Plasma and Oral Fluid Pharmacokinetics and Pharmacodynamics after Oral Codeine Administration

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Background: The ease, noninvasiveness, and safety of oral fluid collection have increased the use of this alternative matrix for drugs-of-abuse testing; however, few controlled drug administration data are available to aid in the interpretation of oral fluid results.

Methods: Single oral codeine doses (60 and 120 mg/70 kg) were administered to 19 volunteers. Oral fluid and plasma were analyzed for free codeine, norcodeine, morphine, and normorphine by solid-phase extraction combined with gas chromatography–mass spectrometry (SPE/GC-MS). Physiologic and subjective effects were examined.

Results: Mean (SE) peak codeine concentrations were 214.2 ± 27.6 and 474.3 ± 77.0 μg/L in plasma and 638.4 ± 64.4 and 1599.3 ± 241.0 μg/L in oral fluid. The oral fluid-to-plasma ratio for codeine was relatively constant (±4) from 1 to 12 h. The mean half-life ($t_{1/2}$) of codeine was 2.2 ± 0.10 h in plasma and 2.2 ± 0.16 h in oral fluid. Significant dose-related miosis and increases in sedation, psychotomimetic effect, and “high” occurred after the high dose. Mean codeine oral fluid detection time was 21 h with a 2.5 μg/L cutoff, longer than that of plasma (12–16 h). Detection times with the proposed Substance Abuse and Mental Health Services Administration cutoff (40 μg/L) were only 7 h. Norcodeine, but not morphine or normorphine, was quantified in both plasma and oral fluid.

Conclusions: The disposition of codeine over time was similar in plasma and oral fluid, but because of high variability, oral fluid codeine concentrations did not reliably predict concurrent plasma concentrations. Oral fluid testing is a useful alternative matrix for monitoring codeine exposure with a detection window of 7–21 h for single doses, depending on cutoff concentrations. These controlled drug administration data should aid in the interpretation of oral fluid codeine results.

Traditional matrices for drugs-of-abuse testing are urine and plasma, but interest in oral fluid monitoring has increased because of the ease and noninvasiveness of specimen collection. Disadvantages of urine testing include concentration variations attributable to changing fluid intake and a greater possibility for adulteration or substitution. Oral fluid collection can be readily observed without loss of privacy, reducing or eliminating the risk of an invalid specimen. Although plasma specimens provide an estimate of the actual circulating concentrations of analytes, blood collection is invasive, causes discomfort, and exposes the individual to the risk of infection. Monitoring of oral fluid may be especially advantageous and important when multiple serial samples are needed or when drug concentrations in children are required.

Earlier studies demonstrated that many therapeutic drugs are transferred rapidly from plasma to saliva by drug transport across biological membranes (1–6). Drug entry into oral fluid is dependent on the physicochemical characteristics of the drug molecule (lipid solubility, degree of ionization, molecular size) and the membrane. It is generally presumed that the drug concentration in oral fluid corresponds to the concentration of free or unbound drug in the plasma, the physiologically active drug component. Lipid-soluble basic drugs that are not extensively bound to plasma proteins preferentially partition into oral fluid.

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fluid. In addition, oral fluid (pH 6.5–7.2) is generally more acidic than plasma; consequently, basic drugs will partition in oral fluid at higher concentrations than in plasma (7, 8).

Oral fluid testing has been used for therapeutic drug monitoring of phenytoin, antipyrine, primidone, ethosuximide, and theophylline concentrations (9–14). Oral fluid testing has also been developed for drugs of abuse, including amphetamines, barbiturates, cannabinoids, cocaine, diazepam, lysergic acid diethylamide, opioids, and phencyclidine (15–17). Therefore, oral fluid may be a viable alternative testing matrix to plasma for drug treatment, workplace drug testing, child custody cases, driving under the influence, and criminal investigations.

Nonstimulated oral fluid samples can be collected by expectoration or draining oral fluid into a tube. Stimulated oral fluid samples have been conventionally obtained by chewing Parafilm wax or Teflon or through exposure of the oral cavity to citric acid candy or crystals. In addition, several oral fluid collection devices and techniques, such as Salivette®, OraSure®, and the Oral-Diffusion-Sink device, have been developed to facilitate and standardize oral fluid sampling. The advantages and disadvantages of several of these new devices that utilize absorbent cotton swabs, rolls, or salt solutions to collect oral fluid have been reviewed recently (18, 19).

Codeine is an opiate commonly prescribed for pain relief and for cough suppression. Although widely used for therapy, codeine is also abused for its euphoric and depressant effects and to prevent opiate withdrawal (20–23). Because of increasing abuse and potential performance-impairing effects, codeine is included in workplace and military drug testing programs. Recently, the Substance Abuse and Mental Health Services Administration (SAMHSA)4 suggested a 40 μg/L screening and confirmation cutoff concentration for federally mandated testing for codeine in oral fluid.

