested/recovered. An n=10 of each device was tested. Following this section are a series of tables that summarize the findings from the in vitro volume recovery experiments.

The Salivette was tested in previous in vivo and in vitro experiments. The in vitro studies shown in table 7 demonstrated that the Salivette rapidly absorbed oral fluid. On average, the device absorbed 1.85 g (mL) of oral fluid and 1.48 g (mL) (82.5%) was recovered from the device that could be used for testing.

The Intercept was not tested in the authors' previous experiments. This device is similar to the Orasure/Epitope, is made by the same company, and has replaced these devices in the market. It is designed to collect approximately 1 mL of oral fluid. The in vitro studies shown in table 8 demonstrated that the device rapidly absorbed the oral fluid. On average, the device absorbed 0.82 g (mL) of oral fluids and 0.64 g (mL) (77.6%) was recovered from the device for testing.

The ORALscreen is made by Avitar and also was not tested in the authors' previous experiments. The in vitro volume studies shown in table 9 demonstrated that, on average, the device absorbed 1.76 g (mL) of oral fluid and 0.58 g (mL) (33.2%) was recovered from the device.

The Finger Collector was tested in the authors' previous in vivo and in vitro experiments. Along with the ORALscreen and the Hooded Collector, this device is made by Avitar. The current in vitro studies shown in table 10 demonstrated that, on average, the device absorbed 1.62 g (mL) of oral fluid and 1.24 g (mL) (76.6%) was recovered from the device.

The Hooded Collector was not tested in previous experiments. The in vitro studies shown in table 11 demonstrated that, on average, the device absorbed 1.68 g (mL) of oral fluid and 0.31 g (mL) (18.2%) was recovered from the device.

In rank order, in vitro oral fluid volume recovery (%) from the devices was:

Salivette > Intercept > Finger Collector > ORALscreen > Hooded Collector

3.3 Conclusions of In Vitro Oral Fluid Absorption (Part 1)

1. The Salivette absorbed its complement oral fluid in less than 1 min. Other devices took up to 5 min. However, anticipated collection times from donors, regardless of device, should be 5 min or less.

2. Less than 1 mL of oral fluid was collected with the Intercept. All other devices collected >1.6 mL. Small specimen volumes create a number of challenges. The limited volume may preclude confirmation of multiple drugs suspected from the screening analysis. It might also eliminate the retest options now available to urine donors and testing agencies. Improved screening and confirmation methods are needed to achieve equivalent cutoffs.

3. "Volume extracted from the device" represents the volume/weight of oral fluid available to be used for drug testing. Less than 1 mL (gr) was recovered from the Intercept, ORALscreen, and Hooded Collector. About 1.2 mL was recovered from the Finger Collector and 1.48 mL from the Salivette. As stated above, a limited specimen volume challenges the laboratory and may limit donor and agency options.

Table 7. In vitro study data for Salivette from Sarstedt Company

#	Time for Absorption (s)	Weight of Empty Tube (g)	Weight of Tube w/2,000 uL of Saliva (g)	Weight of Tube w/Device Removed (g)	Weight of Saliva Absorbed (g)	% Absorbed	Weight Extracted From Device (g)	% Recovery
1	45	11.4395	13.3804	11.4731	1.9073	98.27%	1.1710	61.30%
2	45	11.5388	13.4429	11.5799	1.8630	97.84%	1.5592	83.70%
3	45	11.4873	13.3862	11.5311	1.8551	97.69%	1.1260	86.90%
4	45	11.4240	13.3216	11.4960	1.8256	96.21%	1.5502	84.90%
5	45	11.4075	13.2945	11.4426	1.8519	98.14%	1.5782	85.20%
6	45	11.4154	13.2977	11.4702	1.8275	97.09%	1.5542	85.00%
7	45	11.3357	13.2343	11.3712	1.8631	98.13%	1.5797	84.80%
8	45	11.3792	13.2773	11.4304	1.8469	97.30%	1.5627	84.60%
9	45	11.5905	13.5029	11.6205	1.8824	98.43%	1.6185	86.00%
10	45	11.3488	13.2356	11.3840	1.8516	98.13%	1.5375	83.00%
				Mean	1.85744	97.72%	1.48372	82.54%
				Standard Deviation	0.0242	0.68%	0.1783	7.54%

Table 8. In vitro study data for Intercept from STC Company

#	Time for Absorption (s)	Weight of Empty Tube (g)	Weight of Tube w/2000 uL of Saliva (g)	Weight of Tube w/Device Removed (g)	Weight of Saliva Absorbed (g)	% Absorbed	Weight Extracted from Device (g)	% Recovery
1	180	8.7077	10.5576	9.7464	0.8112	43.85%	0.6394	78.82%
2	180	8.623	10.4050	9.5783	0.8276	46.42%	0.6314	76.29%
3	180	8.5991	10.3199	9.521	0.7989	46.43%	0.6110	76.48%
4	180	8.7557	10.5471	9.7347	0.8124	45.35%	0.6562	80.77%
5	180	8.664	10.4996	9.5562	0.9434	51.39%	0.6639	70.37%
6	180	8.6676	10.3641	9.5771	0.787	46.39%	0.5935	75.41%
7	180	8.5393	10.3489	9.5215	0.8274	45.72%	0.6512	78.70%
8	180	8.6153	10.3835	9.6113	0.7722	43.67%	0.613	79.38%
9	180	8.6126	10.3091	9.5146	0.7945	46.83%	0.6394	80.48%
10	180	8.6391	10.4155	9.5427	0.8728	49.13%	0.6946	79.58%
				Mean	0.82474	46.52%	0.63936	77.63%
				Standard Deviation	0.0500	2.31%	0.0293	3.13%

