The Effects of Collection Methods On Oral Fluid Codeine Concentrations

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Abstract

The use of a variety of alternative biological specimens such as oral fluid for the detection and quantitation of drugs has recently been the focus of considerable scientific research and evaluation. A disadvantage of drug testing using alternative specimens is the lack of scientific literature describing the collection and analyses of these specimens and the limited literature about the pharmacokinetics and disposition of drugs in the specimen. Common methods of oral fluid collection are spitting, draining, suction, and collection on various types of absorbent swabs. The effect(s) of collection techniques on the resultant oral fluid drug concentration has not been thoroughly evaluated. Reported is a controlled clinical study (using codeine) that was designed to determine the effects of five collection techniques and devices on oral fluid codeine concentrations. The collection techniques were control (spitting), acidic stimulation, nonacidic stimulation, and use of either the Salivette™ or the Finger Collector (containing Accu-Sorb™) oral fluid collection devices. Preliminary data were collected from two subjects using the Orasure® device. The in vitro drug recovery was also evaluated for the Salivette and the Finger Collector devices. With the exception of a single time point, codeine concentrations in specimens collected by the control method (spitting) were consistently higher than concentrations in specimens collected by the other methods. The control collection concentrations averaged 3.6 times higher than concentrations in specimens collected by acidic stimulation and 1.3 to 2.0 higher than concentrations in specimens collected by nonacidic stimulation or collection using either the Salivette or the Finger Collector devices. When calculated using oral fluid codeine concentrations from the clinical study, the elimination rate constant, t½, AUC and the peak oral fluid concentrations demonstrated device differences. The slope of the elimination curve for codeine using the acidic collection method exceeded that of the other four methods. As a result, the t½ for the acidic method was significantly less than that of the control method (1.8 vs. 3.0 h, respectively). Oral contamination contributed to the control method having higher AUC than that calculated using the other methods. There was considerable variation in peak codeine concentrations between devices and between individuals within each collection method. When samples were collected simultaneously with the Salivette and the Finger Collector, the mean codeine concentrations were similar. We were able to recover ≥ 500 µL of oral fluid from 81.8% of the clinical samples collected with the Salivette. However, we were able to recover this volume from only 25.5% of the samples collected with the Finger Collector. In addition, the in vitro drug recoveries were lower using the Finger Collector. When oral fluid was collected nearly simultaneously by the control method and by use of the Salivette, mean control codeine concentrations were 2.3 times higher, but the duration of detection was similar for both methods.

Introduction

The use of alternative biological specimens for the detection and quantitation of drugs has recently been the focus of substantial scientific attention and research (1). Oral fluid is a biological specimen with potential for use in drug testing and has several advantages as a testing specimen. Oral fluid can be harvested by simple collection protocols that are noninvasive. Oral fluid can be collected under direct observation, eliminating concerns about sample adulteration and ensuring sample identity. Oral fluid is a blood filtrate and relatively free of blood constituents; therefore, it is easily processed for testing by conventional drug screening and confirmation methods.

Unfortunately, a major disadvantage of oral fluid as a drug testing specimen is the lack of scientific knowledge of the pharmacokinetics and disposition of drugs in oral fluid and the effects of different collection methods on drug concentrations. A variety of methods and devices are available for oral fluid collection. The four most common methods of oral fluid collection are “spitting,” draining, suction, and swab (2). The draining and spitting collection methods target collection of nonstimulated oral fluid, whereas the suction and swab methods involve stimulating oral fluid production. The spitting method may also involve oral fluid stimulation if paraffin wax, Teflon™, rubber bands, or chewing gum are placed in the mouth prior to oral fluid collection. Lemon drops or citric acid crystals can also be placed in the mouth prior to collection to provide a gustatory stimulus for oral fluid production (2-4). Oral fluid collected by stimulation may differ in composition from oral fluid collected by spitting because of changes in oral fluid flow rates. As the oral fluid flow rate increases, the concentration of bicarbonate ions increases. Therefore, oral fluid pH

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will increase, and this may affect the oral fluid drug concentration in a pH-dependent manner.