Although several studies have addressed the disposition of codeine in plasma and oral fluid after single or multiple doses (7, 24–28), there remain many questions on codeine oral fluid pharmacokinetics and on the correlation of drug concentration to concurrent physiologic and behavioral effects. This controlled clinical drug administration study evaluates these relationships and determines the sensitivity and detection time of codeine in oral fluid.

Materials and Methods

HUMAN PARTICIPANTS

Twelve male and 7 female healthy volunteers participated in the controlled drug administration protocol. Of the 19 participants, 14 were African American, 3 were Caucasian, and 2 were Hispanic. Their mean (SE) age was 34.8 ± 1.1 years (range, 23–43 years) with a mean (SE) weight of 78.0 ± 3.4 kg (range, 56.6–106.5 kg). The protocol was approved by the National Institute on Drug Abuse Institutional Review Board. Participants provided written informed consent and were paid for their participation. Screening procedures included a comprehensive physical and psychologic examination. Participants reported a history of cocaine and opioid use, verified by a positive urine test before admission, and were not physically dependent on drugs or medications, with the possible exception of nicotine and caffeine. During the 10-week study, all participants resided on the closed research unit of the Intramural Research Program, National Institute on Drug Abuse.

DRUG ADMINISTRATION

Codeine sulfate for oral human administration was obtained from Roxane Laboratories and was prepared in polished lactose capsules (Amend Drug and Chemical Co.). Participants were admitted to the research unit 20 days before the first scheduled dose to permit previously self-administered drugs to be cleared from the body. Three low doses (60 mg/70 kg) and three high doses (120 mg/70 kg) of codeine sulfate were taken within 7 days (minimum separation of 48 h) in weeks 4 and 8, respectively.

PLASMA AND ORAL FLUID SPECIMENS

Whole-blood samples were withdrawn with a syringe through an indwelling catheter in the antecubital vein and collected in Vacutainer Tubes containing sodium fluoride and acetic acid. Standard green top (sodium lithium) Vacutainer Tubes were supplemented with 25 μL of saturated sodium fluoride and 25 μL of 100 g/L acetic acid per milliliter of blood to improve the stability of cocaine in blood. These specimens were analyzed for cocaine and metabolites in addition to the opiate analytes required for this study. Specimens were placed on ice and centrifuged (2060g for 10 min) to separate the plasma. Plasma was transferred to polypropylene cryotubes and frozen at −20 °C until analysis. Specimens were collected at the following times: predose, 0.08, 0.17, 0.25, 0.50, 1, 2, 4, 8, 12, 24, and 48 h after the first drug administration. Citric acid candy-stimulated oral fluid specimens were obtained at the same time points after the first codeine dose. Oral fluid was also collected with citric acid candy stimulation at the same time points after the second and third doses in four participants to study intrasubject variability (group I). In a second group of four participants (group II), three oral fluid collection methods were used to study drug concentrations with different collect-

4 Nonstandard abbreviations: SAMHSA, Substance Abuse and Mental Health Services Administration; BSTFA, N,O-bis(trimethyl)trifluoroacetamide; TMCS, trimethylchlorosilane; MTBSTFA, N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide; TBDMS, tert-butyldimethylchlorosilane; SPE, solid-phase extraction; GC-MS, gas chromatography–mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; S/P, oral fluid/plasma; Cmax, peak concentration; tmax, time of peak concentration; t1/2, half-life; AUC, area under the curve; ARCI, Addiction Research Center Inventory; SDQ, Single Dose Questionnaire; VAS, Visual Analog Scales; PCAG, Phenobarbital-Chlorpromazine-Alcohol group; and LSD, lysergic acid diethylamide.
tion methods. Oral fluid was collected with citric acid candy stimulation after the first dose, with the Salivette citric acid-treated cotton swab after the second dose, and with the Salivette neutral cotton swab after the third dose. Specimens collected with the Salivette devices were centrifuged in conical tubes to release oral fluid from the cotton. Subsequently, oral fluid was transferred to polypropylene cryotubes and frozen at −20 °C until analysis.

**CHEMICALS AND REAGENTS**

Chemicals were obtained from the following sources: codeine·PO₄, [²H₃]codeine hydrochloride·2 H₂O, norcodeine hydrochloride·3 H₂O, morphine sulfate, [²H₃]morphine hydrochloride·3 H₂O, and normorphine hydrochloride·H₂O were from Sigma Chemicals; N,O-bis(trimethyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) with 1% tert-butyltrimethylchlorosilane (TBDMS) were from Pierce Chemical. Deuterated codeine was used as the internal standard for codeine and norcodeine, and deuterated morphine was used as the internal standard for morphine and normorphine. Solid-phase extraction (SPE) columns (Clean Screen ZSDAU020) and filtration columns (RFF02F4P) were obtained from United Chemical Technologies. Solvents were HPLC grade and purchased from the following sources: methylene chloride, 2-propanol, and acetonitrile were from Mallinckrodt Baker Inc.; and methanol was from Fisher Scientific. Sodium acetate, acetic acid, ammonium hydroxide, and hydrochloric acid were A.C.S. reagent grade and were obtained from the following sources: methylene chloride, 2-propanol, and linearity (r ≥ 0.98) for the four analytes.

