

Dosage de la cocaïne et de ses métabolites dans les cheveux, la salive et les urines

Analysis of cocaine and metabolites in hair, oral fluid and urine

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RÉSUMÉ

Des révisions proposées aux directives fédérales incluant l'utilisation potentielle des cheveux, de la salive ainsi que des urines pour le dépistage des conduites addictives en entreprise ont été publiées en 2004. Cette étude a été conçue pour déterminer le taux de positivité de différents types d'échantillons, dans une population toxicomane et dans une autre niant sa consommation de cocaïne. Cette étude a été menée sur 200 sujets, la moitié admettant une consommation de cocaïne, l'autre moitié la niant. Chaque sujet a donné un échantillon d'urine, de salive et de cheveux prélevés au moment de l'entrevue. Pour chaque sujet, des informations sur leur consommation de stupéfiants, incluant le moment de la dernière prise et la fréquence de consommation, leur appartenance ethnique, leur âge, leur sexe et la couleur de leurs cheveux ont été enregistrés. Les échantillons ont été analysés pour la cocaïne et/ou ses métabolites selon la matrice. Les résultats sont présentés ci-dessous. Les cheveux ont identifié le plus grand nombre de toxico-

SUMMARY

Proposed revisions to the Federal guidelines, which include the potential use of hair and oral fluid, as well as urine, for workplace drug testing were published in 2004. This study was designed to determine the positivity rate in various specimen types, both in a drug using population and a population denying cocaine use. The study enrolled 200 subjects, half of whom admitted to cocaine use, half who did not. Each subject provided a urine sample, an oral fluid and a hair specimen taken from the head at the time of interview. Information on drug use, including time of last use, frequency of use, ethnicity, age, sex and hair color were recorded for each subject. The specimens were analyzed for cocaine and/or its metabolites depending on the matrix and the data is presented.

Hair identified the highest number of drug users in both the admitted using population and those who denied use. Oral fluid and urine gave similar detection rates in both populations, with oral fluid slightly better in the self-reported non-

manes dans les deux populations. La salive et les urines ont donné des taux de détection similaires dans les deux populations, avec une meilleure détection dans la salive pour la population se disant saine et une meilleure détection dans les urines pour la population s'admettant consommatrice de cocaïne.

C'est la première étude où des cheveux, de la salive et des urines ont été collectés simultanément sur une population toxicomane. Le cheveu est la matrice la plus appropriée pour identifier des consommateurs de cocaïne.

using population, and urine slightly better in the self-reported cocaine user population.

This is the first study where hair, oral fluid and urine were collected simultaneously from a drug using population. Hair is the most likely matrix to identify cocaine users.

Introduction

The purpose of our study was to determine which of the three matrices - hair, oral fluid or urine, identified the highest number of drug users in a population admitting to cocaine use, and in a population denying drug use. Proposed revisions to the Federal guidelines, which include the potential use of hair, sweat and oral fluid for workplace drug testing were published in the Federal Register in 2004 (1).

In our study, a hair was considered a positive if it contained at least 500 pg/mg of cocaine and at least 25 pg/mg of benzoylecgonine; or at least 50 pg/mg of cocaethylene or norcocaine, following a positive screening result. An oral fluid specimen was considered positive when at least 8 ng/mL of cocaine or benzoylecgonine was present following a screen positive result of greater than 20 ng/mL. A cumulative result, for example 4 ng/mL of cocaine and 4 ng/mL of benzoylecgonine was not considered positive.

Hair

Human hair grows at approximately 1 - 1.3 cm per month, and has been measured using various biomarkers (2). Hair, due to its growth rate and stability, offers a much longer history of drug exposure than any other matrix. Hair has been increasingly chosen as a workplace test specimen, since, in contrast to urine, the collection is observed, and the possibility of adulteration or substitution of the specimens is reduced. There are many publications regarding the incorporation of cocaine into hair (3-5). Cocaine itself is preferentially incorporated into the hair shaft, although several of its metabolites, including benzoylecgonine, norcocaine and cocaethylene have been detected and analyzed using well established procedures (6,7). For the screening of drugs in hair, both methanol and aqueous extraction procedures have been published (8); and for confirmatory analysis, gas chromatography with mass spectrometric detection is the preferred laboratory procedure (9), although LC/MS and LC/MS/MS are being increasingly employed in testing laboratories.