Table 9. In vitro study data for ORALscreen from Avitar Company

#	Time for Absorption (s)	Weight of Empty Tube (g)	Weight of Tube w/2,000 uL of Saliva (g)	Weight of Tube w/Device Removed (g)	Weight of Saliva Absorbed (g)	% Absorbed	Weight Extracted From Device (g)	% Recovery
1	300	8.6428	10.5194	8.6529	1.8665	99.46%	0.5411	28.99%
2	300	8.6421	10.5008	8.6719	1.8289	98.40%	0.5912	32.33%
3	300	8.7542	10.6205	8.8055	1.8150	97.25%	0.6034	33.25%
4	300	8.6018	10.4084	8.7204	1.6880	93.44%	0.5988	35.47%
5	300	8.5845	10.3770	8.6104	1.7666	98.56%	0.6241	35.33%
6	300	8.5876	10.3560	8.6458	1.7102	96.71%	0.5813	33.99%
7	300	8.5437	10.2863	8.6966	1.5897	91.23%	0.6005	37.77%
8	300	8.6278	10.4188	8.6539	1.7649	98.54%	0.4974	28.18%
9	300	8.6374	10.5361	8.7325	1.8036	94.99%	0.5826	32.30%
10	300	8.6753	10.5033	8.6938	1.8095	98.99%	0.6264	34.62%
				Mean	1.76429	96.76%	0.58468	33.22%
				Standard Deviation	0.0817	2.72%	0.0390	2.94%

Table 10. In vitro study data for Finger Collector from Avitar Company

#	Time for Absorption (s)	Weight of Empty Tube (g)	Weight of Tube w/2,000 uL of Saliva (g)	Weight of Tube w/Device Removed (g)	Weight of Saliva Absorbed (g)	% Absorbed	Weight Extracted From Device (g)	% Recovery
1	300	10.2982	12.0101	10.3503	1.6598	96.96%	1.1181	67.36%
2	300	10.7152	12.4568	10.7755	1.6813	96.54%	1.1437	68.02%
3	300	10.3024	12.0549	10.3295	1.7254	98.45%	1.2803	74.20%
4	300	10.1835	11.9819	10.2295	1.7524	97.44%	1.4265	81.40%
5	300	10.1814	11.8572	10.2004	1.6568	98.87%	1.3559	81.84%
6	300	10.7273	12.2711	10.7749	1.4962	96.92%	1.1865	79.30%
7	300	10.6618	12.2932	10.6972	1.5960	97.83%	1.2603	78.97%
8	300	10.2976	11.5051	10.3262	1.1789	97.63%	0.9219	78.20%
9	300	10.3555	12.1989	10.3901	1.8088	98.12%	1.4345	79.31%
10	300	10.1870	11.9870	10.3403	1.6467	91.48%	1.2699	77.12%
				Mean	1.62023	97.02%	1.23976	76.57%
				Standard Deviation	0.05	2.31%	0.0293	3.13%

Table 11. In vitro study data for Hooded Collector from Avitar Company

#	Time for Absorption (s)	Weight of Empty Tube (g)	Weight of Tube w/2,000 uL of Saliva (g)	Weight of Tube w/Device Removed (g)	Weight of Saliva Absorbed (g)	% Absorbed	Weight Extracted From Device (g)	% Recovery
1	300	8.6708	10.5244	8.8292	1.6952	91.45%	0.3456	20.39%
2	300	8.6839	10.5579	8.7882	1.7697	94.43%	0.3630	20.51%
3	300	8.6292	10.5141	8.8269	1.6872	89.51%	0.2511	14.88%
4	300	8.7432	10.5749	8.8152	1.7597	96.07%	0.3251	18.47%
5	300	8.6192	10.4605	8.6729	1.7876	97.08%	0.1862	10.42%
6	300	8.6894	10.5292	8.7475	1.7817	96.84%	0.3883	21.79%
7	300	8.5921	10.3937	8.6245	1.7692	98.20%	0.4815	27.22%
8	300	8.7548	10.564	8.8076	1.7564	97.08%	0.2857	16.27%
9	300	8.5875	10.3054	8.8005	1.5049	87.60%	0.2445	16.25%
10	300	8.6329	10.2804	8.8992	1.3812	83.84%	0.2219	16.07%
				Mean	1.68928	93.21%	0.30929	18.23%
				Standard Deviation	0.1372	4.87%	0.0890	4.57%

3.4 Study Design and Results of In Vitro Drug Recovery (Part 2)

The in vitro experiments just discussed were designed to determine the volume of oral fluid collected by the devices. The following experiments were designed to determine the in vitro recovery of drugs of abuse and their metabolites from the devices. In the experiments, control drug-free oral fluid was fortified with drugs of abuse at three physiologically relevant concentrations. The samples were then "collected" by each device and assayed for their drug concentration by LC/MS (n=5 at each concentration). The drugs tested were amphetamine, methamphetamine, PCP, morphine, and codeine. The concentrations tested were as follows:

Drug	Concentrations (ng/mL)
Amphetamine	0, 10, 25, and 100
Methamphetamine	0, 10, 25, and 100
PCP	0, 5, 10, and 50
Morphine	0, 25, 50, and 200
Codeine	0, 25, 50, and 200

Pools of oral fluid were fortified at the drug/metabolite concentrations shown above. The pools were then aliquoted into 2 mL volumes and collected by the device. (Recall that devices seldom collected 2 full mLs and the authors were not able to harvest 2 mL from any of the devices.) Additionally, 2 mL portions of the pools were aliquoted as controls. The controls were not "collected" by the device, but rather were extracted and analyzed as "neat" oral fluid. Control samples were analogous to samples collected by spitting in the clinical studies. Quantitation of the drugs/metabolites was performed through concurrent extraction and analysis of a multipoint calibration curve for each analyte. Recovery was calculated by comparing the mean concentration from each device to the mean concentration of controls and reported as percent recovery. The data in the tables assume that each device collected 2 mL of oral fluid. Therefore, the study design was analogous to the real-life situation of collecting an oral fluid specimen with the device and shipping the sample to a laboratory for analysis. The laboratory would not know the exact volume of oral fluid collected by the device and the drug concentration reported would assume a volume based on the device manufacturer's claimed volume.