**GC-MS analysis was performed on a Hewlett-Packard 5890A gas chromatograph interfaced with a Hewlett-Packard 5972 mass-selective detector or a Hewlett-Packard 6890 gas chromatograph interfaced with a Hewlett-Packard 5973 mass-selective detector. The instruments were operated in the splitless mode. Separation of analytes was achieved by HP-1 [12 m × 0.2 mm (i.d.); 0.33-μm film thickness] or Phenomenex ZBI fused-silica capillary column [15 m × 0.2 mm (i.d.); 0.25-μm film thickness] with helium as carrier gas at a flow rate of 1.0 mL/min. The initial column temperature of 70 °C was held for 1.5 min, followed by increases to 175 °C at 30 °C/min, to 250 °C at 23 °C/min, and to 325 °C at 18 °C/min with a 1.0-min final hold time. The mass spectrometer was operated in the electron impact mode with a dwell time of 10 ms/πon. Three ions for each analyte were monitored. The ions for each compound were (quantitative ion indicated in parentheses): [²H₃]codeine, m/z (374), 234, 178; codeine, m/z (371), 234, 178; norcodeine, m/z (429), 254, 292; [²H₃]morphine, m/z (417), 474, 281; morphine, m/z (414), 471, 278; and normorphine, m/z (472), 529, 350. Ion ratios of quality-control samples and participant samples were required to be within ± 20% of those observed for the 10- and 100-ng calibrators for the low and high calibration curves, respectively. The upper limit of linearity for the high calibration curve was 500 μg/L; specimens that exceeded 500 μg/L were diluted with blank oral fluid or plasma and reextracted and analyzed.
The LOD of the method was defined as the lowest concentration at which signal-to-noise ratios of the identifying ions (determined by peak height) were $>3:1$, chromatography and retention time were acceptable, and three of four replicates at specified concentrations had ion ratios within $\pm 20\%$ of those observed for the 10-ng calibrator. The limit of quantification (LOQ) was the lowest concentration that met LOD criteria plus the requirement that three of four replicates quantify within $\pm 20\%$ of the target concentration. The LOD and LOQ of the method were 2.5 $\mu$g/L for all analytes. Interassay precision and accuracy data for codeine and its metabolites were determined for three concentrations (12.5, 25, and 250 $\mu$g/L) in plasma and oral fluid. The interassay CVs for plasma ($n = 10$ runs; 2–3 replicates per run) were as follows: codeine, 13%, 13%, and 7.8%, respectively; norcodeine 13%, 13%, and 13%; morphine 13%, 8.7%, and 13%; normorphine 8.0%, 13%, and 15%. Interassay CVs were as follows for oral fluid: codeine, 8.7%, 7.0%, and 5.1%; norcodeine, 23%, 30%, and 10%; morphine, 11%, 7.7%, and 16%; and normorphine, 29%, 25%, and 18%.

**ORAL FLUID:PLASMA (S/P) AND NORCODEINE:CODEINE (NCOD:COD) RATIOS**

The S/P ratio was calculated by dividing the oral fluid drug concentration by the plasma drug concentration for specimens collected at the same time after codeine administration. NCOD:COD ratios were obtained by dividing norcodeine by codeine concentrations and multiplying by 100.

**PHARMACOKINETIC ANALYSIS**

Peak plasma and oral fluid concentrations ($c_{\text{max}}$) and the time of peak concentration ($t_{\text{max}}$) were obtained directly from the concentration–time data. Elimination half-lives ($t_{1/2}$) were calculated as $t_{1/2} = \ln2/\lambda$, where the elimination rate constant ($\lambda$) was calculated from the slope of the terminal portion of the semilogarithmic concentration–time curve by linear regression analysis. The areas under the plasma or oral fluid concentration–time curves (AUCs) were calculated by the linear trapezoidal rule for 24 h after drug administration. Plasma and oral fluid pharmacokinetic parameters were derived by noncomparative methods with the use of WinNonlin Professional software (Ver. 3.2; Pharsight Co.). Uniform weighting for all data points was used throughout the analysis.

**DATA ANALYSIS**

Data are represented as mean $\pm$ SE. Statistical analysis was performed by SPSS software (Ver. 10.0; SPSS Inc.). For plasma and oral fluid drug concentrations, multivariate ANOVA was used for statistical analysis of the data. For physiologic and subjective effect data, repeated-measures ANOVA was used. Factors were dose (60 and 120 mg/70 kg of codeine) and time (hours after drug administration). Statistical significance was assumed when $P$ was $\leq 0.05$. Separate paired t-tests or post hoc t-tests were performed between placebo and effects at each codeine dose when dose and dose $\times$ time effects were significant.

