Cocaine Disposition in Saliva Following Intravenous, Intranasal, and Smoked Administration

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Abstract

Saliva concentrations of cocaine, benzoylecgonine, ecgonine methyl ester, and anhydroecgonine methyl ester were measured by gas chromatography–mass spectrometry in six healthy male subjects following cocaine administration by the intravenous, intranasal, and smoked routes of administration. Cocaine appeared in saliva rapidly following all routes of administration. Saliva/plasma (S/P) ratios were generally greater than 1, and there was evidence of moderate to extreme contamination of saliva by cocaine immediately following intranasal and smoked routes of administration. Contamination of the oral cavity and saliva cleared rapidly. Saliva obtained 2 h after dosing appeared to be free of contamination and demonstrated S/P ratios comparable with intravenous administration. Benzoylecgonine and ecgonine methyl ester concentrations were consistently low and were only comparable with cocaine concentrations at times when cocaine concentrations had declined to below 100 ng/mL. Anhydroecgonine methyl ester was detectable in saliva following smoked drug administration, but it was quickly cleared. Terminal half-life estimates for cocaine administered by the intranasal and smoked routes were significantly shorter in saliva compared with those measured in plasma. Half-life estimates following intravenous administration tended to be lower for saliva than plasma, but the differences were not significant. The duration of pharmacologic effects was generally the same as or shorter than detection times of cocaine in plasma and saliva. Overall, the study demonstrated the usefulness of saliva as a test matrix for the detection and measurement of cocaine following administration by different routes of administration.

Introduction

The testing of saliva for drugs of abuse has distinct advantages and disadvantages compared with blood analysis. Because it is less invasive, saliva collection may be performed by nonmedical personnel in unusual settings not suitable for blood collection. For example, Peel et al. (1) collected saliva for drug testing in a police station from 56 drivers arrested for suspicion of impaired driving. The drugs detected in that study included alcohol, cannabinoids, diazepam, and cocaine. Another advantage is related to the general principal that pharmacological drug effects are attributed to the fraction of free extracellular drug concentration in blood. The concentration of drug in saliva is related to the free, nonprotein-bound drug in plasma, whereas the drug concentration in blood is the sum of both intracellular and extracellular bound and unbound drug (2). Consequently, saliva drug concentrations should more accurately correlate with pharmacological effects.

Disadvantages of saliva drug testing include the variable nature of saliva pH (3), the influence of collection devices and procedures on drug concentration (4), and the possibility of saliva being contaminated with drug residues in the oral and nasal cavity. A number of factors influence drug concentrations in saliva. The bulk (90%) of mixed saliva in humans is produced under resting conditions by the parotid and submaxillary glands (5,6). Other minor salivary and mucous glands and gingival fluid account for the remaining volume. The amount of saliva produced can vary from 500 to 1500 mL per day (6). Drugs enter saliva primarily by means of passive diffusion from systemic circulation across an epithelial cell membrane barrier. The rate of drug entry into saliva from plasma is governed by the concentration gradient of free un-ionized drug (2,7). Consequently, the physicochemical properties of the drug (pK_a, lipid solubility) may be major determinants of saliva drug concentrations and the resulting saliva/plasma (S/P) ratio.

Saliva testing for cocaine has been reported in a number of studies (8,9). Cocaine is a highly lipid soluble compound with a pK_a of 8.6 (8). Serum protein binding of cocaine appears to be relatively low and concentration dependent. Free cocaine in human serum is reported to increase from a ratio of 0.16 to 0.23 over the concentration range of 1–2500 ng/mL with no effect from the binding of benzoylecgonine, ecgonine methyl ester, norcocaine, and cocaethylene (10). These factors favorably influence the detection of cocaine in saliva following intravenous administration. Cocaine has been reported to appear in saliva almost immediately following intravenous administration, and concentrations over time were significantly correlated with plasma concentrations and with physiological and behavioral effects (11). The shapes of the saliva cocaine concentration curves were similar to the plasma curves resulting in equivalent half-lives of elimination.

Contamination of saliva from residual drug in the oral and nasal cavities can distort the S/P ratio and prevent accurate

estimation of plasma drug concentrations. The administration of cocaine by the smoked and intranasal routes would be expected to produce elevated S/P ratios until cocaine residues in the mouth are cleared. This report examines the effect of different routes of cocaine administration on saliva drug concentrations. Cocaine was administered in crossover studies by the intravenous, smoked, and intranasal routes to six human subjects. Detection times in saliva for cocaine, benzoylecgonine, ecgonine methyl ester, and a pyrolysis product (anhydroecgonine methyl ester) were determined by gas chromatography–mass spectrometry (GC–MS). S/P ratios were compared for the 12 h following drug administration to determine if the effects of oral contamination resolved sufficiently to accurately estimate S/P ratios.

Methods

Chemicals and materials

Cocaine hydrochloride was obtained from Mallinckrodt (St. Louis, MO). Cocaine base was prepared by the neutralization of cocaine hydrochloride with sodium bicarbonate followed by filtration and drying. The purity of the cocaine base was >99% by GC–MS analysis. Benzoylecgonine tetrahydrate and ecgonine methyl ester HCl were obtained from the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD). [$^2H_3$]-Cocaine, [$^2H_3$]-benzoylecgonine tetrahydrate, and [$^2H_3$]-ecgonine methyl ester HCl were purchased from Sigma Chemical (St. Louis, MO). Methanol, methylene chloride, 2-propanol, and acetonitrile (J.T. Baker, Phillipsburg, NJ) were high-performance liquid chromatographic-grade solvents. *N,O*-Bis (trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Pierce Chemical (Rockford, IL). Solid-phase extraction (SPE) columns (Clean Screen® DAU, 200 mg-10 mL and 130 mg-1 mL) and filtration columns (12 mL) were purchased from United Chemical Technologies (Bristol, PA). The SPE elution solvent was prepared fresh daily and consisted of methylene chloride/2-propanol/ammonium hydroxide (80:20:2, v/v/v).