The effect of collection methods and materials on drug concentrations are not well described in the scientific literature. Kato and colleagues (5) reported that cocaine and metabolite concentrations in oral fluid were highly dependent on pH and the manner in which the oral fluid was collected. The authors observed that oral fluid cocaine, benzoylegonine (BE), and ecgonine methyl ester (EME) concentrations were substantially greater in nonstimulated oral fluid than in oral fluid stimulated with citric acid. The potential effects of other collection methods on oral fluid cocaine concentrations or other drug concentrations are not known. Reported is a controlled clinical study designed to determine the effects of selected collection methods and collection devices on oral fluid codeine concentrations and evaluate these methods for ease of collection, donor comfort, and in vitro drug recovery.

Materials and Methods

Chemicals and reagents

Codeine, codeine-d₃, morphine, and morphine-d₃ reference solutions (1 mg/mL in methanol) were obtained from Radian Corp. (Austin, TX). Codeine phosphate and morphine monohydrate were obtained from Alltech-Applied Science (State College, PA). The Pharmacy Department of the University of Utah Health Sciences Center prepared codeine phosphate oral liquid (30 mg/10 mL) for administration to human subjects. Clean Screen® ZSDAU020 extraction columns were purchased from United Chemical Technologies, Inc. (Bristol, PA). The Salivette was purchased from Sarstedt (Newton, NC); the Orasure was obtained from STC Technologies (Bethlehem, PA); and the Finger Collector (Accu-Sorb) was obtained from Avitar Technologies, Inc. (Canton, MA). Trifluoroacetic anhydride (TFAA) was obtained from Pierce Chemical (Rockford, IL); methanol and methylene chloride were obtained from Burdick & Jackson (Muskegon, MI); ultra-high-purity helium was obtained from Mountain Airgas (Salt Lake City, UT). All other chemicals were reagent grade and were obtained from Mallinckrodt Chemical Works (St. Louis, MO). All drug solutions were prepared in gas chromatography–mass spectrometry (GC–MS)-grade methanol or distilled water as indicated.

Human subjects and study protocol

Human subjects were recruited by advertisement and by word-of-mouth at the University of Utah Health Sciences Center to participate in a study approved by the Institutional Review Board. Subjects were required to sign informed consent and be drug-free prior to entering the study. To ensure that the subjects were drug-free, urinalysis drug tests were performed for the following drugs: amphetamines, opiates, BE, cocaine, 11-carboxy-D₉-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 six months or if they had a history of acute or chronic illnesses.

The subjects were admitted to the Clinical Research Center at the University of Utah Health Sciences Center on the evening prior to 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. After administration, the subjects brushed their teeth with toothpaste before any oral fluid was collected. Oral fluid was collected at the following times: predose and 15, 30, and 60 min and 2, 4, 6, 8, 10, 12, and 24 h after drug administration. Subjects were placed into experimental groups depending on the method of oral fluid collection for each subject. For the control group (nonstimulated oral fluid), oral fluid was collected by having the subjects “spit” into 5-mL inert polyethylene tubes (n = 22). 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 1–2 min prior to spitting into inert tubes. For the next five subjects, two devices, the Salivette and the Finger Collector, were used simultaneously to collect stimulated oral fluid specimens. The devices were placed between the cheek and gum (one on each side of the subject’s mouth) for 5 min and then removed. Specimens collected with the Salivette were centrifuged in the conical storage tubes to remove the oral fluid from the cotton roll. Oral fluid was removed from the Finger Collector by vigorously “milking” the Accu-Sorb foam collectors. 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 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 1–2 min), the subject then placed a Salivette between their cheek and gum for 5 min. All specimens were then processed and stored as described.

A similar procedure was followed for the two additional subjects, except an Orasure device was placed between the subject’s cheek and gum for 5 min instead of the Salivette. The oral fluid was removed from the Orasure device by centrifugation, collected in the buffered storage tube, and stored at -20°C until analysis.

Sample preparation and analysis

Drug-free oral fluid was collected from healthy volunteers using the control method, pooled, and stored at -4°C until use. Aliquots (0.5 mL) of calibrators, controls, and samples were extracted by solid phase, derivatized with TFAA, and analyzed on a Finnigan-Mat 4500 GC–MS (Finnigan-Mat, San Jose, CA) using positive-ion chemical ionization as described in a previous method (6). The assay LOQ and LOD were 5 ng/mL and 1 ng/mL, respectively.