**PHYSIOLOGIC AND BEHAVIORAL MEASUREMENTS**

Participants underwent physiologic measurements before and 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 8, and 12 h after placebo and oral codeine administration. Physiologic measurements included pupil size, systolic and diastolic blood pressure, pulse, skin temperature, respiration, blood oxygenation, and core temperature. Each participant also completed self-rating subjective scales before and 0.08, 0.17, 0.25, 0.5, 1, 2, 4, and 8 h after dosing. The Addiction Research Center Inventory (ARCI) (30), Single Dose Questionnaire (SDQ) (31), and Visual Analog Scales (VAS) were used to assess subjective effects. ARCI self-rating subjective subscales consisted of the Morphine-Benzedrine group, Phenobarbital-Chlorpromazine-Alcohol group (PCAG), and Lysergic Acid Diethylamide (LSD) and the Opiate Withdrawal Subscale. The SDQ, modified to provide only subject ratings, consisted of 14 items that were rated on a scale from 0 (no) to 1 (yes) including “feel drug”, “normal”, “skin itchy”, “relaxed”, “coasting”, “nodding”, “high”, “sleepy”, “drunkken”, “nervous”, “drive”, “soap box”, “turning stomach”, and “pleasant sick”. VAS assessed the following effects: “stimulated”, “high”, “anxious”, “sedated”, “down”, and “hungry”. Participants marked a line on a scale ranging from 0 mm (not at all) to 100 mm (extremely) to indicate how they felt at that moment.

**Results**

**CODEINE AND METABOLITE CONCENTRATIONS IN PLASMA**

Codeine was the primary analyte detected in plasma. Mean codeine plasma concentrations-vs-time curves ($n = 16$ for low dose; $n = 14$ for high dose) are illustrated in Fig. 1A. All specimens collected before drug administration were negative for codeine and metabolites. Plasma data were not available for 3 of the 19 participants because of phlebotomy problems. Codeine was initially detected between 0.17 and 1 h, with a mean of 0.5 $\pm$ 0.07 h for the low dose and 0.4 $\pm$ 0.04 h for the high dose. Plasma codeine concentrations peaked between 0.5 and 4 h at a mean of 1.3 $\pm$ 0.22 h and 1.2 $\pm$ 0.18 h for the low and high doses, respectively. The mean plasma codeine $c_{\text{max}}$ was 214.2 $\pm$ 27.6 $\mu$g/L (range, 66.1–413.2) after the low dose and 474.3 $\pm$ 77.0 $\mu$g/L (range, 184.0–1158.1) after the high dose. Plasma codeine concentration was significantly related to dose from 0.5 to 8 h ($P < 0.05$). The mean pharmacokinetic parameters for codeine are shown in Table 1. The mean $t_{1/2}$ of codeine in plasma ($n = 14$) was 2.1 $\pm$ 0.08 h (range, 1.5–2.7 h), and 2.4 $\pm$ 0.18 h (range, 1.4–3.5 h) for low and high doses, respectively. The AUCs for codeine were 734 $\pm$ 66 and 1800 $\pm$ 222 h $\mu$g/L for the 60 and 120 mg/70 kg doses, respectively, also demonstrating a significant dose–concentration relationship ($P = 0.001$).
Morphine and normorphine were not detected in any specimen based on the method LOQ of 2.5 μg/L; however, norcodeine was detected in the plasma after all drug doses except for one participant after the low codeine dose. Mean norcodeine plasma concentrations vs time after oral administration of 60 and 120 mg/70 kg codeine sulfate are illustrated in Fig. 1B. Norcodeine was initially detected from 0.17 and 2 h after codeine administration at a mean of 0.7 ± 0.14 h for the low dose and 0.6 ± 0.08 h for the high dose, slightly later than initial detection of the parent drug. Plasma norcodeine concentrations peaked between 0.5 and 4 h, with a mean peak time of 1.4 ± 0.26 h after the low dose and 1.5 ± 0.17 h after the high dose. The mean $c_{\text{max}}$ for norcodeine was 12.2 ± 1.5 μg/L (range, 4.2–26.1) and 33.4 ± 4.2 μg/L (range, 9.2–63.0) for low and high doses, respectively. There was a significant nonlinear dose–concentration relationship for mean norcodeine in plasma between 0.5 and 8 h ($P < 0.05$). The mean pharmacokinetic parameters for norcodeine in plasma and oral fluid are shown in Table 1. The mean $t_{1/2}$ for norcodeine in plasma was 4.3 ± 0.61 h (range, 2.0–8.6 h) for the low dose and 4.3 ± 0.45 h (range, 2.7–6.9 h) for the high dose; two to three times longer than that of codeine. The mean AUCs for norcodeine in plasma were 84 ± 16 and 232 ± 44 h·μg/L for the low and high doses, respectively, and a significant dose–AUC relationship in plasma was observed ($P < 0.001$). NCOD:COD ratios increased over time during elimination (Fig. 2), indicating a longer $t_{1/2}$ for the metabolite compared with the parent drug. NCOD:COD ratios ranged from 2% to 46% between 0.5 and 8 h after codeine ingestion.