The tables that follow show the recovery versus control for amphetamine, methamphetamine, PCP, morphine, and codeine. Given that amphetamine is a metabolite of methamphetamine and the drugs have a similar chemical structure, one would predict similar recoveries of the two drugs from the devices. This is demonstrated in tables 12 and 13. Recoveries exceeded 50% from the Salivette and the Finger Collector for both amphetamine and methamphetamine at the tested concentrations. Recoveries at the same concentrations from Intercept and the ORALscreen exceeded 30% (except at 100 ng/mL of amphetamine with ORALscreen). Recoveries from the Hooded Collector for amphetamine and methamphetamine were all approximately 25%. Only the recovery of amphetamine at 100 ng/mL exceeded 30% with the Hooded Collector. Recovery of amphetamine and methamphetamine from the devices demonstrated the following pattern:

> Salivette approximately = Finger Collector > Intercept approximately = ORALscreen > Hooded Collector

Table 14 shows that the recovery of PCP approached or exceeded 50% from the Salivette at each tested concentration. Recoveries at the same concentrations from the Intercept were 25% to 37%. The recoveries were similar to those from the Finger Collector, which ranged from (approximately) 23% to 31%. Recoveries from the ORALscreen and the Hooded Collector were very poor (<25%). PCP is far more lipophilic and less basic than amphetamine and methamphetamine. The lipophilic nature of PCP may have contributed to the reduced recovery from the devices. Recovery of PCP from the devices demonstrated the following pattern:

> Salivette > Intercept approximately = Finger Collector > ORALscreen > Hooded Collector

Because morphine is a metabolite of codeine with similar chemical structure, one would predict similar recoveries of these drugs from the devices. Tables 15 and 16 show that recoveries approached 50% for the Finger Collector for both drugs. Recoveries from Salivette and Hooded Collector were in the 37% to 46% range. Recoveries from the Intercept and ORALscreen for morphine and codeine were slightly less at 33% to 39%. Recovery of morphine and codeine from the devices was more consistent than the other drugs and demonstrated the following pattern:

> Finger Collector > Salivette approximately = Hooded Collector > ORALscreen approximately = Intercept

A discussion of the recovery of cocaine, BZE, THC, and THC-COOH from the devices follows.

3.5 Conclusions of In Vitro Drug Absorption (Part 2)

1. Recovery calculations in these experiments were not adjusted for the volume of oral fluids collected by the device, but rather simulated the real-world scenario of a collection and shipment of the device to a laboratory for analysis.

2. Recovery of the tested drugs from the devices tended to be in the range of approximately 30% to 60%. Typical drug recoveries in analytical methods commonly exceed 70% or 80%; occasionally recoveries exceed 90%.

3. The recovery of the lipophilic drug (PCP) was decidedly less than the more hydrophilic drugs from all devices except the Salivette.

4. Intercept is only designed to collect 1 mL of oral fluids. Therefore, the recoveries presented in the tables could be doubled to correct for the analysis volume of 2 mL.

5. Recovery from the devices was poor. The practical implications of poor drug recovery are similar to low-volume recovery. Improved screening and confirmation methods are needed to achieve equivalent cutoffs. This may require more sophisticated methods and instruments and may increase the cost of testing.

6. The "control" shown in tables 12 through 16 simulates oral fluid collected by spitting. This finding was consistent with the authors' clinical study results.

Table 12. Summary of amphetamine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector AMP
10 ng/mL		4.16	3.01	4.96	3.71	2.28
		5.06	3.18	5.20	2.86	2.20
		4.83	3.16	5.19	3.57	2.26
	9.73	6.18	2.86	5.11	4.08	1.41
	9.60	6.02	3.01	5.01	3.01	3.49
Mean	9.67	5.25	3.04	5.09	3.45	2.33
SD	0.09	0.84	0.13	0.11	0.50	0.74
% Control		54.30%	31.48%	52.69%	35.64%	24.06%
25 ng/mL		13.05	8.01	12.83	8.44	7.93
		12.62	8.49	13.69	7.98	4.80
		14.60	8.99	14.88	8.06	3.36
	25.73	16.46	8.81	14.22	6.59	5.70
	23.47	13.71	8.72	13.91	7.18	8.75
Mean	24.60	14.09	8.60	13.91	7.65	6.11
SD	1.60	1.52	0.38	0.75	0.75	2.22
% Control		57.28%	34.97%	56.54%	31.10%	24.84%
100 ng/mL		62.64	33.56	53.55	38.46	37.82
		50.46	34.17	52.83	10.64	36.43
		54.65	34.08	53.85	9.98	28.89
	96.66	61.55	32.96	53.91	9.47	26.31
	99.58	46.82	33.79	52.85	11.09	36.24
Mean	98.12	55.22	33.71	53.40	15.93	33.14
SD	2.07	6.87	0.48	0.53	12.61	5.18
% Control		56.28%	34.36%	54.42%	16.23%	33.77%

Table 13. Summary of methamphetamine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector
10 ng/mL		4.41	3.03	5.37	3.82	2.42
		5.11	3.32	5.47	2.91	2.35
		4.67	3.48	5.41	3.58	2.44
	9.98	6.54	3.12	5.39	4.20	1.52
	9.76	6.25	3.12	5.31	3.12	3.62
Mean	9.82	5.40	3.21	5.39	3.53	2.47
SD	0.08	0.95	0.18	0.06	0.52	0.75
% Control		54.94%	32.73%	54.88%	35.93%	25.17%
25 ng/mL		13.39	8.19	13.70	8.69	8.56
		13.23	8.89	14.43	8.22	5.14
		15.14	9.24	16.12	8.48	3.46
	26.22	17.26	9.09	14.96	6.74	5.92
	24.02	14.22	9.24	14.98	7.63	9.10
Mean	25.12	14.65	8.93	14.84	7.95	6.44
SD	1.56	1.65	0.44	0.89	0.79	2.37
% Control		58.31%	35.55%	59.08%	31.65%	25.63%
100 ng/mL		65.53	34.29	55.58	40.46	38.78
		53.85	35.10	55.65	35.05	26.94
		56.63	35.37	56.32	28.30	27.15
	97.01	64.83	34.44	57.66	24.90	27.19
	99.14	48.42	35.10	57.59	34.30	14.74
Mean	98.07	57.85	34.86	56.56	32.60	26.96
SD	1.51	7.32	0.47	1.01	6.09	8.50
% Control		58.99%	35.55%	57.67%	33.24%	27.49%