Instrumentation

Quantitative analyses were performed on a Hewlett-Packard (Wilmington, DE) 5890A GC with an autosampler (HP7673A) interfaced with a Hewlett-Packard 5972 mass selective detector (MSD). A split/splitless capillary inlet system was operated in the splitless mode with electronic pressure control. An HP-1 fused-silica capillary column (12 m × 0.2-mm i.d., 0.33-µm film thickness) was used for cocaine and analyte analyses according to a published procedure (12).

Research subjects and study protocol

The participants were six adult males who provided written informed consent and were paid for their participation. The protocol was approved by the Francis Scott Key Medical Center Institutional Review Board and adhered to Federal guidelines for the conduct of research with human subjects. All subjects had a history of intravenous and smoked cocaine use. Subjects participated in four experimental sessions in which cocaine or placebo was administered in a randomized, cross-over design. Each session was separated by a minimum of three days. The study was performed over a period of approximately one year in 1992. The design of the study and detailed procedures have been described (13). Four cocaine/placebo combinations were administered as follows: placebo, saline (intravenous), lactose (intranasal); 25 mg cocaine hydrochloride (intravenous), lactose (intranasal); 32 mg cocaine hydrochloride (intranasal), saline (intravenous); and 42 mg cocaine base (smoked), saline (intravenous), lactose (intranasal).

Collection and analysis of saliva specimens

Saliva specimens were collected at various time intervals before and after each drug administration. Saliva was collected under stimulated (citric acid-type sour candy) conditions. The time needed for collection of saliva (5–10 mL) ranged from 3 to 5 min. Mixed saliva was collected in 50-mL, screw-capped polypropylene tubes. After collection, the saliva specimens were frozen at –30°C until the time of analysis which occurred approximately four years later. Cocaine was determined to be stable in saliva over this period by periodic analysis of control samples stored over this time. Recovery of cocaine from control samples typically was >90%.

Specimens were analyzed for cocaine, benzoylecgonine, ecgonine methyl ester, and anhydroecgonine methyl ester according to a published procedure (12). Briefly, saliva specimens (1 mL) were mixed with a solution of internal standards, followed by pH adjustment to pH 4.0 with acetate buffer. The mixture was centrifuged for 5 min. Specimens were decanted onto conditioned SPE columns and extracted. Cocaine analytes were eluted, the solvent was evaporated, and the residue was reconstituted in acetonitrile. Derivatizing reagent (20 µL of BSTFA with 1% TMCS) was added and the vials were sealed and heated at 60°C for 30 min. One microliter was injected for GC–MS analysis. Duplicate standard curves were processed along with specimens. The range of the standard curve was 1.1–500 ng/mL for each analyte. Multilevel control samples were processed in duplicate with each batch of specimens. The limits of detection by this method for cocaine, benzoylecgonine, and ecgonine methyl ester were approximately 1 ng/mL. Mean determinations (n = 8) for analytes in the control samples (100 ng/mL target concentration) and between-run coefficients of variation were as follows: cocaine, 100.4 ng/mL, 1.0%; benzoylecgonine, 105.1 ng/mL, 10.1%; ecgonine methyl ester, 102.4 ng/mL, 5.3%; and anhydroecgonine methyl ester (50 ng/mL target concentration), 46.8 ng/mL, 5.2%.

Pharmacokinetic analyses

Plasma and saliva data were fitted by nonlinear regression analysis with WinNonlin® (Scientific Consulting, Apex, NC) software. Pharmacokinetic parameters of cocaine, benzoylecgonine, ecgonine methyl ester, and anhydroecgonine methyl ester were obtained by use of model-independent methods. Plasma area under the curve (AUC) was calculated by the trapezoidal rule from 0 to 12 h after dose administration. The elimination rate constant (λ_z) was estimated by linear regression of the last 2–5 plasma or saliva concentration data points of the

terminal postdistribution phase. The terminal half-life ($T_{1/2}$) was estimated from $0.693/\lambda_z$. The AUC(0-∞) was calculated as follows:

$$AUC\ (0–\infty) = AUC\ (0–t) + C_t/\lambda_z$$

where t represents the time of the last data point and C_t represents the last point plasma concentration.

Statistics

Differences between pharmacokinetic parameters obtained from saliva and plasma were analyzed for statistical significance using a single-factor analysis of variance (ANOVA). The ANOVA was used to test the null hypothesis that there was no significant difference at an alpha level of 0.05.

Results

Saliva concentrations of cocaine and metabolites after intravenous, intranasal, and smoked routes of administration

Saliva cocaine concentrations and S/P ratios were highest in the first specimen (0.08 h) collected after intravenous cocaine administration (Table I). Peak concentrations ranged from 258 to 1303 ng/mL, and S/P ratios ranged from 1.3 to 10.1. Peak cocaine concentrations and S/P ratios following smoked and intranasal administration were frequently higher than those following intravenous administration as a result of contamination of the oral cavity. Subject C failed to inhale the complete cocaine dose by the smoking route and, consequently, exhibited lower saliva and plasma cocaine concentrations. Saliva cocaine concentrations declined rapidly after drug administration for all subjects by all routes and usually approached the limit of detection (LOD) of the assay (approximately 1 ng/mL) within 12 h.