**CODEINE AND METABOLITES IN ORAL FLUID**

The time course of codeine concentration in oral fluid was similar to that seen in plasma (Fig. 1A). Generally, codeine concentrations were higher in oral fluid than in plasma ($P < 0.05$ for both doses from 0.5 to 8 h). Codeine was initially detected in oral fluid between 0.08 and 1.0 h with a mean of 0.5 ± 0.05 h for the low dose and 0.4 ± 0.05 h for the high dose, comparable to initial detection in plasma. Oral fluid concentrations peaked at 0.5–4 h with a mean of 1.7 ± 0.23 h and 1.6 ± 0.14 h with concentrations of 638.6 ± 64.4 μg/L (range, 184.0–1288.8 μg/L) and 1599.3 ± 241.0 μg/L (range, 619.6–3350.2 μg/L) after the 60 and 120 mg/70 kg doses, respectively. There were significant dose–concentration relationships for codeine.

**Table 1. Mean pharmacokinetic parameters for codeine and norcodeine in plasma and oral fluid.**

<table>
<thead>
<tr>
<th></th>
<th>Dose, mg/70 kg</th>
<th>n</th>
<th>$c_{\text{max}}$a μg/L</th>
<th>$t_{\text{max}}$a h</th>
<th>$t_{1/2}$a h</th>
<th>AUC$_{24}$a,b μg·h/L</th>
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<tr>
<td>Codeine</td>
<td>Plasma</td>
<td>60</td>
<td>214.2 ± 27.6</td>
<td>1.3 ± 0.22</td>
<td>2.1 ± 0.08</td>
<td>734 ± 66</td>
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<td></td>
<td></td>
<td>120</td>
<td>474.3 ± 77.0</td>
<td>1.2 ± 0.18</td>
<td>2.4 ± 0.18</td>
<td>1800 ± 222</td>
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<td></td>
<td>Oral fluid</td>
<td>60</td>
<td>638.6 ± 64.4</td>
<td>1.7 ± 0.23</td>
<td>2.5 ± 0.21</td>
<td>2612 ± 408</td>
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<td></td>
<td></td>
<td>120</td>
<td>1599.3 ± 241.0</td>
<td>1.6 ± 0.14</td>
<td>1.8 ± 0.19</td>
<td>5827 ± 766</td>
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<tr>
<td>S/P ratio</td>
<td>60</td>
<td>14</td>
<td>3.4 ± 0.6</td>
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<td>3.4 ± 0.6</td>
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<td></td>
<td></td>
<td>120</td>
<td>4.1 ± 0.8</td>
<td></td>
<td></td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>Norcodeine</td>
<td>Plasma</td>
<td>60</td>
<td>12.2 ± 1.5</td>
<td>1.4 ± 0.26</td>
<td>4.3 ± 0.61</td>
<td>84 ± 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>33.4 ± 4.2</td>
<td>1.5 ± 0.17</td>
<td>4.3 ± 0.45</td>
<td>232 ± 34</td>
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<tr>
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<td>Oral fluid</td>
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<td>2.1 ± 0.31</td>
<td>7.9 ± 2.17</td>
<td>144 ± 40</td>
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<td>120</td>
<td>46.7 ± 17.0</td>
<td>2.4 ± 1.19</td>
<td>4.6 ± 1.11</td>
<td>279 ± 97</td>
</tr>
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</table>

*a Data are mean ± SE.

*b AUC$_{24}$, AUC for 24 h.
in oral fluid collected from 0.5 to 4 h after oral administration \((P < 0.05)\). The mean \(t_{1/2}\) of codeine in oral fluid was 2.5 ± 0.21 h (range, 1.0–3.8 h) for the low dose and 1.8 ± 0.19 h (range, 1.2–3.1 h) for the high dose. The AUCs for codeine in oral fluid were 2612 ± 408 and 5827 ± 766 h·µg/L for the low and high doses, respectively, demonstrating a significant dose–AUC relationship for codeine in oral fluid \((P < 0.001)\).

Morphine and normorphine were not detected in oral fluid at the LOQ of 2.5 µg/L in any specimen. Norcodeine was not detected in oral fluid in 2 of 19 participants after the low dose of codeine despite paired plasma sample concentrations of 3.9–12.5 µg/L. Mean initial detection times for norcodeine in oral fluid were 1.0 ± 0.13 h after administration of the low dose and 0.8 ± 0.12 h after administration of the high dose. The mean norcodeine \(t_{\text{max}}\) in oral fluid occurred later than the plasma \(t_{\text{max}}\), and was 2.1 ± 0.31 and 2.4 ± 0.33 h after administration of the low and high doses, respectively. The mean norcodeine \(c_{\text{max}}\) was 17.1 ± 3.1 µg/L (range, 3.9–58 µg/L) after the 60 mg/70 kg dose and 46.7 ± 17.0 µg/L (range, 10.3–191.2 µg/L) after the 120 mg/70 kg dose of codeine sulfate. The mean disposition profile of norcodeine in oral fluid followed a time course similar to that in plasma (Fig. 1B). The mean \(t_{1/2}\) of norcodeine in oral fluid was 7.9 ± 2.17 h for the low dose (range, 1.6–30.9 h) and 4.6 ± 1.11 h for the high dose (range, 2.1–9.5 h) and was longer than that of codeine. The AUCs for norcodeine in oral fluid were 144 ± 40 and 279 ± 97 h·µg/L for the low and high doses, respectively. The NCOD:COD ratio in oral fluid increased over time, ranging from 0.3% to 31% between 0.5 and 8 h after administration.