Table 14. Summary of phenylcyclidene (PCP) recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector
5 ng/mL		2.86	1.28	1.09	0.88	0.87
		2.49	1.34	1.1	0.78	0.54
		2.94	1.25	0.99	0.47	0.26
	4.54	1.95	1.22	1.23	0.71	0.39
	5.27	2.89	1.24	1.23	0.66	0.21
Mean	4.91	2.63	1.27	1.13	0.70	0.45
SD	0.52	0.42	0.05	0.10	0.15	0.27
% Control		53.54%	25.81%	23.00%	14.27%	9.26%
10 ng/mL		4.19	3.07	2.7	1.3	0.89
		4.44	3.41	2.98	1.57	1.09
		4.94	3.14	2.62	1.6	1.25
	9.31	4.64	3.14	2.6	1.88	1.32
Mean	9.15	4.49	2.85	2.52	1.6	0.93
SD	9.23	4.54	3.12	2.68	1.59	1.10
% Control	0.11	0.28	0.20	0.18	0.21	0.19
		49.19%	33.82%	29.08%	17.23%	11.87%
50 ng/mL		25.29	18.36	12.46	11.78	10.86
		24.95	17.77	11.68	10.64	8.98
		24.71	16.92	13.99	9.98	5.33
	44.64	27.9	15.18	14.66	9.47	7.77
	46.14	23.96	16.08	17.26	11.09	6.93
Mean	45.39	25.36	16.86	14.01	10.59	7.97
SD	1.06	1.50	1.28	2.17	0.91	2.09
% Control		55.88%	37.15%	30.87%	23.34%	17.57%

Table 15. Summary of morphine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector
25 ng/mL		11.76	8.55	13.50	8.53	13.19
		12.65	10.05	12.91	10.98	11.35
		14.21	9.59	12.84	7.18	11.45
		10.33	10.16	13.31	8.54	11.11
	26.42	11.80	9.69	12.56	11.84	13.83
Mean	26.42	12.15	9.61	13.02	9.41	12.19
SD	0.00	1.42	0.64	0.38	1.93	1.24
% Control		45.99%	36.37%	49.30%	35.63%	46.13%
50 ng/mL		19.70	17.65	26.75	18.24	17.92
		19.89	17.67	26.79	21.94	0 (error, not counted)
		22.35	17.88	28.07	23.58	21.52
		22.17	19.12	25.77	21.50	15.92
	53.14	19.79	19.16	25.67	18.97	23.39
Mean	53.14	20.78	18.30	26.61	20.84	19.69
SD	0.00	1.35	0.77	0.97	2.20	3.38
% Control		39.10%	34.43%	50.07%	39.22%	37.04%
200 ng/mL		83.63	64.97	103.18	78.64	106.08
		74.19	64.20	94.65	81.50	89.49
		79.22	66.02	101.62	62.94	90.13
		80.30	73.90	96.19	80.23	74.33
	203.60	74.40	68.12	95.40	61.84	70.87
Mean	203.60	78.35	67.44	98.21	73.03	86.18
SD	0.00	4.04	3.90	3.90	9.77	14.12
% Control		38.48%	33.12%	48.24%	35.87%	42.33%

Table 16. Summary of codeine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector
25 ng/mL		11.88	9.05	13.0564	8.54	13.53
		13.09	10.39	12.6499	11.07	11.62
		14.67	9.89	12.4976	7.07	11.71
		10.66	10.37	13.233	8.19	11.32
	27.41	12.37	10.11	12.3354	11.29	14.63
Mean	27.41	12.53	9.96	12.75	9.23	12.56
SD	0.00	1.49	0.55	0.38	1.86	1.45
% Control		45.71%	36.33%	46.52%	33.68%	45.82%
50 ng/mL		20.48	18.72	26.7147	17.46	18.58
		20.79	18.50	26.5704	20.92	0 (error, not counted)
		23.22	19.03	28.2516	22.33	22.19
		23.11	20.02	25.9359	21.14	16.63
	54.84	20.72	20.08	25.9112	18.52	24.26
Mean	54.84	21.67	19.27	26.68	20.08	20.41
SD	0.00	1.38	0.74	0.95	2.01	3.44
% Control		39.51%	35.14%	48.65%	36.61%	37.23%
200 ng/mL		85.02	67.64	102.1194	76.03	103.98
		77.34	66.75	94.8489	78.05	88.22
		81.78	67.20	102.1507	60.77	87.35
		83.23	76.62	97.9424	77.53	72.27
	209.55	77.60	70.54	97.7931	60.77	68.08
Mean	209.55	81.00	69.75	98.97	70.63	83.98
SD	0.00	3.42	4.12	3.14	9.03	14.31
% Control		38.65%	33.29%	47.23%	33.71%	40.08%

3.6 Study Design and Results of In Vitro Drug Recovery (Part 3), Volume Corrected

The previous experiments evaluated the devices in a simulated donor collection scenario where the volume of oral fluid collected would likely vary. Consequently, the concentration of drug reported from the device would vary. The experiments reported in this section were similar to those reported above except the device was allowed to collect its normal oral fluid volume, and a *consistent volume* (0.5 mL) of oral fluid was harvested from the device for testing. This allowed for an estimate of the actual drug recovery from each device. The Hooded Collector is no longer manufactured and was not included in all of the following evaluations. Due to the limited number of these devices, cocaine and BZE recoveries were not included in Part 2, but are included below. With the exception of the constant volume collection, the procedures for analysis and data interpretation were identical to those described in Part 2. Control drug-free saliva was fortified with drugs of abuse at three concentrations, and the fortified samples were collected with each device and assayed for each drug of abuse by LC/MS (n=5 at each concentration). The drugs tested were amphetamine, methamphetamine, PCP, morphine, codeine, cocaine, BZE, THC, and THC-COOH. The concentrations tested were as follows:

Drug	Concentrations (ng/mL)
Amphetamine	0, 10, 25, and 100
Methamphetamine	0, 10, 25, and 100
PCP	0, 5, 10, and 50
Morphine	0, 25, 50, and 200
Codeine	0, 25, 50, and 200
Cocaine	0, 5, 10, and 100
BZE	0, 5, 10, and 100
THC	0, 1, 2.5, and 10
THC-COOH	0, 2.5, 5, and 25

The drug recovery data from the devices are shown in tables 17 through 24. Recovery was calculated by comparing the mean drug concentration from the devices to the mean concentration of its respective control and reported as percent recovery. As described earlier, the controls were not collected by the device, but rather were extracted and analyzed as neat oral fluid.