Benzoylecgonine and ecgonine methyl ester usually appeared in saliva between 0.08 h and 1 h after cocaine administration. Peak concentrations occurred at 0.17 to 4 h and were consistently lower than peak cocaine concentrations. Typically, cocaine concentrations exceeded metabolite concentrations over the first 2 h after drug administration. Thereafter, cocaine concentrations declined rapidly and sometimes dropped below metabolite concentrations within 4–6 h. S/P ratios of benzoylecgonine ranged from 0.1 to 63.7.

Anhydroecgonine methyl ester was detected in saliva specimens collected following cocaine administration by the smoked route. Concentrations were highest immediately after smoking and declined rapidly. Concentrations generally declined to the LOD of the assay within 1 h of smoking cocaine. Artifact production of anhydroecgonine methyl ester occurred when cocaine concentrations exceeded approximately 3000 ng/mL, but it was not detectable when samples were diluted.

Pharmacokinetics estimates based on saliva drug concentrations

Estimates of half-life and AUC measures for cocaine and metabolites in saliva were obtained by noncompartmental analysis (Table II). For comparison, half-life and AUC estimates for these analytes in plasma from the same subjects were also included (13). Generally, half-life estimates for cocaine from saliva were lower than those obtained from plasma. However, the differences were only significant ($p < 0.05$) for cocaine half-lives determined following cocaine administration by the intranasal and smoking routes. Half-lives for benzoylecgonine from saliva also tended to be lower than corresponding plasma estimates, but these differences were not significant.

AUC estimates for cocaine in saliva appeared to be equivalent to those in plasma by the intravenous and smoked routes. However, the mean AUC for cocaine in saliva by the intranasal route was significantly higher ($p < 0.05$). Mean cocaine AUC ratios plus or minus the standard error of the mean (SEM) of saliva to plasma by the intravenous, intranasal, and smoking routes were 1.8 ± 0.61, 68.27 ± 58.27, and 2.28 ± 0.95, respectively. The mean benzoylecgonine AUC for saliva was significantly lower ($p < 0.05$) compared with plasma for intravenous cocaine. Mean AUCs for benzoylecgonine by the intranasal and smoking routes also tended to be lower for saliva compared with plasma, but the differences were not significant.

Half-life estimates and AUC measurements were determined for ecgonine methyl ester in saliva, but they could not be compared with corresponding plasma estimates because of the low abundance of this metabolite in plasma (13). Generally, both half-life estimates and AUC measures for ecgonine methyl ester in saliva were similar to corresponding estimates for benzoylecgonine in saliva.

Half-life estimates and AUC measurements were obtained for anhydroecgonine methyl ester after the smoking route in four of six subjects. Concentrations were insufficient in the remaining subjects for estimates of these parameters.

Discussion

Saliva has been proposed as a useful matrix for therapeutic drug monitoring of drugs that exist in plasma primarily in the un-ionized state (5). Drugs are distributed from plasma to other body compartments including saliva by passive diffusion, which is a process limited by the availability of free, un-ionized, nonprotein-bound drug (2,7). This free, un-ionized fraction distributes into other compartments at rates determined by the drug's physicochemical properties and the nature of the membrane barrier. The concentration of basic drugs in saliva is influenced by the acidity of saliva relative to plasma. Saliva pH typically ranges from 6.2 to 7.4 with the higher pHs occurring upon increased stimulation and saliva flow. These pH changes can have substantial influences on the basic drug concentration ($pK_a > 5.5$) in saliva and likely represent a source of considerable individual variability. The increase in salivary pH that occurs with stimulation appears to suppress the diffusion of cocaine into saliva. Kato et al. (4) reported significantly higher concentrations of cocaine in nonstimulated saliva compared with stimulated saliva. In that study, cocaine

Table I. Saliva Concentrations of Cocaine and Cocaine Analytes and Saliva/Plasma Ratios* Following Cocaine Administration to Six Male Subjects by the Intravenous, Intranasal, and Smoked Routes