**CODEINE AND NORCODEINE S/P RATIOS**

The S/P codeine concentration ratios are illustrated as a function of time after drug administration in Fig. 3A. Except for early in the time course during codeine absorption and distribution, S/P ratios were >1. Codeine S/P ratios increased up to 1 h and then were relatively constant from 1 to 12 h, with a mean ratio of 4.0 ± 0.5 (range, 1.1–17.2) for the low dose and 4.1 ± 0.5 (range, 0.6–16.4) for the high dose. In addition, the mean codeine AUC S/P ratios were 3.4 ± 0.6 (range, 1.3–7.6) for the low dose and 4.1 ± 0.8 (range, 1.0–10.4) for the high dose. As shown in Fig. 4, oral fluid codeine concentrations were significantly correlated to plasma codeine concentrations from 0.5 to 12 h after dosing \((r = 0.22; P <0.0001)\).

Higher variability in norcodeine oral fluid concentrations produced greater variability in norcodeine S/P ratios. Mean norcodeine S/P ratios for the low dose (1.7 ± 0.3) tended to be higher than those of the high dose (1.0 ± 0.2; Fig. 3B). Norcodeine S/P ratios were lower than concurrent codeine S/P ratios.

**EFFECTS OF COLLECTION METHOD ON CODEINE AND METABOLITE ORAL FLUID CONCENTRATIONS**

In group I \((n = 4)\), citric acid candy was used to stimulate oral fluid secretion after administration of three 60 mg/70 kg doses of codeine (Fig. 5A). Although variability in codeine oral fluid concentration was observed, there were...
no significant differences in the 24-h AUCs of these doses. In group II (n = 4), three different collection methods were evaluated: citric acid candy stimulation after the first dose, Salivette with citric acid-treated cotton swab after the second dose; and Salivette with neutral cotton swab after the third dose (Fig. 5B). Codeine concentrations in oral fluid collected with citric acid sour candy tended to be higher than those in oral fluid collected with the other methods up to 2 h after drug administration; however, the differences were not statistically significant. After 2 h, codeine concentrations in oral fluid were consistent across collection methods.

**DETECTION TIMES OF CODEINE IN PLASMA AND ORAL FLUID**

Detection times for codeine in plasma and oral fluid varied by participant, dose, and cutoff concentration. Mean detection times for codeine in plasma with a 2.5 µg/L cutoff were 12.4 ± 1.70 and 16.6 ± 2.57 h for the low and high doses, respectively (Table 2). Codeine in oral fluid collected with citric acid candy was detectable for 21.1 ± 1.70 h after the low dose and 21.6 ± 1.38 h after the high dose with a 2.5 µg/L cutoff. Codeine could be detected for 5–9 h longer in oral fluid than in plasma when the LOQ cutoff was used. Codeine in oral fluid was detected for only 7 h with a 40 µg/L cutoff concentration, the suggested SAMHSA confirmatory test cutoff.

Norcodeine had shorter detection times than codeine in both matrices. Mean detection times for norcodeine based on the LOQ were 9.4 ± 2.07 and 10.3 ± 1.77 h in plasma and 6.0 ± 0.74 and 9.4 ± 1.64 h in oral fluid after the low and high doses, respectively.

**PHYSIOLOGIC EFFECTS**

The effects of 60 and 120 mg/70 kg doses of oral codeine on physiologic measures are illustrated in Fig. 6A. Statistically significant dose-related miosis was observed at 4 h after 60 mg/70 kg and from 1 to 4 h after 120 mg/70 kg oral codeine (P < 0.05). Pulse, respiration, diastolic and systolic blood pressures, skin and core temperatures, and blood oxygenation were not significantly affected by single 60- or 120-mg doses of oral codeine.

**SUBJECTIVE EFFECTS**

Mean responses on the PCAG (sedation) and LSD (psychotomimetic effect) scales of the ARCI were significantly increased compared with placebo (P < 0.05) after 120 mg of codeine for 1–2 h and 1–4 h, respectively (Fig. 6, B and C). In addition, “feel drug”, “relaxed”, and “high” self-rating scales of the SDQ were significantly increased from placebo (P < 0.05) from 0.5 to 2 h after the high dose (Fig. 6, D–F). No significant differences were found for the other ARCI, SDQ, or VAS measures at any time after single 60 or 120 mg/70 kg oral codeine doses.

**Discussion**

Oral codeine induced dose-related decreases in pupil size, but did not significantly affect other physiologic measures. These results are consistent with those of other
studies. Walker and Zacny (32) found dose-related miosis and no effects on other physiologic measures after single 60- and 120-mg doses of oral codeine. Cone (24) reported peak miotic effects within 0.75–1.0 h after 60 and 120 mg of intramuscular codeine in one participant. In this study, PCAG and LSD scales of the ARCI and “feel drug,” “relaxed,” and “high” scales of the SDQ were increased after 120 mg/70 kg oral codeine. The PCAG scale reflects feelings of sedation and apathy, and the LSD scale reflects feelings of dysphoria and sensory and somatic disturbance (30, 33). The SDQ relies on participants’ own personal history of exposure to various drugs. In another study (32), oral codeine did not affect ARCI scores, but produced significant increases in “dry mouth” and a trend for increased “skin itch” and “numb” feelings after 120 mg of oral codeine. A drug-induced “high” was also reported after a 120-mg intramuscular dose of codeine in one participant (24).

