Published in final edited form as:

Ther Drug Monit. 2010 October ; 32(5): 628–637. doi:10.1097/FTD.0b013e3181f2b729.

Pharmacokinetics of Cocaine and Metabolites in Human Oral Fluid and Correlation with Plasma Concentrations following Controlled Administration

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Abstract

Oral fluid is an attractive alternative matrix for drug testing, with a non-invasive and directly observed collection, but there are few controlled cocaine administration studies to guide interpretation.

Materials and Methods—While residing on a closed research unit for up to 10 weeks under constant medical supervision, 19 participants were administered 75 mg/70 kg subcutaneous cocaine and 14 received 150mg/70 kg. The disposition of cocaine, benzoylecgonine (BE) and ecgonine methyl ester (EME) into oral fluid was determined by gas chromatography-mass spectrometry for 0.08–48h after administration.

Results—In oral fluid collected by citric acid candy stimulated expectoration, cocaine first appeared in oral fluid 0.08–0.32h after dosing and was rapidly eliminated with half-lives of 1.1–3.8h. BE and EME were first detected 0.08–1.0h after dosing, with longer half-lives of 3.4–13.8 (BE) and 2.4–15.5h (EME) ($p < 0.05$). Oral fluid and plasma concentrations were significantly correlated for cocaine, BE and EME ($p < 0.0001$). There were no significant differences ($p > 0.05$) in first and last detection times with the 8 $\mu\text{g/L}$ cutoff proposed by the Substance Abuse and Mental Health Services Administration or the 10 $\mu\text{g/L}$ cutoff from the European initiative, Driving Under the Influence of Drugs, Alcohol and Medicines. Metabolite:cocaine ratios increased after cocaine administration, potentially helpful for interpreting time of last use. Comparison of oral fluid collection via citric acid candy stimulated expectoration, citric acid treated Salivette® and neutral cotton Salivette® devices did not reveal significant differences between devices for areas under the curve for cocaine, BE or EME ($p > 0.05$).

Discussion and Conclusion—These results provide additional evidence for interpreting cocaine and metabolite concentrations in oral fluid and oral fluid's usefulness as an alternative matrix for drug testing.

Keywords

cocaine; oral fluid; clinical study; pharmacokinetics

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Introduction

Blood is the classic matrix for correlating drug concentrations to pharmacological effects, yet sampling is invasive and drug half-lives are relatively short, in comparison to other matrices. Oral fluid collection is non-invasive, easily observed, and has little potential for adulteration¹⁻³. In addition, the detection window of basic drugs is longer than in blood due to ion trapping in the more acidic oral fluid³. Collection of oral fluid is simple, either as expectoration into a tube or sampling with one of a large number of commercially available collection devices. There are limitations associated with oral fluid testing, including low specimen volume, contamination of the oral cavity after oral or smoked administration, cultural aversion to expectoration, and dry mouth following stimulant or cannabis use³. Although oral fluid point-of-collection devices provide viable options for detection of licit and illicit drugs, additional issues are associated with this new technology. An elution buffer improves drug recovery from the collection device, although cocaine and metabolites are usually not problematic, but also dilutes drug concentrations and may interfere with chromatographic analysis, especially liquid chromatography procedures⁴.

Oral fluid refers to the mixture of secretions from the major and minor salivary glands and gingival crevices⁵. Oral fluid is 99% water, a filtrate of plasma with less than 1% of its proteins⁶. Passage of compounds from plasma to oral fluid occurs primarily by passive diffusion that is dependent upon plasma pH and protein binding, drug lipophilicity, molecular weight, structure and pKa, and salivary pH and flow rate⁶. As only unbound or free drug passes into oral fluid, there is evidence for some drugs that oral fluid concentrations correlate with free drug plasma concentrations and to subjective and physiological measurements⁷. Collection methods that stimulate flow of oral fluid alter salivary pH, reducing drug concentrations due to increased saliva volume and a higher pH from increased bicarbonate excretion⁸.

Cocaine, a psychoactive drug from the coca leaf, is primarily metabolized by the liver to two major, inactive metabolites, benzoylecgonine (BE) and ecgonine methyl ester (EME)⁹. Cocaine is taken for its intense euphoric and stimulatory effects¹⁰, despite well-documented cardiotoxic effects¹¹. In recent years, there were approximately two million past month users of cocaine in both the US and Europe¹²⁻¹³.

Oral fluid testing provides an excellent monitoring tool for driving under the influence of drugs (DUID) cases and workplace, drug treatment and parolee programs². In 2004, as a result of growing interest in this alternative matrix, the Substance Abuse and Mental Health Services Administration (SAMHSA) proposed technical guidelines for oral fluid testing for federally-mandated workplace drug testing¹⁴. Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID), a European initiative that promotes research and scientific support to reduce European Union road deaths has proposed guidelines for the DRUID program¹⁵. In Talloires, France, international experts addressed guidelines for research on drugged driving and suggested drugs and drug concentrations that should be included in for oral fluid testing for DUID¹⁶. Although the SAMHSA guidelines have yet to be approved, oral fluid testing in the non-regulated sector in the US is growing rapidly, and monitoring of this matrix is firmly in place in Victoria, Australia and throughout Europe^{3, 17}.