Oral Fluid

Oral fluid is a useful specimen for the determination of recent drug use, and in some cases drugs detected may be directly related to human performance. The main advantage of oral fluid over urine for workplace testing is that the collection of the specimen is observed, circumventing the additional expense of testing for adulterants in the laboratory (10). There are several review papers regarding the analysis of drugs in oral fluid (11), and there are a number of published articles specific to the disposition of cocaine in plasma and saliva following administration (12,13). Generally, cocaine and its metabolite benzoylecgonine are the predominant drugs detected in saliva. Oral fluid is an excellent specimen for analysis when recent drug use is suspected, such as in "for cause" or roadside testing.

Urine

Urine has been the accepted specimen for Workplace Drug Testing for many years, however, problems with adulteration and substitution of samples, due to collection not being observed, have prompted the need for other, or "alternative" matrices. These problems caused laboratories to increase their workload by testing for oxidants, nitrites or other adulterants (14,15). Urine is the most widely tested substance, and generally contains higher levels of drug metabolites than the other matrices. It is useful for random testing, and in probation and parole settings.

Materials and Methods

Subjects

The study enrolled 200 subjects, from the Drug and Alcohol Recovery Team (DART) in Fullerton, Orange County, Southern California. The study was approved under Immunalysis Institutional Review Board IRB # 2004-05-001. All subjects participating in the study were made aware of the purpose of the study, and signed a consent form. While information on drug use, including time of last use, frequency of use, ethnicity, age, sex and hair color were recorded for each subject,

names, addresses or other identifying information was not collected on the interview sheet. Complete anonymity was established during the sample collection and laboratory testing procedures.

One hundred of the enrolled subjects admitted cocaine use in the recent past, the others denied drug use. Each subject provided a urine sample, an oral fluid and a hair specimen taken from the head at the time of interview. Of the 100 volunteers who admitted cocaine use, 19% were females and 81% were male. 60% of the subjects were black, 23% were white, 11% were Hispanic and 6% described themselves as "other". The subjects ranged in age from 21 to 65 years old, and frequency of use ranged from daily to once a month.

Of the 100 volunteers who did not admit to cocaine use, 28% were female and 72% were male. 53% were white; 21% black; 14% considered themselves "other" and 12% were Hispanic. The age range was the same as for the drug users.

Experimental

Hair and oral fluid screening kits were obtained from Immunalysis Corporation (Pomona, CA). The Cocaine Direct ELISA Kit (Catalog # 229-0480) was used for screening the hair specimens and the Cocaine and Benzoyllecgonine Direct ELISA Kit (Catalog # 212-0480) was used for screening oral fluid specimens. Both were used according to the manufacturer's package insert instructions. For confirmatory procedures, deuterated d_3 -cocaine, d_3 -benzoyllecgonine (BZE) and d_3 -cocaethylene internal standards as well as unlabelled drug standards were obtained from Cerrilliant (Round Rock, TX). Solid phase mixed mode cation exchange - hydrophobic phase extraction columns (CSDAU020) were obtained from United Chemical Technologies Inc. (Bristol, PA). Clin-II 35 mg mixed mode, cation exchange - hydrophobic phase extraction columns were purchased from SPEWare (San Pedro, CA). The derivatizing agents pentafluoropropanol (PFPOH), pentafluoropropionic anhydride (PFPA) heptafluorobutyric anhydride (HFBA) were obtained from Pierce Chemical Co. (Rockford, IL). Trifluoroethanol was obtained from Aldrich (Milwaukee, WI). All solvents were HPLC grade or better, and all chemicals were ACS grade.

Hair

Screening Assay

The hair was cut into small segments (3-5 mm), and an aliquot of 10 mg was weighed. The hair was washed briefly with methanol (2 mL /10 min), the solvent was decanted and the hair was allowed to dry. To the hairs, 0.025 M monobasic phosphate buffer with 0.1% bovine serum albumin (BSA) (pH 2.7, 0.5 mL) was added,

the tubes were capped and incubated at 60°C for one hour. 0.5 M dibasic phosphate buffer (pH 9.0; 50 μ L) was added to neutralize the acid environment and the liquids were transferred to corresponding clean glass tubes. All specimens, calibrators and controls were then diluted 1:5 by adding 400 μ L of phosphate buffer saline (PBS with 0.1% BSA, pH 7.0) to 100 μ L of extract. An aliquot of the diluted hair extract (20 μ L), along with Horseradish peroxidase enzyme labeled cocaine derivative (100 μ L) was added to the individual micro-plate well coated with a cocaine specific antibody. After incubation (60 min, room temperature), the micro-plate wells were washed with deionized water (6 x 300 μ L). Tetramethyl benzidine (TMB, 100 μ L) was added to each well and the plate incubated in the dark for 30 minutes. The reaction was stopped using 1N hydrochloric acid (100 μ L) and the plate read at 450 nm with a reference wavelength of 620 nm.