Codeine is primarily metabolized by glucuronidation to codeine 6-glucuronide, N-demethylation to norcodeine, and O-demethylation to morphine in humans (28, 34, 35). Norcodeine and morphine also undergo glucuronidation. In the current study, we analyzed free codeine and free metabolites in plasma and oral fluid after single oral administration of 60 and 120 mg/70 kg of codeine to evaluate the relationship of free codeine and its metabolites in plasma and oral fluid. Codeine was the primary analyte in plasma and oral fluid; the metabolite, norcodeine, was also detected in both matrices. Morphine and normorphine were not identified in any specimens with a 2.5 μg/L LOQ. O’Neal et al. (25) analyzed free codeine and free morphine in plasma and oral fluid by GC-MS with a 5 μg/L LOQ after 30 mg of oral liquid codeine. Participants (n = 17) had previously received three daily doses of 30 mg of codeine for 5 days, followed by a 24-h washout period. The authors also reported codeine as the primary analyte in plasma and oral fluid; the metabolite, norcodeine, was also detected in both matrices. Morphine and normorphine were not identified in any specimens with a 2.5 μg/L LOQ. O’Neal et al. (25) analyzed free codeine and free morphine in plasma and oral fluid by GC-MS with a 5 μg/L LOQ after 30 mg of oral liquid codeine.

### Table 2. Comparison of codeine and norcodeine detection times in plasma and oral fluid after oral codeine administration.

<table>
<thead>
<tr>
<th></th>
<th>GC-MS cutoff, μg/L</th>
<th>Dose, mg/70 kg</th>
<th>n</th>
<th>Detection time, a h</th>
<th>Codeine</th>
<th>Norcodeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2.5</td>
<td>60</td>
<td>14</td>
<td>12.4 ± 1.70</td>
<td>9.4 ± 2.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>15</td>
<td>16.6 ± 2.57</td>
<td>10.3 ± 1.77</td>
<td></td>
</tr>
<tr>
<td>Oral fluid</td>
<td>2.5</td>
<td>60</td>
<td>16</td>
<td>21.1 ± 1.70</td>
<td>6.0 ± 0.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>18</td>
<td>21.6 ± 1.38</td>
<td>9.4 ± 1.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>60</td>
<td>16</td>
<td>7.2 ± 1.30</td>
<td>NA a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>18</td>
<td>7.3 ± 0.36</td>
<td>NA a</td>
<td></td>
</tr>
</tbody>
</table>

* Data are mean ± SE.
* Method LOQ.
* The suggested SAMHSA cutoff concentration.
* NA, not applicable.

Fig. 6. Mean time course (n = 19) of effects after 60 mg/70 kg codeine (○), 120 mg/70 kg codeine (▲), and placebo (□).

Effects: (A), pupil size; (B), PCAG group (ARCI), a measure of sedation; (C), LSD (ARCI), a measure of psychotomimetic effect; (D), “feel drug”; (E), “relaxed”; (F), “high”. D–F are included in the SDQ. Each point represents the mean rating across all participants.
Concentrations of 183.9 and 307.6 μg/L for codeine from Edward J. Cone. Thus, norcodeine is more polar than codeine. The oil/water partition coefficient in the more acidic oral fluid. In addition, ion trapping in the more acidic oral fluid. In addition, a significant correlation was observed at earlier time points. Chen et al. (35) reported mean S/P ratios of 3.7 that remained consistent over time from 2 to 12 h after 30 mg of oral codeine, similar to our findings. In addition, a significant correlation \( r = 0.809 \) between saliva and plasma codeine concentrations was observed after 2 h, suggesting that oral fluid codeine concentrations could be used to predict plasma concentrations. However, because of oral contamination, no correlation was observed at earlier time points. Chen et al. (35) also reported a positive correlation between oral fluid and plasma codeine concentrations \( r = 0.80; P < 0.01 \) after 30 mg of oral codeine (n = 8) with a codeine AUC S/P of 2.95 ± 0.72, comparable to what we observed. In our study, although oral fluid codeine concentrations were significantly correlated to plasma codeine concentrations, we concluded that it was not possible to accurately predict a plasma codeine concentration from an oral fluid codeine concentration because of high intra- and intersubject variability.