Although the science of oral fluid testing is rapidly advancing, limited controlled drug administration studies with oral fluid concentration data are available to improve interpretation of oral fluid test results. The aim of this research was to characterize the pharmacokinetics of cocaine and its two major metabolites, BE and EME, in oral fluid following controlled subcutaneous (sc) cocaine administration. A secondary goal was to determine oral fluid/plasma ratios in simultaneously collected specimens and suggest

whether plasma concentrations could be predicted based on those found in oral fluid. Finally, the effect of oral fluid collection method on cocaine, BE and EME concentrations was evaluated.

Materials and Methods

HUMAN PARTICIPANTS

Healthy male and female volunteers provided written informed consent to participate in this Institutional Review Board-approved study. Subjects were compensated for their time. Screening procedures included comprehensive medical and psychological evaluations in addition to urine drug screening for cocaine, opiates, phencyclidine, amphetamines, cannabinoids and barbiturates. Positive drug screening results were not grounds for exclusion from the study. All participants had a documented history of cocaine and opioid use, but were not physically dependent on either drug. Ethical and safety concerns required documented self-reported history of cocaine use and a positive cocaine urine test within the previous 30 days for inclusion in this study. Participants resided on a closed research unit for up to 10 continuous weeks under constant medical supervision.

DRUG ADMINISTRATION

Characterization of cocaine distribution into human hair was a secondary objective of this study. Participants were required to reside on the research unit 20 days prior to the first scheduled drug administration to allow for clearance of previously self-administered drugs from hair. Cocaine hydrochloride was administered subcutaneously up to three times during week three (75mg/70kg low doses) and up to three times during week seven (150mg/70kg high doses). Within the two dosing weeks, injections were separated by at least 48h.

SPECIMEN COLLECTION

Oral fluid was collected 0.25h prior to and 0.08 0.17, 0.25, 0.5, 1, 2, 4, 8, 12, 26, 28, 32, and 48h after each cocaine dose. In the first cocaine session each week, oral fluid excretion was stimulated by citric acid candy and collected by expectoration into 50mL polypropylene tubes. Specimens were centrifuged and frozen (-20°C) until analysis. Five participants received two additional cocaine doses each week to enable comparison of drug concentrations in oral fluid collected by three different methods. During the second session oral fluid was collected with Sarstedt (Numbrecht, Germany) Salivette[®] citric acid treated cotton swabs and in the third, Salivette[®] neutral cotton swabs. Salivette[®] devices were centrifuged in conical vials, oral fluid was transferred to polypropylene cryotubes and stored at -20°C until analysis.

CHEMICALS AND REAGENTS

Cocaine hydrochloride for human administration was obtained from Mallinckrodt (St. Louis, MO, USA) and prepared in saline for sc injection. Cocaine, BE, and EME standards and deuterated internal standards ($-d_3$) were purchased from Cerilliant Corp. (Round Rock, TX, USA). N,O-bis (trimethyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylchlorosilane (TBDMCS) were from Pierce Chemical (Rockford, IL, USA). Methanol (Fisher Scientific, Fair Lawn, NJ, USA), methylene chloride, 2-propanol, and acetonitrile (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) were high-performance liquid chromatography (HPLC)-grade chemicals. Sodium acetate, acetic acid, ammonium hydroxide, and hydrochloric acid were reagent grade and purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Solid-phase extraction (SPE) columns (Clean Screen

ZSDAU020) and fritted filters (RFV02F4P) were from United Chemical Technologies (Bristol, PA, USA).

QUANTIFICATION OF COCAINE, EME AND BE IN ORAL FLUID AND PLASMA

Quantitative analysis for cocaine, EME and BE in oral fluid and plasma was performed according to previously published analytical methods ^{8, 18–21}. Briefly, 100ng internal standard cocaine-d₃, BE-d₃ and EME-d₃ and 3mL 2.0M sodium acetate buffer (pH 4.0) were added to 1mL oral fluid or plasma. After mixing and centrifugation, the filtrate was decanted onto preconditioned solid phase extraction columns. Columns were washed (H₂O, 0.1M hydrochloric acid, methanol), dried, and analytes eluted with methylene chloride, 2-propanol, and 14.5M ammonium hydroxide (80:20:2, v/v/v) into tubes containing 20 µL MTBSTFA and 1% TBDMCS. After evaporation to dryness, the extract was reconstituted with 500 µL acetonitrile, vortex-mixed and dried. Following reconstitution with 20 µL acetonitrile, mixing and centrifugation, samples were derivatized with 20 µL of MTBSTFA with 1% TBDMCS and heated at 80 C for 15–20 min. After cooling, 20 µL BSTFA with 1% TMCS was added, vials were capped, and extracts heated at 80°C for 45 min. The derivatized extract (2 µL) was analyzed by electron impact gas chromatography mass spectrometry (GCMS) in selective ion monitoring mode ²¹.