The recoveries of amphetamine and methamphetamine and are shown in tables 17 and 18. Recoveries exceeded 85% for both drugs, at all tested concentrations and with all devices. Given that the analytical accuracy of determining the control and device concentrations was $\pm$10%, calculated recoveries of 85% should be considered good and those >90% excellent. No pattern was evident in the recovery from the devices except all were good and those from the ORALscreen were excellent.

Table 19 shows that the recovery of PCP exceeded 80% from the Intercept at concentrations of 5 ng/mL, 10 ng/mL, and 50 ng/mL of oral fluid. Recoveries at the same concentrations from the Salivette were 76% to 82%. Recoveries from the ORALscreen and the Finger Collector were poor for PCP (44% to 54%). As with the previous study, PCP recoveries were less than the more hydrophilic drugs such as amphetamine, methamphetamine, and the opiates. Recovery of PCP from the devices demonstrated the following pattern:

Intercept > Salivette > ORALscreen > Finger Collector

The recoveries of morphine and codeine are shown in tables 20 and 21. They show that recovery of the two drugs from the Intercept exceeded 80%. Recoveries at all concentrations from the Salivette were 78% to 86%. Recoveries from the ORALscreen exceeded 72% and those from the Finger Collector were approximately 60% to 63%. As with the PCP results, recovery of morphine and codeine from the devices demonstrated the following pattern:

Intercept > Salivette > ORALscreen > Finger Collector

The recoveries of cocaine and BZE are shown in tables 22 and 23. The recoveries of these drugs were not evaluated in Part 2 because there was a limited supply of Hooded Collectors. The tables show that recovery of cocaine exceeded 61% and that of BZE drugs exceeded 87% from all devices. BZE is more polar than cocaine and its recovery exceeded cocaine with four of the devices at all concentrations. This supports the observation that hydrophilic drugs are more easily recovered from the devices. Recovery of cocaine demonstrated the following pattern:

Intercept > Salivette > ORALscreen > Hooded Collector > Finger Collector

Recovery of BZE demonstrated the following pattern:

ORALscreen > Salivette approximately = Hooded Collector approximately = Finger Collector > Intercept

Determining the recovery of THC and THC-COOH from the devices presented significant challenges. Multiple attempts were made to evaluate the recovery of these cannabinoids, but no successful data were obtained for either analyte in the in vitro recovery studies (Part 2). The initial challenge was to prepare a fortified pool of THC and THC-COOH oral fluid at the specified concentrations. It is well documented that cannabinoids adhere to absorptive surfaces such that measured concentrations seldom reflect fortified concentrations of control materials.

The authors were successful in preparing fortified pools from which the measured concentrations reflected the fortified concentrations for THC-COOH. However, they were not successful in preparing a similar THC pool. Tables were not prepared for THC because measured concentrations were about one-third of fortified concentrations. In addition, the experiments indicated that the recovery of THC from the devices was very poor (approximately 25%). Therefore, the THC concentrations approached the LOQ of the analysis where the quantitative accuracy becomes more problematic. Further, the authors were limited in the number of times that they could attempt these experiments because the Hooded Collector was no longer available.

This is unfortunate because THC is the primary cannabinoid in the oral fluid of users. The data reported for THC-COOH from the Hooded Collector are based on n=3 collections.

Table 24 shows that the recovery of THC-COOH approached 50% from the Intercept. The recovery from the Salivette improved as a function of increasing THC-COOH concentration and approached 50% at 25 ng/mL. Regardless of THC-COOH concentration, recoveries from the Finger Collector, ORALscreen, and Hooded Collector were very poor and unacceptable by analytical standards.

Recovery of THC-COOH from the devices demonstrated the following pattern:

Intercept > Salivette > > > Finger Collector, ORALscreen, and Hooded Collector

3.7 Conclusions of In Vitro Drug Absorption (Part 3), Volume Corrected

1. Data from these studies reflect the actual drug recovery from the devices because the recovery percentages were volume controlled and control corrected.

2. Recovery of amphetamine, methamphetamine, morphine, and codeine from the devices was in the range of approximately 60% to nearly 90%.

3. The recovery of the lipophilic drug (PCP) was decidedly less than the hydrophilic drugs from all devices except the Salivette and Intercept.

4. Recovery of cocaine and BZE from the devices was in the range of 60% to more than 97%. The recovery of BZE exceeded that of the other drugs tested.

5. Recovery studies are needed for THC. Like PCP, THC is lipophilic and may have poor recovery from the devices. Studies will need to be well conceived to avoid the multiple complications encountered in this research.

6. The recovery of THC-COOH was poor from all of the devices. A 50% recovery would have the effect of raising the analytical cutoff concentration twofold. Recoveries from the Salivette were unacceptable at lower concentrations and poor even at higher concentrations. THC-COOH was essentially not recovered from the Finger Collector, ORALscreen, and the Hooded Collector.

7. Data from the Part 3 studies showed that recovery of drugs/metabolites from the devices for the most part was acceptable. However, the accuracy of the concentrations reported from the devices suffered because the devices do not control the volume of oral fluid collected or available for testing (Part 2).

Table 17. Summary of amphetamine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen
10 ng/mL		8.37	8.49	8.58	9.04
		8.32	8.58	8.15	8.74
		8.48	8.43	8.59	8.85
	9.68	8.57	8.55	8.44	9.15
	9.92	8.22	8.95	8.9	9.08
Mean	9.80	8.39	8.60	8.53	8.97
SD	0.17	0.14	0.20	0.27	0.17
% Control		85.63%	87.76%	87.06%	91.55%
25 ng/mL		22.44	22.02	22.29	25.12
		22.42	23.35	22.77	25.13
		22.76	22.97	23.06	24.01
	26.21	22.3	22.1	22.12	24.77
	26.15	22.71	24.9	23.25	24.08
Mean	26.18	22.53	23.07	22.70	24.62
SD	0.04	0.20	1.17	0.49	0.55
% Control		86.04%	88.11%	86.70%	94.05%
100 ng/mL		93.21	95.37	95.96	102.36
		95.88	96.91	93.5	103.96
		96.5	95.1	94.75	104.52
	110.9	95.34	94.63	103.18	102.94
	110.3	95.02	92.84	95.28	98.94
Mean	110.60	95.19	94.97	96.53	102.54
SD	0.42	1.24	1.47	3.82	2.18
% Control		86.07%	85.87%	87.28%	92.72%