In vitro recovery study

Morphine and codeine were added to drug-free oral fluid in the following concentrations: 0.0, 10.0, 25.0, 100.0, 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. The recovery of codeine and morphine from each device was determined by comparison to oral fluid specimens of the same concentration.
Pharmacokinetic analysis

The areas under the pharmacokinetic curves (AUC) were computed for the interval 0–24 h by the trapezoidal rule (7). The elimination rate constant (k) was estimated by linear regression of the oral fluid concentration data points after 2 h. The terminal half-life (t₁/₂) was estimated from 0.693/k.

Results

All predose oral fluid samples were codeine free. The time course of codeine elimination from oral fluid for subjects using each of the five collection methods is shown in Figure 1. Because contamination from the oral codeine dose has been shown to produce elevated oral fluid codeine concentrations in the first 1 to 2 h after administration (6), only mean concentrations from 2 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 codeine concentrations detected in the 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.

Differences in the duration of the detection time of codeine were also observed for the five oral fluid collection methods (Table I). Because oral contamination appeared to be a significant problem for the 15 and 30 min controlled collections, the 0.25 h time point was excluded when the average concentration ratio (control/alternate) was calculated for each subject group. In the control group, codeine was detected in all of the 12-h specimens, and only 3 of 22 specimens had codeine 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) and two (40%) positive specimens at 24 h. Using the acidic oral fluid stimulation method, no specimens collected at 24 h contained codeine, and 4 of 5 specimens collected at 12 h were also negative.

Pharmacokinetic parameters, including estimates for the elimination rate constant, t₁/₂, and AUC, were calculated from the codeine concentrations found using each collection methods. These data are shown in Table II. The table illustrates that substantially different pharmacokinetic parameters were obtained using the oral fluid codeine concentrations from the different collection methods. As illustrated in Figure 1, the slope of the elimi-
nation 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 substantially less than the \( t_{1/2} \) for the control method, 1.8 and 3.0 h, respectively. The \( t_{1/2} \) was also less than the \( t_{1/2} \) for the other methods, but the difference was less dramatic. Because of the higher codeine concentrations observed for the control method, especially within the first 30 min after drug administration, the control method AUC was substantially greater than the calculated AUC for the acidic and nonacidic collection methods. However, there was considerable interindividual variation in peak codeine concentrations observed between the collection methods and within each collection method group. For example, a concentration range of 82 to 1690 ng/mL was observed for acidic stimulation collection and 298 to 16,500 ng/mL for the control collection method.

The in vitro recovery studies suggested there might be a difference in codeine absorption between the Salivette and the Finger Collector. The percent recoveries of codeine and morphine were 8.3% and 6.8% less than control, respectively, for the Salivette and 46.7% and 39.1% less than control, respectively, for the Finger Collector at concentrations from 10 to 200 ng/mL (Figure 2). A 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.

Because oral fluid specimens were collected from the same subjects at the same time with the two devices, an in vivo comparison of the collection devices was also made. As illustrated in Figure 3 and Table I, 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, we were able to recover \( \geq 500 \mu L \) 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 \( \leq 300 \mu L \). We were unable to analyze 9 of the 55 clinical specimens collected with the Finger Collector (all from the same three subjects) because no oral fluid could be milked from the foam applicator and the sample volume for three of these specimens was \( \leq 50 \mu L \).

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 4. 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 the codeine concentrations in the oral fluid collected with the Salivette. The 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 the codeine concentrations in the oral fluid collected with the Salivette. Although a decreasing trend of 4.0 to 1.6 was observed through the experimental session (Table III). The first time point (0.25 h) was excluded when the average ratio was calculated for each subject group. Even though the
control codeine concentrations were typically more than twice the Salivette codeine 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 t1/2, for each group was similar (2.3 and 2.5 h), but the AUC for the control group was more than three times the AUC for the Salivette subject group.

Results from the experiment in which oral fluids were collected by the control method and almost simultaneously with the Orasure device are shown in Table IV. Differences in codeine concentrations for the two collection methods were also observed in this experiment. Because only two subjects were included in this experiment, data for each are shown instead of mean values. These data should be considered preliminary because only two subjects were tested, the control codeine concentration were considerably higher than in the previous experiment, and the ratio of control to device oral fluid concentrations was quite variable (Table IV). 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, and there was no way to determine the volume of oral fluid collected by device.