Subject	Dose/ route	Time (h)	Cocaine (ng/mL)	S/P Ratio	BZE (ng/mL)	S/P Ratio	EME (ng/mL)	AEME (ng/mL)
C	25 mg/ IV	0	0	–†	0	–	0	0
		0.08	851	4.6	0	–	2	0
		0.17	153	0.9	0	–	2	0
		0.25	194	1.2	3	0.1	6	0
		0.33	117	0.7	3	0.1	7	0
		0.50	169	1.4	15	0.2	14	0
		0.75	187	2.1	28	0.3	21	0
		1.00	78	1.2	20	0.2	20	0
		1.50	66	1.6	21	0.2	24	0
		2.00	29	1.0	19	0.2	24	0
		3.00	32	2.0	25	0.2	33	0
		4.00	3	0.3	17	0.2	20	0
		6.00	0	–	4	0	13	0
		12.00	0	–	1	0	6	0
C	32 mg/ IN	0	0	–	0	–	0	0
		0.08	603	40.2	0	–	0	0
		0.17	250	8.1	0	–	0	0
		0.25	307	6.4	0	–	0	0
		0.33	291	6.1	0	–	0	0
		0.50	87	1.6	0	0	0	0
		0.75	99	1.7	2	0.1	5	0
		1.00	65	1.2	6	0.1	10	0
		1.50	91	1.4	25	0.2	23	0
		2.00	80	1.5	28	0.2	24	0
		3.00	31	1.0	21	0.2	23	0
		4.00	23	1.6	24	0.2	24	0
		6.00	2	0	12	0.1	14	0
		12.00	0	0	11	0.3	12	0
C	42 mg/ SM	0	0	–	0	–	0	0
		0.08	94	3.9	0	–	0	5
		0.17	–	0	–	–	–	–
		0.25	32	1.7	0	–	0	1
		0.33	14	1.0	0	–	0	0
		0.50	10	0.9	0	0	0	0
		0.75	6	0.8	0	0	0	0
		1.00	3	0.5	0	0	0	0
		1.50	0	–	0	–	0	0
		2.00	0	0	0	0	0	0
		3.00	0	0	0	–	0	0
		4.00	0	0	0	–	0	0
		6.00	0	–	0	–	0	0
		12.00	0	–	0	–	0	0
D	25 mg/ IV	0	0	–	0	–	0	0
		0.08	1303	–	0	–	0	0
		0.17	633	3.0	0	0	0	0
		0.25	–	–	–	–	–	–
		0.33	325	4.0	0	0	1	0
		0.50	313	4.2	12	0.2	12	0
		0.75	175	3.2	11	0.1	18	0
		1.00	199	4.4	18	0.2	22	0
		1.50	129	3.7	14	0.1	21	0
		2.00	93	3.9	11	0.1	32	0
		3.00	84	5.6	33	0.4	43	0
		4.00	52	6.5	34	0.4	50	0

* Abbreviations: S/P, saliva plasma ratio; IV, intravenous; IN, intranasal; SM, smoked; BZE, benzoylecgonine; EME, ecgonine methyl ester; AEME, anhydroecgonine methyl ester.
† Specimen was not collected, or determination of the S/P could not be made.

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Subject	Dose/ route	Time (h)	Cocaine (ng/mL)	S/P Ratio	BZE (ng/mL)	S/P Ratio	EME (ng/mL)	AEME (ng/mL)
		6.00	32	6.4	43	0.5	48	0
		12.00	0	–†	0	0	1	0
D	32 mg/ IN	0	3	–	0	–	0	0
		0.08	7141	264.5	7	–	0	0
		0.17	8024	200.6	13	–	0	0
		0.25	–	–	–	–	–	–
		0.33	59600	1324.4	178	–	44	20
		0.50	4829	100.6	0	–	0	0
		0.75	1073	22.8	0	0	0	0
		1.00	839	15.5	5	0.1	10	0
		1.50	265	5.1	27	0.2	34	0
		2.00	98	2.2	13	0.1	31	0
		3.00	118	3.6	48	0.4	59	0
		4.00	36	1.4	22	0.2	29	0
		6.00	26	1.8	46	0.5	46	0
		12.00	0	–	1	0	2	0
D	42 mg/ SM	0	0	–	0	–	0	0
		0.08	7737	24.9	0	–	16	775
		0.17	3520	16.6	0	–	18	264
		0.25	1504	8.2	0	0	35	77
		0.33	534	3.7	0	0	110	0
		0.50	579	4.7	6	0.1	24	20
		0.75	452	4.5	17	0.2	45	15
		1.00	413	5.2	28	0.3	34	11
		1.50	154	2.6	7	0.1	22	0
		2.00	114	2.1	14	0.1	27	0
		3.00	86	3.0	28	0.3	50	0
		4.00	24	1.0	13	0.1	22	0
		6.00	26	1.5	13	0.2	21	0
		12.00	0	0	0	0	0	0
E	25 mg/ IV	0	0	–	0	–	0	0
		0.08	357	1.3	0	0	0	0
		0.17	147	0.6	0	0	0	0
		0.25	105	0.5	0	0	0	0
		0.33	68	0.4	0	0	0	0
		0.50	48	0.3	0	0	0	0
		0.75	57	0.6	0	0	0	0
		1.00	34	0.4	2	0	0	0
		1.50	38	0.7	0	0	2	0
		2.00	24	0.6	0	0	0	0
		3.00	13	0.7	0	0	0	0
		4.00	1	0.1	0	0	0	0
		6.00	0	–	0	–	0	0
		12.00	0	–	0	–	0	0
E	32 mg/ IN	0	0	–	0	–	0	0
		0.08	49	0.8	0	–	0	0
		0.17	123	1.7	0	–	0	0
		0.25	173	2.1	0	–	0	0
		0.33	150	1.9	0	0	0	0
		0.50	185	2.0	0	0	0	0
		0.75	62	0.7	0	0	0	0
		1.00	26	0.3	0	0	0	0
		1.50	151	2.1	3	0	1	0
		2.00	92	1.3	20	0.1	11	0

* Abbreviations: S/P, saliva plasma ratio; IV, intravenous; IN, intranasal; SM, smoked; BZE, benzoylecgonine; EME, ecgonine methyl ester; AEME, anhydroecgonine methyl ester.
† Specimen was not collected, or determination of the S/P could not be made.