INSTRUMENTATION

Splitless injections were made onto an Agilent 5890 GC interfaced to an Agilent 5972 mass selective detector or an Agilent 6890 GC interfaced to a 5973 mass selective detector. Chromatographic separation was achieved with a Phenomenex ZB1 (15 m × 0.2-mm i.d., 0.25 µm film thickness) or HP-1 (12 m × 0.2-mm i.d., 0.33 µm film thickness) capillary column. Instrument parameters were described in detail previously ^{8, 18–21}. Two calibration curves, 2.5–50 µg/L and 50–1000 µg/L, were employed to extend the assay's dynamic range. Limits of quantification (LOQ) were 2.5 µg/L for cocaine, EME and BE.

STATISTICAL AND PHARMACOKINETIC ANALYSES

Visual inspection of data and evaluation by Kolmogorov-Smirnov tests indicated non-normal data distribution. Therefore, statistical comparisons were conducted via non-parametric Mann-Whitney tests using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Comparisons were considered significant if $p < 0.05$. Area under the curve (AUC_{0-last}) and terminal elimination half-lives were calculated employing a noncompartmental model using WinNonlin version 5.2 (Pharsight Inc., Mountain View, CA, USA). Correlations of cocaine, BE and EME oral fluid to plasma concentrations were performed using least-squares regression analysis in Prism version 5.02 (Graphpad Software Inc., La Jolla, CA, USA).

COCAINE AND METABOLITE ORAL FLUID/PLASMA RATIOS

Oral fluid-to-plasma ratios (S/P) for the parent drug and two metabolites were determined in simultaneously collected specimens. Ratios were only calculated for time points when analytes were quantifiable in both matrices. LOQ in plasma were 2.5 µg/L ²¹.

Results

HUMAN PARTICIPANTS

Demographics of the 19 participants are listed in Table 1. Five subjects departed after completing the low dose session only. Withdrawals were due to medical, behavioral, or family issues. Blood (plasma) was not collected from one and two subjects during the high

and low dose sessions, respectively, due to catheter failure. Five participants completed two additional sessions each week for evaluation of oral fluid collection methods.

COCAINE AND METABOLITES IN ORAL FLUID

Overall oral fluid results—445 oral fluid specimens were collected after sc cocaine from 19 participants. SC cocaine was employed for these studies due to medical risks inherent in cocaine administration by more rapid, intravenous and smoked routes of administration. Cocaine was found in higher concentrations than BE and EME, although most specimens contained all three analytes (Figure 1). Proposed SAMHSA guidelines for cocaine testing in oral fluid suggest a cutoff concentration of 8 µg/L for cocaine or BE, with no provisions for EME¹⁴. European DRUID testing guidelines and Talloires recommendations employ a 10 µg/L cutoff for cocaine or BE and also do not include EME^{15–16}. Figure 2 depicts detection rates 0.08 to 48h after oral cocaine employing the LOQ, SAMHSA and DRUID/Talloires guidelines. Inclusion of both cocaine and BE in SAMHSA or DRUID/Talloires oral fluid testing guidelines increases detection rates 0.08–0.25 and 24–48h compared to employing cocaine or BE alone (Figure 2). EME alone was not found in any specimen after the high dose, although depending on cutoff concentration, EME was identified as the only cocaine analyte in 1–3 specimens after low dose cocaine.

Times of first detection—Cocaine initially appeared in oral fluid as early as 0.08h and as late as 0.32h after dosing (Table 2). Cocaine T_{first} typically occurred in the first specimen collected. BE and EME initially appeared in oral fluid 0.08–1.0h after cocaine.

Times of last detection—Table 2 lists times of last detection (T_{last}) for cocaine, BE and EME determined at the assay's LOQ, proposed SAMHSA and DRUID cutoffs. Cocaine was eliminated most rapidly from oral fluid (low dose: 8.9–14h, high dose: 13.4–17.2h) compared to BE (low dose: 26.8–36.2h, high dose: 39.0–46.9h) and EME (low dose: 18.5–28.0, high dose: 26.9–36.0h). BE T_{last} at SAMHSA and DRUID cutoffs were significantly longer than EME after both doses, but was significantly longer following low but not high dose cocaine at the LOQ. Between dose comparisons revealed that cocaine and EME T_{last} at LOQ, SAMHSA and DRUID cutoffs were equivalent after low and high doses. BE T_{last} calculated using SAMHSA and DRUID cutoffs were significantly longer after high dose compared to low dose. Within dose comparisons of cocaine T_{last} only found significant differences between LOQ and DRUID cutoffs after low dose cocaine, while all cutoffs were equivalent after high dose cocaine. After low dose cocaine BE and EME T_{last} were significantly different comparing LOQ to SAMHSA and DRUID cutoffs. After high dose cocaine there were not any differences between BE T_{last} calculated with any of the cutoff concentrations. EME T_{last} were significantly different comparing LOQ to DRUID cutoffs after high dose cocaine. In no instance for cocaine, BE or EME were there any differences comparing T_{last} calculated using SAMHSA versus DRUID cutoff concentrations.