Norcodeine S/P ratios were lower than those of codeine, most likely because of differences in \( pK_a \) and polarity. Drugs enter oral fluid primarily via passive diffusion, a process that facilitates the passage of nonionized drugs (3, 36–38). For acidic or basic drugs, the degree of ionization in the oral fluid and plasma is highly dependent on the \( pK_a \) of the drug and the pH of the environment (8, 11–14). The pH of oral fluid is lower than that of plasma. Norcodeine, with a \( pK_a \) of 5.7 (39) compared with the \( pK_a \) of 8.2 for codeine (39), would be less ionized in plasma than codeine and thus less subject to ion trapping in the more acidic oral fluid. In addition, norcodeine is more polar than codeine. The oil/water (1-octanol/water) coefficient for codeine (37 °C, pH 7.4) is 2.90, and that for norcodeine is 0.11 (personal communication from Edward J. Cone). Thus, norcodeine is ~26 times less lipophilic than codeine, limiting the passive diffusion of norcodeine from plasma to oral fluid. In addition, NCOD:COD ratios increased over time in both plasma and oral fluid, indicating a longer half-life for the metabolite than the parent compound.

A major drawback to the use of oral fluid for drug monitoring is the variability of oral fluid pH. Muklow et al. (11) reported that alteration of oral fluid flow rate and pH produced twofold changes in oral fluid drug concentrations with wide inter- and intrasubject variation. Oral fluid pH is dependent on salivary flow rates; salivary gland stimulation increases bicarbonate concentration and oral fluid pH.

Our mean elimination half-lives for codeine in plasma and oral fluid were comparable to previously reported estimates. A plasma \( t_1/2 \) of 2.4 ± 0.76 h (females) and 2.9 ± 0.32 h (males) was reported after the last of fifteen 30-mg consecutive codeine oral doses over 5 days (40). A similar mean plasma \( t_1/2 \) of 2.36 h in 19 male participants after a 60- or 120-mg codeine dose was noted by Rollins et al. (41). After 30-mg oral codeine, O’Neal et al. (25) observed a mean codeine \( t_1/2 \) of 2.6 ± 0.32 h for plasma and 2.9 ± 0.28 h for oral fluid. Using the same dosing regimen, Chen et al. (35) determined a mean \( t_1/2 \) of 3.24 ± 0.34 h for plasma and 3.11 ± 0.45 h for oral fluid.

O’Neal et al. (18) reported higher codeine concentrations in nonstimulated oral fluid (expectoration) compared with stimulated oral fluid. Oral fluid codeine concentrations collected with the Salivette device or with acidic stimulation (a lemon drop) were lower than those collected by expectoration (control), 77% and 30% of control, respectively. Our study also compared codeine concentrations in oral fluid collected by three different methods, the first method using citric acid candy stimulation, the second a citric acid-impregnated Salivette device, and the third a neutral Salivette cotton swab. At early collection time points, codeine concentrations tended to be higher after citric acid candy stimulation than with either Salivette device; however, these differences did not reach statistical significance. Expectoration after acidic stimulation did produce a higher volume of oral fluid. Collection with the Salivette devices yielded smaller volumes of oral fluid and required an additional centrifugation step to separate oral fluid from the cotton swabs. However, the use of collection devices is considered less objectionable to participants and staff and produces a cleaner oral fluid sample.

Codeine detection times in plasma of 12–16 h (LOQ of 2.5 μg/L), as reported in this study, are in agreement with those reported by O’Neal et al. (25). A shorter mean plasma detection time of 5 h was observed by Vree et al. (28) with a 5 μg/L cutoff after a 30-mg dose. In our study, the mean detection times for codeine in oral fluid were longer than in plasma. Thus, the advantages of monitoring codeine use in oral fluid include a longer detection window compared with plasma, less invasive sampling and discomfort, and a lower risk of infection. With the
higher SAMHSA-recommended cutoff of 40 µg/L for codeine in oral fluid, a mean detection time of only 7 h was found after the 60 and 120 mg/70 kg doses. Use of this cutoff for monitoring codeine use could enable the identification of far fewer positive specimens than other types of drug testing, i.e., urine. Therefore, a lower oral fluid cutoff for codeine is recommended.

In conclusion, we found that codeine was the major analyte detected in plasma and oral fluid. Initial detection times for codeine in oral fluid were comparable to those observed for plasma, peak concentrations were significantly higher, and mean detection times were 5-9 h longer than in plasma. Norcodeine also was detected in both matrices; however, morphine and normorphine were not measurable at the GC-MS LOQ of 2.5 µg/L after single 60 and 120 mg/70 kg doses. Although it is difficult to predict plasma codeine concentrations from simultaneously collected oral fluid specimens because of intra- and intersubject variability, codeine S/P ratios were relatively constant after 1-2 h. Thus, the advantages of using oral fluid as a test matrix for codeine exposure compared with plasma include higher concentrations, longer detection windows, less invasive sampling and discomfort, and lower risk of infection. Oral fluid is a useful alternative matrix to monitor opiate use for drug treatment, workplace testing, infection. Oral fluid is a useful alternative matrix to less invasive sampling and discomfort, and lower risk of

We thank Bonnie Ladaga and Richard Taylor for expert assistance with the statistical analysis and pharmacodynamic assessments.

References

31. Fraser HF, Van Horn GD, Martin WR, Wolbach AB, Isbell H. Method