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Subject	Dose/ route	Time (h)	Cocaine (ng/mL)	S/P Ratio	BZE (ng/mL)	S/P Ratio	EME (ng/mL)	AEME (ng/mL)
		3.00	61	1.1	43	0.2	15	0
		4.00	22	0.7	16	0.1	6	0
		6.00	5	0.2	4	0	0	0
		12.00	–†	–	–	–	–	–
E	42 mg/ SM	0	0	–	0	–	0	0
		0.08	0	15.4	0	–	0	51
		0.17	657	6.3	0	–	0	9
		0.25	147	1.6	0	0	0	0
		0.33	65	0.8	0	0	0	0
		0.50	51	0.7	0	0	0	0
		0.75	39	0.7	0	0	0	0
		1.00	18	0.4	0	0	0	0
		1.50	14	0.4	0	0	0	0
		2.00	5	0.2	0	0	0	0
		3.00	0	0	0	0	0	0
		4.00	0	0	0	0	0	0
		6.00	0	0	0	0	0	0
		12.00	0	0	0	0	0	0
F	25 mg/ IV	0	0	–	0	–	0	0
		0.08	258	1.5	0	–	0	0
		0.17	131	0.9	0	0	0	0
		0.25	100	0.6	4	0.1	0	0
		0.33	73	0.5	7	0.2	0	0
		0.50	76	0.7	13	0.2	20	0
		0.75	116	1.3	23	0.3	27	0
		1.00	61	0.9	17	0.2	16	0
		1.50	62	1.0	20	0.2	19	0
		2.00	54	0.9	26	0.3	37	0
		3.00	39	0.9	24	0.3	31	0
		4.00	16	0.5	20	0.2	26	0
		6.00	0	0	10	0.1	4	0
		12.00	0	0	22	0.8	6	0
F	32 mg/ IN	0	0	–	0	–	0	0
		0.08	0	0	0	–	0	0
		0.17	10	0.1	0	–	0	0
		0.25	28	0.3	0	–	0	0
		0.33	29	0.3	0	0	0	0
		0.50	35	0.4	0	0	13	0
		0.75	75	0.9	11	0.2	29	0
		1.00	55	0.7	17	0.2	34	0
		1.50	47	0.8	23	0.2	35	0
		2.00	35	0.7	28	0.2	22	0
		3.00	50	1.0	45	0.3	40	0
		4.00	29	0.6	35	0.2	44	0
		6.00	11	0.3	31	0.3	18	0
		12.00	0	0	14	0.3	10	0
F	42 mg/ SM	0	0	–	0	–	0	0
		0.08	5016	34.1	0	–	0	116
		0.17	755	5.5	0	–	0	14
		0.25	311	2.1	0	0	0	7
		0.33	160	1.3	3	0.1	2	0
		0.50	112	1.3	7	0.1	4	0
		0.75	96	1.3	12	0.3	23	0
		1.00	62	1.0	11	0.2	32	0
		1.50	79	1.4	17	0.3	39	0

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Subject	Dose/ route	Time (h)	Cocaine (ng/mL)	S/P Ratio	BZE (ng/mL)	S/P Ratio	EME (ng/mL)	AEME (ng/mL)
		2.00	36	0.7	10	0.2	30	0
		3.00	26	0.7	14	0.3	39	0
		4.00	2	0.1	8	0.2	23	0
		6.00	1	0	15	0.5	21	0
		12.00	0	0	3	0.3	0	0
I	25 mg/ IV	0	0	–†	0	–	0	0
		0.08	533	3.3	2	–	0	0
		0.17	251	1.6	4	1.3	0	0
		0.25	122	1.0	6	0.3	0	0
		0.33	112	1.0	17	0.5	3	0
		0.50	102	1.2	15	0.3	4	0
		0.75	248	3.3	32	0.5	15	0
		1.00	121	2.1	30	0.3	14	0
		1.50	99	2.3	32	0.3	19	0
		2.00	68	2.1	32	0.3	20	0
		3.00	31	1.8	25	0.2	14	0
		4.00	32	3.2	39	0.4	18	0
		6.00	14	7.0	27	0.3	11	0
		12.00	4	–	19	0.5	0	0
I	32 mg/ IN	0	0	–	0	–	0	0
		0.08	71125	17781.3	859	–	32	0
		0.17	147436	8672.7	1912	–	84	0
		0.25	78568	2909.9	1018	–	67	0
		0.33	125380	3799.4	1931	–	154	0
		0.50	34141	794.0	637	63.7	86	0
		0.75	18945	386.6	589	10.2	106	0
		1.00	4351	85.3	217	2.3	72	0
		1.50	281	15.6	27	0.2	21	0
		2.00	175	17.5	47	0.4	40	0
		3.00	48	1.3	24	0.2	25	0
		4.00	75	2.4	35	0.3	29	0
		6.00	46	23.0	37	0.4	21	0
		12.00	18	–	18	0.4	1	0
I	42 mg/ SM	0	0	–	0	–	0	0
		0.08	12582	63.5	0	0	0	303
		0.17	3332	22.7	0	0	0	70
		0.25	974	4.6	9	0.6	2	19
		0.33	455	2.7	12	0.2	3	5
		0.50	326	2.5	15	0.2	7	3
		0.75	326	3.2	29	0.2	13	0
		1.00	252	3.1	37	0.3	19	0
		1.50	108	2.1	31	0.2	18	0
		2.00	176	3.8	49	0.3	29	0
		3.00	38	1.4	28	0.2	16	0
		4.00	24	1.5	28	0.2	15	0
		6.00	28	2.3	51	0.4	19	0
		12.00	5	–	21	0.3	0	0
J	25 mg/ IV	0	0	–	0	–	0	0
		0.08	842	10.1	3	–	0	0
		0.17	155	1.9	0	0	0	0
		0.25	140	1.6	0	0	0	0
		0.33	88	1.1	0	0	0	0
		0.50	181	2.9	9	0.2	7	0
		0.75	192	3.6	8	0.2	23	0
		1.00	146	3.2	6	0.1	2	0

* Abbreviations: S/P, saliva plasma ratio; IV, intravenous; IN, intranasal; SM, smoked; BZE, benzoylecgonine; EME, ecgonine methyl ester; AEME, anhydroecgonine methyl ester.
† Specimen was not collected, or determination of the S/P could not be made.