Pharmacokinetics of cocaine and metabolites in oral fluid—Mean maximum oral fluid concentrations (C_{max}), times of maximum concentration (T_{max}), terminal half-lives and areas under the curves ($AUC_{0-\text{last}}$) are listed in panel A of Table 3. C_{max} and $AUC_{0-\text{last}}$ were highest for cocaine. After both doses, mean BE and EME C_{max} were approximately 10% of mean cocaine C_{max} . Cocaine, BE and EME C_{max} were significantly dose-related. T_{max} demonstrate that cocaine concentrations peaked significantly earlier than those of the two metabolites. Cocaine half-lives were significantly shorter than those of BE and EME, indicating that elimination of cocaine from oral fluid is faster than BE and EME. BE half-lives were significantly longer than EME after high dose cocaine. Cocaine, BE and EME $AUC_{0-\text{last}}$ were significantly dose-related. BE:cocaine and EME:cocaine ratios in oral fluid increased over 48h from 0.5 to 1220% and 0.7 to 820%, respectively (Figure 3).

Comparison of oral fluid to plasma cocaine, BE and EME concentrations—

Cocaine and metabolites' pharmacokinetics in plasma following sc administration were described previously for most of these participants²¹. Statistical comparisons were performed to determine if there were significant differences between matrices in T_{last} , C_{max} , T_{max} , or AUC_{0-last} for cocaine, BE and EME (Table 3, panel B). Median plasma cocaine T_{last} were 11.5h (range: 4.0–24.1h) and 9.8h (range: 4.0–47.6h) for low and high doses, respectively, 48.0h (range: 24.0–48.0h) for BE T_{last} and 24.0h (range 4.0–48.0h) for EME T_{last} after low and high dose cocaine. There were no significant differences between T_{last} in plasma and oral fluid for cocaine, BE or EME. Cocaine and EME C_{max} were significantly higher in oral fluid than plasma after both doses. BE displayed the opposite trend, with significantly higher plasma concentrations than those in oral fluid. T_{max} occurred significantly later in oral fluid compared to plasma for cocaine after both doses; BE T_{max} following the high dose was the only instance in which T_{max} occurred significantly later in plasma for either metabolite. Cocaine half-lives were significantly prolonged in plasma compared to oral fluid after both doses, while BE and EME half-lives were similar in plasma and oral fluid. AUC_{0-last} demonstrated similar patterns as observed for C_{max} .

Median S/P ratios are shown from 0.08–24h after low and high cocaine doses in Figure 4. There were numerous oral fluid-plasma pairs with cocaine, BE or EME concentrations less than 2.5 µg/L that were not included in S/P ratio analysis. Generally cocaine negative specimen pairs occurred 24–48h after cocaine administration: 122 pairs were negative for cocaine in both oral fluid and plasma, 17 were negative in oral fluid only and 14 were negative for cocaine in plasma only. Eleven specimen pairs were negative for BE in both oral fluid and plasma, 70 were negative in oral fluid only and two were negative in plasma only, typically occurring 0.08–0.5 and 24–48h after cocaine. Negative EME specimens followed a similar time-course as BE: 64 specimen pairs were both oral fluid and plasma negative, 55 were negative in oral fluid only and 32 were negative in plasma only.

Oral fluid and plasma concentration profiles appear similar for cocaine, BE and EME (Figure 1) although the S/P ratios are highly variable between subjects following sc cocaine (Figure 4). Generally, median cocaine and EME S/P ratios were greater than one from 0.5–24h after both doses, while BE S/P ratios were typically less than one. Oral fluid and plasma concentrations of cocaine, BE and EME appear significantly correlated with $R^2=0.27, 0.49$ and 0.35 , respectively; all $p<0.0001$ (Figure 5). Equations of the fitted lines were: $y=2.8x + 178.5$ for cocaine, $y=0.4x + 0.1$ for BE and $y=2.4 - 0.8$ for EME.

Comparison of oral fluid collection methods—Figure 6 shows mean cocaine, BE and EME oral fluid concentrations from five participants during studies that evaluated citric acid candy stimulated expectoration, citric acid treated Salivette and neutral cotton Salivette collection methods. Comparison of AUC_{0-last} did not reveal any significant differences between the three collection methods for cocaine, BE or EME.