Table 18. Summary of methamphetamine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen
10 ng/mL		8.54	8.77	9.08	9.8
		8.89	8.94	8.69	9.38
		8.76	8.9	8.94	9.37
	9.94	8.74	8.74	8.83	9.85
	9.93	8.77	9	8.83	9.58
Mean	9.94	8.74	8.87	8.87	9.60
SD	0.01	0.13	0.11	0.15	0.23
% Control		87.97%	89.28%	89.32%	96.59%
25 ng/mL		23.72	26.33	23.51	26.18
		23.84	24.24	24.46	26.6
		24.2	23.93	24.41	25.6
	26.93	23.18	22.71	24.35	26.63
	27.02	24.1	26.12	25.11	25.66
Mean	26.98	23.81	24.67	24.37	26.13
SD	0.06	0.40	1.54	0.57	0.49
% Control		88.26%	91.44%	90.34%	96.88%
100 ng/mL		97.37	92.79	100.94	109.69
		97.41	95.99	100.97	105.09
		101.47	96.78	100.3	109.98
	112.52	100.1	94.04	108.3	108.1
	110.7	99.25	98.76	100.25	102.72
Mean	111.61	99.12	95.67	102.15	107.12
SD	1.29	1.77	2.34	3.45	3.13
% Control		88.81%	85.72%	91.53%	95.97%

Table 19. Summary of PCP recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen
5 ng/mL		4.19	4.49	2.38	2.82
		4.2	4.48	2.19	2.96
		4.34	4.63	2.16	2.79
	5.27	3.8	4.46	2.46	3.02
	5.17	4.11	4.7	2.38	2.5
Mean	5.22	4.13	4.55	2.31	2.82
SD	0.07	0.20	0.11	0.13	0.20
% Control		79.08%	87.20%	44.33%	53.98%
10 ng/mL		7.66	8.34	4.53	5.2
		7.59	8.7	4.6	5.43
		7.72	8.65	4.6	5.22
	9.92	7.3	8.42	4.32	5.35
	9.87	7.5	8.81	4.35	5.5
Mean	9.90	7.55	8.58	4.48	5.34
SD	0.04	0.16	0.20	0.14	0.13
% Control		76.34%	86.75%	45.28%	53.97%
50 ng/mL		43.44	42.74	23.7	25.34
		41.94	45.72	24.4	27.17
		40.36	44.02	25.37	27.41
	50.79	40.56	44.73	24.86	25.34
	50.55	40.7	45.75	24.94	26.39
Mean	50.67	41.40	44.59	24.65	26.33
SD	0.17	1.30	1.26	0.63	0.98
% Control		81.71%	88.00%	48.66%	51.96%

Table 20. Summary of morphine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen
25 ng/mL		21.36	20.82	16.18	19.38
		21.15	21.31	16.21	19.34
		20.62	21.17	17.09	18.83
	26.46	20.68	21.98	16.09	19.28
	26.62	20.3	21.28	17.14	19.69
Mean	26.54	20.82	21.31	16.54	19.30
SD	0.11	0.43	0.42	0.53	0.31
% Control		78.46%	80.30%	62.33%	72.74%
50 ng/mL		46.36	48.21	38.19	44.43
		48.38	49.02	35.67	43.85
		46.43	49.84	35.8	43.66
	60.46	47.57	50.99	38.11	45.3
	60.4	48.54	50.02	36.39	44.93
Mean	60.43	47.46	49.62	36.83	44.43
SD	0.04	1.04	1.05	1.23	0.70
% Control		78.53%	82.10%	60.95%	73.53%
200 ng/mL		167.07	161.97	122.44	149.02
		165.26	169.18	128.94	153.3
		162.03	161.86	133.69	148.59
	200.25	156.69	168.63	120.61	156.47
	198.98	163.68	173.05	127.27	151.78
Mean	199.62	162.95	166.94	126.59	151.83
SD	0.90	3.96	4.89	5.23	3.24
% Control		81.63%	83.63%	63.42%	76.06%

Table 21. Summary of codeine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen
25 ng/mL		22.92	22.75	16.36	19.33
		22.98	23.49	16.49	19.47
		22.26	23.5	17.28	18.79
	26.92	22.44	24.38	16.29	19.24
	27.89	22.21	23.39	17.45	19.83
Mean	27.41	22.56	23.50	16.77	19.33
SD	0.69	0.36	0.58	0.55	0.38
% Control		82.33%	85.76%	61.21%	70.54%
50 ng/mL		50.66	53.81	38.58	43.71
		52.01	54.76	36.25	43.09
		50.5	55.88	35.79	44.12
	62.28	51.42	55.97	38.39	46.01
	62.18	52.34	54.88	36.62	45.34
Mean	62.23	51.39	55.06	37.13	44.45
SD	0.07	0.81	0.89	1.28	1.20
% Control		82.57%	88.48%	59.66%	71.43%
200 ng/mL		176.58	180.73	120.67	156.17
		176.53	186.78	128.17	162.85
		173.37	178.92	133.82	156.3
	200.78	168.78	181.5	120.8	168.64
	202.1	173.37	186.66	127.22	164.26
Mean	201.44	173.73	182.92	126.14	161.64
SD	0.93	3.19	3.60	5.54	5.38
% Control		86.24%	90.81%	62.62%	80.24%