Table I continued. **Saliva Concentrations of Cocaine and Cocaine Analytes and Saliva/Plasma Ratios* Following Cocaine Administration to Six Male Subjects by the Intravenous, Intranasal, and Smoked Routes**

Subject	Dose/ route	Time (h)	Cocaine (ng/mL)	S/P Ratio	BZE (ng/mL)	S/P Ratio	EME (ng/mL)	AEME (ng/mL)
		1.50	128	3.2	1	0	20	0
		2.00	83	2.5	16	0.3	33	0
		3.00	81	2.8	8	0.2	19	0
		4.00	33	1.3	5	0.1	14	0
		6.00	34	1.8	17	0.3	26	0
		12.00	0	0	1	0	1	0
J	32 mg/ IN	0	0	–†	0	–	0	0
		0.08	3129	173.8	0	–	0	0
		0.17	36140	1290.7	390	–	0	0
		0.25	2921	85.9	22	–	0	0
		0.33	3438	83.9	0	–	7	0
		0.50	232	5.7	0	0	9	0
		0.75	155	3.9	0	0	34	0
		1.00	71	1.5	0	0	69	0
		1.50	122	2.9	10	0.1	44	0
		2.00	101	2.6	13	0.1	45	0
		3.00	77	2.3	13	0.1	58	0
		4.00	78	3.3	31	0.4	45	0
		6.00	42	2.1	23	0.3	34	0
		12.00	2	0.2	6	0.2	18	0
J	42 mg/ SM	0	0	–	0	–	0	0
		0.08	4075	39.2	0	–	0	142
		0.17	618	5.7	0	0	0	14
		0.25	291	3.4	0	0	0	5
		0.33	138	1.7	1	0	5	1
		0.50	124	1.8	7	0.1	3	0
		0.75	95	1.7	9	0.2	28	0
		1.00	121	2.6	16	0.3	0	0
		1.50	90	2.0	0	0	17	0
		2.00	10	0.3	0	0	0	0
		3.00	42	1.5	1	0	6	0
		4.00	8	0.4	0	0	0	0
		6.00	5	0.3	0	0	0	0
		12.00	0	0	0	0	0	0

* Abbreviations: S/P, saliva plasma ratio; IV, intravenous; IN, intranasal; SM, smoked; BZE, benzoylecgonine; EME, ecgonine methyl ester; AEME, anhydroecgonine methyl ester.
† Specimen was not collected, or determination of the S/P could not be made.

AUC ratios in saliva varied from 3.0 to 9.5 for six male subjects who received intravenous doses of cocaine. It was concluded that the method of collection of saliva is an important determinant of cocaine concentration.

The present study examined the potential effects of salivary contamination by the intranasal and smoking routes of administration. During intranasal cocaine administration, subjects inhaled cocaine through their nostrils. Large drug particles clung to the mucus membranes of the nostrils and turbinates where direct vascular absorption could occur. Smaller drug particles penetrated deeper into the respiratory tree and were trapped by mucus in the nasopharynx, trachea, bronchi, and terminal bronchioles. Tracheobronchial mucus was carried by epithelial cilia into the oropharynx. Consequently, cocaine became detectable in saliva following intranasal administration from both vascular absorption and subsequent salivary secretion and from respiratory contamination. Cocaine was also deposited in the mouth during smoking, which produced direct contamination of saliva. In the present study, the extent of contamination was highly variable by the intranasal route of administration. Subjects D, I, and J demonstrated extreme saliva cocaine contamination following intranasal administration and Subject C demonstrated moderate contamination. However, specimens of Subjects E and F had equivalent S/P ratios to those observed for intravenous administration. The smoked route of administration also produced contamination of saliva, but the degree of contamination was less than that observed for the intranasal route. Jenkins et al. (14) also reported saliva cocaine contamination when drug was administered by a computer-assisted smoking device. In the present study, intranasal cocaine contamination persisted longer than contamination by the smoked route. Elevated S/P ratios were apparent following the smoked route for approximately 0.25 h compared with 2 h for the intranasal route.

Despite contamination of saliva by cocaine as a result of intranasal and smoked routes of administration, S/P ratios obtained after 2 h appeared normal and were comparable with S/P ratios obtained by the intravenous route. Benzoylecgonine and

ecgonine methyl ester concentrations were consistently low and were only comparable with cocaine concentrations at times when cocaine concentrations had declined below 100 ng/mL. Anhydroecgonine methyl ester was detectable in saliva following smoked-drug administration but was quickly cleared. The pattern of cocaine and cocaine analyte disposition in saliva was consistent with an earlier report of cocaine administration by the intravenous and smoked routes of administration (14). It seems likely that the rapid clearance of cocaine contamination from saliva occurred as a result of ion trapping in the mouth because of saliva acidity. At a typical saliva pH of 6.4, the ratio of ionized cocaine to un-ionized cocaine would be > 150, based on the Henderson–Hasselbach equation. The rapid dissolution of cocaine in saliva would allow effective clearance by swallowing. Consequently, saliva measurements after 2 h appeared to be determined almost solely by passive diffusion into saliva from blood, despite initial elevated S/P ratios from oral contamination.