Discussion

There is interest in oral fluid as an alternative matrix for drug testing in DUID cases and workplace, drug treatment and parolee programs, since oral fluid can be obtained non-invasively, does not require same sex collectors or specialized collection facilities and is not easily adulterated². Furthermore, oral fluid cocaine concentrations may correlate with pharmacodynamic effects, suggesting that oral fluid might be an alternative to whole blood or serum for assisting interpretation of impairment in DUID cases^{3,7}. However, there are only two reports that investigated cocaine and metabolites' pharmacokinetics in oral fluid following controlled drug administration^{22–23}. Controlled drug administration studies also are vital to evaluate analyte cutoff concentrations for oral fluid testing; only one report

evaluated proposed SAMHSA cocaine oral fluid cutoffs ²⁴. The present controlled cocaine administration study was conducted to further investigate cocaine and metabolite pharmacokinetics in oral fluid and to investigate performance of oral fluid testing guidelines proposed by SAMHSA, DRUID and the Talloires meeting ^{14–16}. We also evaluated three oral fluid collection methods.

Cocaine appeared in oral fluid 0.08–0.32h after sc cocaine administration and peaked after 0.2–2.1h. This is the first work to clearly present $T_{\max}$ data for cocaine in oral fluid following a single controlled dose administration. Cocaine was rapidly eliminated from oral fluid with a half-life of approximately 3h, similar to reports after intravenous (0.4–7.2h) ^{22, 25}, intranasal (0.6–4.2h) ²², and smoked (0.2–5.9h) ^{22, 25} cocaine, but longer than after oral cocaine (1–2h) ²³. Large variability was noted in earlier studies that based half-lives upon 7 or fewer participants ^{22–23}. Cocaine was the predominant analyte found in oral fluid after single sc, intravenous, intranasal and smoked cocaine doses ^{22, 25}. Interestingly, Jufer et al. reported that EME predominated in oral fluid following repeated oral cocaine doses, possibly due to metabolite accumulation during repeated dosing ²³.

BE and EME $T_{\max}$ in oral fluid occurred 1–8 and 2–4h after sc administration, respectively, similar to 1–3h reported following repeated oral cocaine doses ²³. BE and EME $T_{\max}$ were not reported after controlled intravenous, intranasal or smoked cocaine administration. BE half-lives after sc cocaine were 3.4–13.8h, consistent with 4.8–9.2h following repeated oral cocaine dosing ²³, 1.4–9.3 after intravenous, 1.0–10.7 after intranasal, and 0.5–6.5h following smoked administration ²². EME oral fluid half-lives of 2.4–15.5h following sc cocaine appear similar to reports after oral (3.9–5.4h) ²³, intravenous (1.2–4.6h) ²², intranasal (0.8–12.1h) ²² and smoked cocaine (1.0–5.7h) ²². Again, comparison is difficult due to variability and the small number of participants in previous studies ^{22–23}.

SAMHSA, DRUID and Talloires guidelines specify cutoff concentrations for cocaine and BE. We found that inclusion of both cocaine and BE in these guidelines increased sensitivity up to 80% compared to employing cocaine or BE alone, providing 50% or greater detection rates 0.08–24 and 0.08–32h after low and high dose, respectively (Figure 2, panels E and F). SAMHSA, DRUID and Talloires guidelines do not include EME. In our study there were only three of 445 specimens that contained EME alone; therefore, inclusion of EME for oral fluid testing does not appear advantageous.

Measurement of BE in oral fluid offers the advantage of a wider window of drug detection than cocaine. In general, use of a typical 100 mg dose of cocaine could be detected in oral fluid for either cocaine or BE for approximately 1–2 days. Using the lower LOQ cutoffs, detects lower cocaine doses, but the current SAMHSA or DRUID/Talloires oral fluid testing cutoffs, appear sensitive enough to detect most single cocaine uses for a similar timeframe as blood. During high dose cocaine sessions, T_{last} in oral fluid were similar for cocaine, BE and EME regardless of cutoff concentration. There were not any differences between T_{last} calculated using SAMHSA or DRUID/Talloires guidelines for cocaine, BE or EME after either cocaine dose.

Metabolite:cocaine time-course plots suggest that these ratios might be useful for interpreting time of last use. We observed that BE:cocaine and EME:cocaine ratios generally increased over time, indicating that a specimen containing high concentrations of cocaine relative to BE and EME suggests recent cocaine use. Granted this finding is based upon observations from single cocaine administration studies that demonstrated considerable inter-subject variability, but data in previous reports also suggest that metabolite:cocaine ratios could prove useful for estimating time of last use for intravenous, intranasal and

smoked cocaine usage^{22, 25}. Although, multiple dosing or binge use may produce results differing from single administration studies.