Table 22. Summary of cocaine recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector
5 ng/mL		4.23	4.69	3.02	3.41	3.23
		4.42	4.37	3.06	3.47	3.22
		4.3	4.37	2.99	3.26	3.34
	4.73	4.22	4.62	3.05	3.31	3.26
	4.62	4.33	4.28	3.11	3.33	3.06
Mean	4.68	4.30	4.47	3.05	3.36	3.22
SD	0.08	0.08	0.18	0.05	0.08	0.10
% Control		91.98%	95.53%	65.16%	71.79%	68.92%
10 ng/mL		8.11	8.54	5.32	6.08	5.84
		8.13	8.07	5.18	5.57	6.27
		7.85	8.88	5.55	5.94	6.26
	8.87	7.96	8.4	5.74	6.5	6.67
	8.85	8.51	9	5.57	6.04	5.46
Mean	8.86	8.11	8.58	5.47	6.03	6.10
SD	0.01	0.25	0.37	0.22	0.33	0.46
% Control		91.56%	96.82%	61.76%	68.01%	68.85%
100 ng/mL		75.57	76.95	50	55.81	55.39
		77.99	84.02	51.63	58.88	51.78
		77.4	83.47	56.8	58.56	54.78
	86.95	78.29	81.71	52.25	56.6	52.51
	84.08	79.18	80.98	50.28	59.97	51.99
Mean	85.52	77.69	81.43	52.19	57.96	53.29
SD	2.03	1.35	2.79	2.74	1.71	1.67
% Control		90.84%	95.22%	61.03%	67.78%	62.32%

Table 23. Summary of BZE recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector
5 ng/mL		5.03	4.67	4.98	4.99	4.81
		4.42	4.67	5.11	5.04	5.08
		5.1	4.56	4.97	5.03	5.14
	5.28	4.84	4.83	4.81	5.01	4.94
	5.29	4.93	4.84	4.7	4.92	4.68
Mean	5.29	4.86	4.71	4.91	5.00	4.93
SD	0.01	0.27	0.12	0.16	0.05	0.19
% Control		92.03%	89.20%	92.98%	94.57%	93.28%
10 ng/mL		9.69	8.96	9.5	10.19	9.92
		9.36	8.52	9.36	8.99	10.06
		9.36	9.5	9.27	9.57	9.63
	10.02	9.52	8.98	9.65	9.95	9.74
	10.04	9.6	9.79	9.22	9.98	9.07
Mean	10.03	9.51	9.15	9.40	9.74	9.68
SD	0.01	0.15	0.50	0.18	0.47	0.38
% Control		94.78%	91.23%	93.72%	97.07%	96.55%
100 ng/mL		90.65	78.83	90.22	94.48	91.68
		90.8	88.19	86.23	98.14	88.54
		91.77	83.87	91.59	92.62	89.67
	97.92	91.54	85.03	87.47	95.26	79.1
	98.37	90.6	83.97	91.99	94.89	79.8
Mean	98.15	91.07	83.98	89.50	95.08	85.76
SD	0.32	0.54	3.37	2.54	1.99	5.87
% Control		92.79%	85.57%	91.19%	96.88%	87.38%

Table 24. Summary of THC-COOH recovery

	Control	Salivette	Intercept	Finger Collector	ORALscreen	Hooded Collector
2.5 ng/mL		0.06	0.76	0	0	0
		0.31	0.86	0	0	0
		0.13	0.95	0	0	0
	1.89	0.39	0.90	0	0	
	1.89	0.26	0.96	0	0	
Mean	1.89	0.23	0.89	0.00	0.00	0.00
SD	0.00	0.13	0.08	0.00	0.00	0.00
% Control		12.17%	46.86%	0.00%	0.00%	0.00%
5 ng/mL		1.26	2.41	0	0.02	0
		1.03	2.34	0	0.34	0
		1.67	2.33	0	0.08	0
	4.74	2.25	2.25	0	0.08	
	4.46	1.38	2.42	0.00	0.03	
Mean	4.60	1.52	2.35	0.00	0.11	0.00
SD	0.20	0.47	0.07	0.00	0.13	0.00
% Control		33.00%	51.09%	0.00%	2.39%	0.00%
25 ng/mL		12.91	11.44	0.37	1.08	0.471
		12.63	14.1	0.05	0.8	0.471
		12.26	13.53	0.15	0.17	0.485
	25.59	10.9	14.54	0	0.44	
	24.7	10.9	13.69	0.05	0.44	
Mean	25.15	11.92	13.46	0.12	0.59	0.48
SD	0.63	0.96	1.20	0.15	0.36	0.01
% Control		47.41%	53.53%	0.49%	2.33%	1.89%

4. REPORT OF SPECIMEN VALIDITY— IMMUNOGLOBULIN G (IgG) CONCENTRATION

4.1 Introduction

For oral fluid to be accepted as a viable specimen for drug testing, it must be possible to ensure that a valid and representative specimen was collected. There also must be objective methods to ensure that the specimen is valid and representative. For example, several methods can be used to ensure that urine specimens have not been diluted or substituted, such as determining sample temperature and determinations of pH, specific gravity, and creatinine. At a series of meetings (most recently held at the Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Drug Testing Advisory Board, Chevy Chase, MD, December 6, 2001), it was suggested that an oral fluid specimen was valid if it contained IgG at a concentration ≥0.5 μg/mL. As part of this research, oral fluid samples were randomly tested for IgG concentration. Preliminary data suggested that the IgG standard was ineffective. In a series of preliminary studies, oral fluid collected from normal human subjects was diluted at an average of 1:30 and found to still contain IgG at concentrations ≥0.5 μg/mL. In addition, volunteers rinsed their mouths with 5 mL to 10 mL of water just prior to oral fluid collection and the IgG concentrations in these samples averaged approximately 2.0 μg/mL.

4.2 Study Design and Results

Table 25 presents IgG concentrations from 100 oral fluid samples. These samples were randomly selected for analysis from those collected by spitting in the clinical studies. Note that the mean concentration is approximately 3 mcg/mL. If one assumes a normal distribution of IgG concentrations, two standard deviations from the mean would only go down to a concentration of 1.82 μg/mL—far in excess of the 0.5 μg/mL standard. Therefore, a subject would have to have an IgG concentration 4.28 standard deviations below the mean to fail the proposed oral fluid specimen validity criterion.

Table 26 shows the effect of in vitro dilution of oral fluid specimens on IgG concentration. These samples were randomly selected for analysis from those collected by spitting in the clinical studies and were tested by immunoassay for IgG concentration. These data verify those reported earlier, which show that IgG concentrations decreased as the dilution increased. However, even with a 1:10 dilution of the specimens, the mean concentration was still 1.55 standard deviations above the proposed validity standard. The 1:10 dilution resulted in only two specimens having an IgG concentration of 0.5 μg/mL, and none less than that concentration. A linear decrease in IgG concentration with corresponding increases in dilution was not observed. This was due to the lack of linearity of the immunoassay and saturation of the kit above 2 μg/mL to 2.5 μg/mL. Because the assay linearity plateaus above 2 μg/mL to 2.5 μg/mL, oral fluid IgG concentrations at, or above, that concentration are suspect and dilutions simply reduce the sample concentration into the dynamic range of the assay.