Determination of kinetic parameters for cocaine in saliva appears to have been the subject of only two earlier reports. Cone et al. (9) reported equivalent half-lives for saliva and plasma cocaine following intravenous-drug administration. Jenkins et al. (14) reported a shorter half-life for cocaine in saliva compared with plasma after smoking, but a longer half-life after intravenous administration was reported. In the present study, half-life estimates for cocaine in saliva and plasma were determined from the terminal elimination phase and were based on the last 2–5 data points. This approach was adopted to circumvent use of early cocaine measures that could have been affected by oral contamination. Half-life estimates for cocaine administered by the intranasal and smoked routes were significantly shorter for saliva compared with plasma. Half-life estimates following intravenous administration tended to be lower for saliva than plasma, but the differences were not significant.

Concentrations of cocaine in saliva after intravenous administration have been significantly correlated with physiological and behavioral effects and with corresponding plasma concentrations (9). In the present study, plasma specimens and pharmacologic measures were collected simultaneously with saliva specimens. The results of the analyses of plasma and a description of the pharmacologic effects have been reported (13). This presented the unique opportunity to compare detection times of cocaine in saliva and plasma with drug effects in the same subjects. Table III summarizes the time course of

Table II. Estimated Pharmacokinetic Parameters for Cocaine, Benzoylecgonine*, Ecgonine Methyl Ester*, and Anhydroecgonine Methyl Ester in Saliva and Plasma

		Cocaine					BZE					EME		AEME	
Subject	Route	Plasma† $T_{1/2}$	Saliva $T_{1/2}$	Plasma† AUC(0–∞)	Saliva AUC(0–∞)	AUC(0–∞)$_s$/ AUC(0–∞)$_p$	Plasma† $T_{1/2}$	Saliva $T_{1/2}$	Plasma† AUC(0–∞)	Saliva AUC(0–∞)	Saliva AUC(0–∞)$_s$/ AUC(0–∞)$_p$	Saliva $T_{1/2}$	Saliva AUC(0–∞)	Saliva $T_{1/2}$	Saliva AUC(0–∞)
C	IV	1.16	0.52	223.88	316.92	1.42	3.93	2.53	1140.74	114.87	0.10	4.62	216.92	–‡	–
D	IV	2.37	2.49	199.85	870.02	4.35	4.18	1.45	980.97	179.13	0.18	1.18	366.55	–	–
E	IV	1.32	0.37	280.50	147.52	0.53	4.10	–	1284.65	–	–	–	–	–	–
F	IV	14.89	1.03	945.88	261.52	0.28	4.20	2.66	934.67	247.79	0.27	–	–	–	–
I	IV	0.91	3.12	197.41	484.7	2.46	5.13	9.30	1214.04	566.26	0.47	3.02	136.94	–	–
J	IV	9.65	7.18	500.13	874.54	1.75	6.75	1.56	803.97	111.44	0.14	1.42	196.59	–	–
Mean		5.05	2.45	391.27	492.54	1.80	4.72	3.50	1059.84§	243.90§	0.23	2.56	229.25	–	–
SEM		2.39	1.05	120.27	128.03	0.61	0.44	1.34	74.88	77.01	0.06	0.80	48.81	–	–
C	IN	1.73	0.62	333.39	374.81	1.12	3.87	10.69	1395.31	333.70	0.24	12.11	383.25	–	–
D	IN	6.13	1.10	338.98	13523.93	39.90	5.60	1.87	1423.36	376.48	0.26	1.82	362.73	–	–
E	IN	7.42	0.94	575.42	361.84	0.63	5.58	0.95	1748.36	95.00	0.05	0.77	33.20	–	–
F	IN	10.18	1.37	836.29	230.39	0.28	4.72	5.44	1460.77	412.23	0.28	5.50	345.99	–	–
I	IN	0.61	4.19	153.24	54851.9	357.91	4.54	5.77	1299.20	1397.9	1.08	1.49	304.40	–	–
J	IN	9.35	1.45	475.54	4627.0	9.73	7.18	3.37	1182.94	273.48	0.23	5.92	557.46	–	–
Mean		5.90§	1.61§	452.14§	12328.31§	68.27	5.25	4.68	1418.32§	481.47§	0.36	4.60	331.17	–	–
SEM		1.61	0.53	96.53	8758.65	58.27	0.47	1.43	77.65	188.87	0.15	2.14	85.04	–	–
C	SM	1.73	–	30.34	22.33	0.74	–	–	2.48	–	–	–	–	–	–
D	SM	8.12	5.87	532.13	2041.92	3.84	5.69	6.46	1107.81	201.54	0.18	5.71	348.71	0.63	121.49
E	SM	12.21	0.22	468.28	295.54	0.63	1.82	–	285.25	–	–	–	–	–	–
F	SM	13.76	2.22	803.57	705.35	0.88	3.71	2.36	440.96	129.36	0.29	4.98	297.43	0.07	12.05
I	SM	3.27	2.41	335.72	2136.5	6.36	6.07	4.69	1985.30	558.5	0.28	2.79	157.45	0.04	34.41
J	SM	12.78	2.41	543.91	661.84	1.22	5.64	0.51	687.11	24.52	0.04	1.04	48.66	0.03	13.73
Mean		8.64§	2.63§	452.32	977.25	2.28	4.59	3.50	751.48	228.48	0.20	3.63	213.06	0.19	45.42
SEM		2.10	0.91	104.89	366.4	0.95	0.73	1.30	290.30	115.85	0.06	1.06	68.09	0.14	25.86