In five participants, we investigated oral fluid collection methods including citric acid candy stimulated expectoration, citric acid treated Salivette® and neutral cotton Salivette® collection devices. Comparison of AUC_{0-last} for cocaine, BE and EME revealed no significant concentration differences between these collection methods. Kato et al. demonstrated that oral fluid cocaine concentrations collected via expectoration were higher without citric acid stimulation²⁶. The explanation for our results failing to find any differences between collection devices remains unclear, but equivalent concentrations obtained with the neutral Salivette® and citric acid stimulated expectoration may be due to drug adsorption to the collection device. It also may be possible that insertion of the neutral Salivette® collection device stimulates salivary flow similar to citric acid candy stimulated expectoration and citric acid treated Salivette® device producing similar cocaine and metabolite oral fluid concentrations for these three collection methods. Furthermore, the small sample size, 5 participants for the device comparison studies, may also limit our findings.

We found that oral fluid and plasma concentrations of cocaine were significantly correlated, and for the first time, showed similar correlations for BE and EME. There were significant correlations between simultaneously collected cocaine and metabolite oral fluid and plasma concentrations, although care is warranted regarding this finding since linear regression analysis is designed for independent data and is not ideal for repeated measures time-course data presented here. Furthermore, inter- and intra-subject variability observed in these data suggest that reliable prediction of plasma concentrations from oral fluid concentrations is unreliable (Figure 5). These data provide important evidence for the interpretation of oral fluid tests. S/P ratios reflect higher cocaine and EME oral fluid concentrations compared to plasma, while the reverse is shown for BE. In general, we found oral fluid AUC_{0-last}/plasma AUC_{0-last} ratios appeared to fall in range with those previously reported for cocaine and BE following intravenous, intranasal, smoked and oral cocaine, suggesting that sc cocaine was an effective model for studying cocaine pharmacokinetics²²⁻²³. We found cocaine oral fluid AUC_{0-last}/plasma AUC_{0-last} ratios after sc administration ranging from 1.3–12.1, that appear similar to those following intravenous (0.3–4.4)²², smoked (0.6–6.4)²² and oral cocaine (3.8–13.2)²³. Cocaine S/P ratios after intranasal administration varied widely ranging from 0.3–358, most likely due to contamination occurring from this route of drug delivery²². We observed BE oral fluid AUC_{0-last}/plasma AUC_{0-last} ratios of 0.1–0.8, similar to 0.1–1.1 reported following intravenous, intranasal, smoked and repeated oral cocaine²²⁻²³. EME oral fluid AUC_{0-last}/plasma AUC_{0-last} ratios of 2.3–5.1 were previously reported after repeated oral cocaine²³, similar to our results of 1.0–23.0. Passage of drugs from plasma into oral fluid primarily occurs by passive diffusion of nonionized drugs, making lipophilicity and pKa key factors for excretion of drugs into and elimination from oral fluid^{3, 5}. For weak bases like cocaine and EME, ion trapping into oral fluid occurs because oral fluid pH is typically acidic relative to the neutral pH of blood³.

Formal comparisons of T_{first} in plasma and oral fluid were not performed because cocaine and metabolites routinely occurred in the first sample collected at 0.08h after dosing. However, it does appear that BE and EME T_{first} might be slightly delayed in oral fluid compared to plasma, as some of the first specimens did not contain measureable metabolites. We found that cocaine half-lives were significantly longer in plasma than in oral fluid, consistent with data following smoked²² and intranasal cocaine^{22, 25}; but inconsistent with half-lives reported following intravenous administration^{22, 25}. BE and EME half-lives were similar in plasma and oral fluid. Observed S/P ratios greater than one indicate that cocaine and EME undergo ion trapping, suggesting that oral fluid might yield longer windows of

detection than plasma. The observation of longer half-lives in plasma compared to oral fluid suggests that cocaine T_{last} would occur later in plasma than oral fluid. Ultimately however, our results demonstrate that cocaine, BE and EME T_{last} determined using the LOQ were similar for plasma and oral fluid and that oral fluid provides similar detection windows as plasma.

Conclusion

We observed that cocaine, BE and EME appeared in oral fluid shortly after sc administration and cocaine was eliminated more rapidly than BE and EME. We found that oral fluid and plasma concentrations of cocaine, BE and EME were significantly correlated, although there was considerable inter- and intra-subject variability making reliable prediction of plasma concentrations from oral fluid concentrations difficult. Comparison of SAMHSA cutoff concentrations of 8 µg/L versus DRUID/Talloires cutoffs of 10 µg/L, did not reveal significant effects on BE and EME T_{first} , or T_{last} for cocaine, BE or EME. Plasma and oral fluid detection windows were similar in plasma and oral fluid. Metabolite:cocaine ratios increase after cocaine administration and might prove useful for interpreting times of last use. We did not observe any significant differences in cocaine, BE or EME oral fluid concentrations when collected by citric acid candy stimulated expectoration, citric acid treated Salivette® and neutral cotton Salivette® collection devices. These results will improve interpretation of oral fluid tests in DUID cases and workplace, drug treatment and parolee programs.