Table 26 shows the potential effect of in vivo dilution of oral fluid specimens on IgG concentration. Five subjects provided an oral fluid specimen by spitting. They then rinsed their mouths with 50 mL of tap water and provided a second specimen, again by spitting. They performed one additional 50 mL rinse and provided a third specimen. The specimens were then tested by immunoassay for IgG. The results verify the data presented earlier and those shown in tables 17 and 27, which indicated that concentrations decreased as a function of rinsing. However, even with 2 mL x 50 mL (total 100 mL) dilution of the oral fluids in the oral cavity, the mean concentration was still well above the 0.5 μg/mL proposed dilute specimen standard. The mean concentration after the two washes remained 2.61 standard deviations above the 0.5 μg/mL criterion.

4.3 Conclusions

From the data in tables 25 through 27 and those of the earlier study, it is apparent that having an oral fluid IgG concentration of ≥0.5 μg/mL does not ensure that the specimen is valid (has not been diluted). Therefore, this criterion does not guard against even the most obvious mechanism of subverting a drug test—diluting the specimen and thereby diluting its drug concentration to avoid detection. Table 26 shows that in vitro dilution of the specimen by up to 1:10 resulted in a mean IgG concentration still approximately 3 times the 0.5 μg/mL criterion. Table 27 shows that in vivo dilution of the specimen with multiple tap water rinses of the oral cavity only lowered the IgG concentration 25.5% and no specimens failed the ≥0.5 μg/mL criterion.

Additional research is needed to identify a chemical marker to ensure the validity of oral fluid specimens collected from donors. Based on the data presented, the current criterion is ineffective for use in the criminal justice system. In addition, the criterion neglects the clinical condition of the donor. For example, an immunocompromised donor might have difficulty passing the dilution criterion. This would also be true of a donor taking immunosuppressant medications. Further, a donor with a medical condition that increases IgG concentration could pass the criterion even after severely diluting his or her specimen.

Table 25. IgG concentrations (μg/mL)

Sample ID	Concentration	Sample ID	Concentration	Sample ID	Concentration	Sample ID	Concentration
100	3.16	126	3.20	152	3.09	178	2.35
101	3.49	127	3.27	153	3.26	179	3.31
102	2.94	128	3.10	154	3.27	180	3.48
103	2.94	129	2.21	155	2.34	181	2.86
104	2.89	130	2.55	156	3.02	182	3.18
105	3.18	131	2.80	157	2.35	183	2.06
106	2.53	132	3.23	158	2.89	184	3.08
107	2.96	133	2.18	159	3.35	185	3.37
108	2.85	134	2.63	160	3.16	186	3.4
109	3.09	135	2.72	161	3.40	187	3.65
110	3.35	136	3.47	162	3.02	188	3.62
111	2.70	137	4.00	163	1.85	189	2.76
112	3.87	138	2.88	164	2.77	190	2.72
113	3.55	139	2.90	165	3.47	191	3.27
114	2.96	140	1.52	166	3.14	192	3.59
115	3.41	141	0.99	167	3.54	193	3.92
116	1.96	142	1.99	168	3.09	194	2.81
117	3.74	143	1.56	169	3.32	195	2.13
118	2.98	144	3.19	170	3.21	196	3.06
119	3.65	145	2.90	171	3.46	197	3.46
120	3.18	146	2.50	172	2.75	198	3.56
121	3.60	147	1.49	173	2.56	199	3.33
122	3.11	148	3.17	174	2.58	200	2.59
123	4.06	149	3.56	175	3.20		
124	1.98	150	3.34	176	2.78	Mean	2.98
125	2.29	151	3.57	177	3.24	SD	0.58

Table 26. The effect of dilution on IgG concentration

Sample ID	Group A (no dilution) OD	Concentration	Group B (no dilution) OD	Concentration	Group C (1/5 dilution) OD	Concentration	Group C (1/10 dilution) OD	Concentration
123 A	1.463	3.3	1.352	3.0	1.256	2.7	1.042	2.1
121 A	1.133	2.4	1.134	2.4	0.975	1.9	0.877	1.7
119 A	1.347	2.9	1.155	2.4	1.038	2.1	0.890	1.7
115 A	1.472	3.3	1.255	2.7	1.035	2.1	0.817	1.5
113 A	1.333	2.9	1.227	2.6	1.140	2.4	0.888	1.7
110 A	1.249	2.7	0.950	1.9	0.734	1.3	0.442	0.5
101 A	1.079	2.2	1.123	2.3	0.836	1.6	0.607	1.0
126 A	1.155	2.4	1.114	2.3	1.078	2.2	0.989	2.0
120 A	1.426	3.2	1.252	2.7	0.937	1.8	0.898	1.7
105 A	1.230	2.6	0.804	1.5	0.603	0.9	0.432	0.5
Concentrations in µg/mL								
Mean		2.8		2.4		1.9		1.4

Table 27. The effect of rinsing on IgG concentration

	ID	OD	Concentration
Pre-Rinse	001A	1.583	2.77
	002A	1.892	3.43
	003A	1.458	2.50
	004A	1.591	2.79
	005A	1.478	2.54
	006A	1.215	1.98
Mean			2.67
Std Dev			0.47
% Decrease			
50 mL Rinse	001B	1.635	2.88
	002B	1.778	3.19
	003B	0.966	1.45
	004B	1.664	2.94
	005B	1.004	1.53
	006B	1.221	1.99
Mean			2.33
Std Dev			0.77
% Decrease initial			12.7%
100 mL Rinse	001C	1.545	2.69
	002C	1.708	3.04
	003C	1.079*	1.52
	004C	1.667	2.95
	005C	1.341*	2.11
	006C	1.36	2.29
Mean			1.99
Std Dev			0.57
% Decrease			25.5%
Concentration in μg/mL			

*Analyzed on a different calibration curve.

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