* Abbreviations: BZE, benzoylecgonine; EME, ecgonine methyl ester; AEME, anhydroecgonine methyl ester; $T^1/_2$, terminal excretion half-life; AUC (0–∞), area under the curve from zero time to infinity; IV, intravenous; IN, intranasal; SM, smoked; SEM, standard error of the mean.

† Data were obtained from reference 13.

‡ Pharmacokinetic estimates could not be made.

§ Means were significantly different ($p < 0.05$) for saliva compared with plasma.

detectability of cocaine in saliva and plasma compared with physiological effects (pupil diameter, heart rate) and behavioral measures (visual analogue scale of subject-reported high [VAS] and subjective rating scales of feeling drug [Subject Feel] and liking drug [Subject Liking]). Cocaine was detectable in saliva and plasma for 3–6 h at an arbitrary cutoff concentration of 25 ng/mL of cocaine. Detection times for saliva appeared to be slightly longer than plasma, which is consistent with S/P ratios > 1 as demonstrated in this study and earlier reports (9,11,14–16). The duration of pharmacologic effects was generally the same as or shorter than detection times of cocaine in plasma and saliva.

This report addressed the time course of cocaine and related analytes in saliva following administration of a single dose of cocaine. A previous report by Kato et al. (4) evaluated cocaine concentrations in saliva following multiple doses of cocaine administered by the intravenous route. Three doses were administered consecutively with each being separated by 3 h. Approximately 15% cocaine carryover (comparison of precocaine concentrations to peak concentrations) occurred from the first to the second and from the second to the third dose. Of greater significance was the steady accumulation of benzoylecgonine and ecgonine methyl ester in saliva with multiple dosing. These results suggested that future studies should be performed to determine if ratios of cocaine metabolites to cocaine in saliva would be useful for distinguishing individuals who consume a single dose of cocaine from those who have self-administered multiple doses over a short period of time.

Conclusion

This study demonstrated the usefulness of saliva as a test matrix for the detection and measurement of cocaine following administration by frequently used routes. The short detection time of cocaine in saliva paralleled the appearance and disappearance of pharmacologic effects. Based on these findings, it is anticipated that test methodology will be developed that will use saliva as a relatively noninvasive test matrix for cocaine detection in populations that are engaged in safety-sensitive activities, postaccident investigations, and roadside testing.

Table III. Duration of Cocaine's Pharmacologic Effects and Detection Times of Cocaine in Saliva and Plasma Following Single Doses of Cocaine by Different Routes of Administration

Route of administration	Subject	Cocaine saliva detection time (h)*	Cocaine plasma detection time(h)*,†	Pupil diameter change (h)†	Heart rate change (h)†	VAS ("high") change (h)†	Subject feel drug change (h)†	Subject liking change (h)†
IV‡	C	3	2	0.17	4.0	0.5	0.5	0.33
	D	6	1.5	0.33	0.17	0.08	1.5	0
	E	1.5	2	2.0	0.5	0	0	0
	F	3	6	12	1.5	0	0	0
	I	4	2	0.17	0.33	0.17	12	1.5
	J	6	4	0.33	0.75	1.0	0.75	0.75
Mean		3.92	2.92	2.50	1.21	0.29	2.46	0.43
SEM		0.74	0.71	1.92	0.59	0.16	1.92	0.25
IN	C	3	6	NC	NC	1.5	1.5	1.5
	D	6	4	0.25	0.5	2.0	2.0	2.0
	E	3	4	12	0.75	0	0	0
	F	4	6	0	1.0	0.02	0.17	0.17
	I	6	4	0	0.02	0	0	0
	J	12	3	0.5	0.17	2.0	0.75	1.5
Mean		5.67	4.5	2.55	0.49	0.92	0.74	0.86
SEM		1.38	0.50	2.16	0.16	0.42	0.35	0.37
SM	C	0.25	0.05	NC	NC	NC	0.17	0.17
	D	6	3	0.5	0.33	2.0	3.0	3.0
	E	0.75	2	12	0.05	0	0	0
	F	3	4	1.0	0.08	0.08	0	0
	I	6	3	0.5	0.25	0.25	0.33	12
	J	3	3	0.05	0.17	0.75	0	1.5
Mean		3.17	2.51	2.81	0.18	0.62	0.58	2.78
SEM		1.01	0.56	2.10	0.05	0.34	0.49	1.91

* Saliva and plasma detection times were determined as the time from drug administration to time of last cocaine measurement that was ≥25 ng/mL.
† Physiological and behavioral times were determined as the time from drug administration that the change remained ≥110% of pre-cocaine baseline measures. Data were obtained from reference 13.
‡ Abbreviations: IV, intravenous; SEM, standard error of the mean; IN, intranasal; NC, not calculated; SM, smoked.

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Manuscript received March 28, 1997;
revision accepted May 15, 1997.