Acknowledgments

This research was supported by funds from the National Institutes of Health, Intramural Research Program, National Institute on Drug Abuse.

We would like to thank the participants and clinical research staff. Funding for this research was from the National Institutes of Health, Intramural Research Program, National Institute on Drug Abuse.

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Figure 1.

Median concentrations of cocaine, benzoylecgonine and ecgonine methyl ester in oral fluid and plasma after a single 75mg/70kg ($n=19$ for oral fluid, 17 for plasma) or 150mg/70kg ($n=14$ for oral fluid, 13 for plasma) subcutaneous cocaine dose. Bars are interquartile ranges. Asterisks indicate that all subsequent specimens contained less than 2.5 $\mu\text{g/L}$ cocaine. Dotted lines indicate the following cutoff concentrations: limits of quantification (2.5 $\mu\text{g/L}$), Substance Abuse and Mental Health Services Administration (SAMHSA, 8 $\mu\text{g/L}$) and Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID, 10 $\mu\text{g/L}$).

Figure 2.

Detection rates for cocaine, benzoylecgonine, cocaine and/or benzoylecgonine, ecgonine methyl ester in oral fluid calculated using the assay limit of quantification (LOQ), Substance Abuse and Mental Health Services Administration (SAMHSA) cutoff and Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) cutoff after 75mg/70kg or 150mg/70kg subcutaneous cocaine dose. N=19 and 14 participants for 75mg/70kg or 150mg/70kg cocaine doses, respectively. Data are not visible if detection rates are identical to those of the higher DRUID cutoff.

Figure 3. Median benzoyllecgonine (BE):cocaine and ecgonine methyl ester (EME):cocaine ratios (x100) in oral fluid after a single 75mg/70kg (n=19) or 150mg/70kg (n=14) subcutaneous cocaine dose. Bars are interquartile ranges. Only paired positive specimens were included in analysis.

Figure 4.

Median oral fluid:plasma ratios for cocaine, benzoylecgonine and ecgonine methyl ester after a single 75mg/70kg (n=19) or 150mg/70kg (n=14) subcutaneous cocaine dose. Bars are interquartile ranges. Only paired positive specimens were included in the analysis.

Figure 5.

Correlation of oral fluid to plasma cocaine, benzoylecgonine and ecgonine methyl ester concentrations in simultaneously collected specimens from 19 participants between 0.08 and 48h after 75 and 150mg/70kg subcutaneous cocaine. The regression was calculated by least-squares regression analysis. Only paired positive specimens were included in the analysis.

Figure 6.

Median oral fluid concentrations of cocaine, benzoylecgonine and ecgonine methyl ester after 75mg/70kg subcutaneous cocaine comparing collection via citric acid stimulated expectoration, Salivette® with citric acid – treated cotton and Salivette® with neutral cotton (n=5 participants, 181 specimens total). Bars are interquartile ranges.

Table 1

Participant demographics

Total participants	19
African American	8 males 7 females
Caucasian	2 males
Hispanic	2 males
Age (years)	34.5±5.1 (range: 23–43)
Weight (kg)	77.4±14.0 (range: 56.6–106.5)

Median (range) detection windows for cocaine, benzoylecgonine (BE) and ecgonine methyl ester (EME) in oral fluid for 48h following 75 and 150 mg/70kg subcutaneous cocaine. 75mg/70kg cocaine (N=19 participants)

Table 2

	T_{first}^a	T_{last}^b	Hours (range)			
			T_{first}	T_{last}	T_{first}	T_{last}
	$\geq LOQ^c$ [2.5 $\mu\text{g/L}$]		$\geq SAMHSA^d$ [8.0 $\mu\text{g/L}$]		$\geq DRUID^e$ [10.0 $\mu\text{g/L}$]	
Cocaine	0.08 (0.08–0.32)	11.5 ^{2,4,5} (4.1–24.1)	0.08 (0.08–0.32)	8.0 ^{4,5} (4.0–24.1)	0.08 (0.08–0.32)	8.0 ^{2,4,5} (4.0–11.6)
BE	0.2 (0.1–0.6)	32.0 ^{1,2,4,6} (4.1–71.6)	0.5 (0.2–1.0)	28.0 ^{*,1,4,6} (4.1–71.6)	0.5 (0.2–1.0)	28.0 ^{*,2,4,6} (4.1–71.6)
EME	0.2 (0.1–0.6)	28.0 ^{1,2,5,6} (4.1–71.6)	0.5 (0.2–0.6)	24.1 ^{1,5,6} (4.1–32.0)	0.5 (0.2–1.0)	24.1 ^{2,5,6} (4.1–32.0)
150mg/70kg cocaine (N=14 participants)						
Cocaine	0.08 (0.08–0.08)	17.7 ^{4,5} (7.9–28.5)	0.08 (0.08–0.08)	9.8 ^{4,5} (7.9–28.5)	0.08 (0.08–0.08)	8.0 ^{4,5} (7.9–28.5)
BE	0.2 (0.1–1.0)	47.0 ⁴ (24.0–72.0)	0.5 (0.2–1.0)	32.0 ^{*,4,6} (24.0–72.0)	0.5 (0.2–1.0)	32.0 ^{*,4,6} (24.0–72.0)
EME	0.2 (0.1–1.0)	32.0 ^{2,5} (7.9–72.0)	0.4 (0.2–1.0)	28.0 ^{5,6} (7.9–72.0)	0.5 (0.2–1.0)	26.0 ^{2,5,6} (7.9–72.0)