Discussion

Data from all experiments, except those from the control collection method, were from a limited number of subjects. The data also showed considerable intersubject variability and were for a single drug, codeine. Therefore, they should be verified with additional experiments before making definitive conclusions about the collection methods and devices. However, the data demonstrate that a number of factors need to be considered when using oral fluids as a drug testing specimen. Several fluids combine to constitute what is commonly referred to as “saliva,” “whole saliva,” or “oral fluids” (8). Oral fluids are excreted by the major salivary glands with some contribution from minor glands and gingival crevices, whereas saliva contains desquamated epithelial cells, food debris, bacteria, gingival crevicular fluid, leukocytes, and mucoprotein (2). The terms “saliva,” “whole saliva,” and “oral fluids” have frequently been used interchangeably in the literature (8). Because the terms have been imprecisely used, applying the previously published data to a current interpretation should be done with caution. Salivary pH is normally in the range of 6.2 and 7.4. However, stimulating saliva production may change its composition and affect the salivary pH. As the saliva flow rate increases, the concentration of bicarbonate increases. Consequently, saliva pH increases and affects the saliva drug concentration in a pH-dependent manner (9).

There are a variety of oral fluid collection methods and devices available that harvest either nonstimulated or stimulated oral fluid. A thorough understanding of the effects these methods and devices on oral fluid collection and subsequent drug concentration is critical in order to interpret testing results. For example, Kato et al. (5), reported oral fluid concentrations of cocaine, BE, and EME were substantially higher in nonstimulated oral fluid compared with concentrations 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, BE and EME, were 6.0 and 5.5, respectively. The authors concluded that cocaine and metabolite concentrations in saliva are highly dependent on pH (not measured in this study) and the manner in which the oral fluid is collected. Previously, we reported that a predictable relationship exists between oral fluid and plasma codeine concentrations for 2 to 12 h after oral administration (6). 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 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 we used the term “nonstimulated oral fluid” the act of spitting is usually a sufficient stimulus to produce a flow of approximately 0.5 mL/min. A mechanical stimulus such as chewing wax, parafilm, rubber bands, or (as used in this study) sugarless gum stimulates a flow of approximately 1 to 3 mL/min (8). Citric acid stimulation may produce flows of from 5 to 10 mL/min (9). The Salivette and the Finger Collector devices collect oral fluid through use of an absorbent material (10,11). This method also results in some stimulation of oral fluid production (2). It is important to note that subjects in this study were instructed not to chew either device to stimulate saliva production. 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 (mechanical stimulus) 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 and Salivette specimens from four subjects allowed for a direct comparison of these two collection methods (Table III). The concentration differences observed in these subjects were in general agreement with the results shown in Table I. However, the mean control concentrations and the average ratio of control/Salivette (C/S) 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 and 5290 ng/mL. Because of the small number of subjects in the control group, results from this subject had an appreciable effect on the mean concentrations, C/S ratio, and the AUC.

The Salivette and the Finger Collector devices were compared in vitro and in vivo experiments. 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. We were able to recover ≥300 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 ≤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 screening and GC–MS methods with less sensitivity.

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 (12). However, if it is left in the mouth for the required 2 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 a 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).

Of the six 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. The subjects indicated that the Salivette produced a dry sensation in their mouth, and the Finger Collector gave them a very moist feeling. Neither feeling was described by the subjects as being unpleasant. Subjects also indicated that the Orasure device left a salty aftertaste. This was attributed to the buffer salts on the cotton pad.

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 the device were not required. Removal of oral fluid from the Finger Collector was often difficult and not readily performed by simply “milking” the

| Table IV. Codeine Concentrations in Oral Fluid Collected by the Control Method and the Orasure Device with Corresponding Ratios of Control/Orasure Concentrations* |
|-----------------|-----------------|-------|-----------------|-----------------|-------|
| Time (h) | Subject 1 | | Subject 2 | | |
| | Orasure | Control | Ratio† | Orasure | Control | Ratio† |
| 0.25 | 668 | 8410 | 12.6 | 342 | 5440 | 15.9 |
| 0.5 | 295 | 3070 | 10.4 | 223 | 2970 | 13.3 |
| 1 | 256 | 607 | 2.4 | 127 | 493 | 3.9 |
| 2 | 107 | 202 | 1.9 | 105 | 229 | 2.2 |
| 4 | 36 | 137 | 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 | — |

* n = 2
† Ratio = Control concentration/Orasure concentration.
Accu-Sorb material in the device between the thumb and forefinger as suggested by the manufacturer. This difficulty may have contributed to the low sample volumes recovered in this study.

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