^a time of first detection,

^b time of last detection,

^c limit of quantification,

^d Substance Abuse and Mental Health Services Administration and

^e Driving Under the Influence of Drugs, Alcohol and Medicines,

* significant difference between low and high dose; significant within dose differences:

¹ LOQ vs SAMHSA,

² LOQ vs DRUID,

³ SAMHSA vs DRUID,

⁴ Cocaine vs BE,

⁵ Cocaine vs EME and

⁶ BE vs EME. All comparisons by Mann-Whitney test, $p < 0.05$.

Table 3

Median (range) pharmacokinetic parameters for cocaine, benzoylecgonine (BE) and ecgonine methyl ester (EME) in A) oral fluid and B) plasma after subcutaneous cocaine.

A) Oral Fluid								
	Dose (mg/70 kg)	N	C _{max} ^a (µg/L)	T _{max} ^b (h)	T _{1/2} ^c (h)	AUC _{0-last} ^d (h × µg/L)	AUC _{0-oral fluid} /AUC _{0-plasma} ^e	
Cocaine	75	19	1092 * ^{1,2,4} (406–3006)	1.0 ^{1,2,4} (0.2–2.1)	3.0 ^{1,2,4} (2.2–3.8)	3546 * ^{1,2,4} (1412–9267)	N/A ^f	
	150	14	2600 * ^{1,2,4} (1193–8495)	1.0 ^{1,2,4} (0.5–2.0)	2.6 ^{1,2,4} (1.1–3.8)	8826 * ^{1,2,4} (4901–23697)	N/A	
Benzoylecgonine	75	19	133.6 * ^{1,4} (81.8–440.6)	2.1 ¹ (1.0–8.0)	7.4 ¹ (3.4–12.0)	1388 * ^{1,4} (609–4545)	N/A	
	150	14	280.0 * ^{1,4} (132.6–757.0)	2.0 ^{1,4} (2.0–4.0)	9.1 ^{1,3} (4.4–13.8)	3519 * ^{1,4} (1351–7174)	N/A	
Ecgonine methyl ester	75	19	131.5 * ^{2,4} (48.5–1329.7)	2.1 ² (1.0–4.0)	5.6 ² (2.4–15.5)	1251 * ^{2,4} (436–10061)	N/A	
	150	14	283.6 * ^{2,4} (141.6–949.6)	2.0 ² (2.0–4.0)	5.9 ^{2,3} (2.4–8.4)	2666 * ^{2,4} (1443–6979)	N/A	
B) Plasma								
Cocaine	75	17	305.4 ⁴ (108.6–434.1)	0.5 ⁴ (0.2–1.0)	4.5 ⁴ (3.3–5.9)	1010 ⁴ (656–2167)	3.2 (1.3–12.1)	
	150	13	654.9 ⁴ (253.5–1153.9)	0.5 ⁴ (0.1–1.0)	3.9 ⁴ (2.9–7.4)	2106 ⁴ (1325–2744)	4.7 (2.3–9.0)	
Benzoylecgonine	75	17	320.0 ⁴ (180.1–411.2)	4.0 (2.0–8.0)	8.1 (5.3–11.1)	5009 ⁴ (3787–6430)	0.32 (0.14–0.78)	
	150	13	635.5 ⁴ (336.3–832.0)	4.0 ⁴ (2.0–11.5)	8.6 (6.2–12.8)	9307 ⁴ (5998–14256)	0.36 (0.10–0.77)	
Ecgonine methyl ester	75	17	51.2 ⁴ (29.8–67.3)	2.1 (1.0–8.0)	6.7 (3.7–13.8)	711 ⁴ (400–1023)	1.6 (0.9–22.9)	
	150	13	117.7 ⁴ (70.1–338.9)	2.0 (1.0–11.5)	4.5 (3.0–12.0)	1364 ⁴ (814–2591)	1.8 (1.1–8.6)	

^a maximum concentration,

^b time of maximum concentration,

^c terminal half-life,

^d area under the curve_{0-last},

^e area under the curve_{0-last} oral fluid to plasma ratios,

^f not available. All statistical comparisons by Mann-Whitney test; p<0.05. Significant difference:

* low vs high dose,

¹ cocaine vs BE,

- ² cocaine vs EME,
- ³ BE vs EME,
- ⁴ oral fluid vs plasma.