In order to determine the limit of detection (LOD) of the assay, 16 replicates of hair specimens spiked with low levels of cocaine were analyzed using the ELISA procedure described. The minimum detectable concentration of cocaine was determined to be 15 pg/mg. The intra-day precision of the assay was determined by spiking 16 replicates of drug free hair at 50%, 100%, 150% and 200% of the recommended screening cut-off concentration for cocaine. At 500 pg/mg the coefficient of variation (CV) of the assay was 3.04%. Inter-day precision was determined by the analysis of eight replicates at each of the concentrations on three different days. The coefficient of variation at 500 pg/mg was 4.74%. The specificity of the kit was determined by analyzing structurally similar drugs to cocaine in the ELISA format. The percentage cross-reactivity with respect to cocaine response at 500 pg/mg was 9% for benzoyllecgonine, 133% for cocaethylene and 5.5% for *m*-hydroxybenzoyllecgonine. Benzoyllecgonine isopropyl ester displayed the highest degree of cross-reactivity at 200%. Other cocaine related compounds, e.g. nor-cocaine, ecgonine, ecgonine methyl ester were less than 5% cross-reactive to the antibody.

Confirmation Assay

Presumptive positive samples identified using the screening assay were carried forward to confirmation using gas chromatography-mass spectrometry (GC/MS) operating in electron impact mode. A separate aliquot of hair was weighed out (2 - 10 mg, depending on the numerical screening result) and methanol (1 mL) was added. The samples were incubated for 1 - 5 minutes at room temperature, and the methanol was discarded. The internal standard solution containing d_3 -cocaine, d_3 -benzoyllecgonine (BZE) and d_3 -cocaethylene was added to the hair samples to give concentrations of 500, 50 and 50 pg/mg respectively.

Low controls (200 pg/mg, for cocaine, 20 pg/mg for metabolites), a cut-off calibrator, and high controls (625 pg/mg for cocaine, 62.5pg/mg for metabolites), as well as a hydrolysis control at 5000 pg/mg of cocaine only, in order to monitor any cocaine conversion to BZE were included in every batch. A separate internal standard for norcocaine was not included since the retention time of cocaethylene and norcocaine were so close. The norcocaine was quantified using deuterated cocaethylene as the internal standard. An interference control comprising bupivacaine (10 µg/mL), mepivacaine, etidocaine, prilocaine, lidocaine, ephedrine and pseudoephedrine (5 µg/mL) was also included to ensure the reliability of peak identification.

0.1M hydrochloric acid (1 mL) was added to each of the washed hair specimens, and the tubes were heated at 75°C for two hours. The acid was decanted into clean glass tubes, 0.1 M phosphate buffer (pH 6.0, 1 mL) and 0.1 M sodium hydroxide was added until the pH was 6.0 - 6.5.

Solid-phase mixed mode cation exchange/hydrophobic extraction columns were placed into a vacuum manifold. Each column was conditioned with methylene chloride: isopropanol: ammonium hydroxide (80:20:1.8; 2 mL), methanol (3 mL) and 0.1 M phosphate buffer (pH 6.0; 2 mL). The samples were allowed to flow through the columns without vacuum. The columns were washed with deionized water (2 mL), 0.1M hydrochloric acid (2 mL) and methanol (3 mL), then allowed to dry at 15 psi for 5 min. The drugs were finally eluted using fresh methylene chloride : isopropanol : ammonium hydroxide (80:20:1.8; 2 mL) and the extracts were evaporated to dryness under nitrogen (15 psi, 60°C). Pentafluoropropanol (PFPOH; 50 µL) and pentafluoropropionic anhydride (PFPA; 50 µL) were added. The tubes were capped and heated (75°C, 15 min). The extracts were again evaporated to dryness and reconstituted in pyridine/ethyl acetate (1:4) (50 µL). The extracts were transferred to auto-sampler vials for GC/MS analysis.

Analytical Conditions (GC/MS)

An Agilent Technologies 6890 gas chromatograph coupled to a 5973 mass selective detector (MSD) operating in electron impact mode was used for analysis (GC/MS). The gas chromatographic column was 5% phenyl-95% methyl silicone DB-5, 0.20 mm ID, 0.33 µm film thickness, 12 m length (J & W Scientific, an Agilent Company, Palo Alto, CA) and the injection temperature was 250°C. The injection mode was splitless. The oven was programmed from 80°C for 30 seconds; ramped at 30°C/min to 220°C; then ramped at 10°C/min to 240°C, and finally at 50°C/min to 300°C, where it remained for two minutes. The transfer line

was held at 280°C. The ions monitored were 185 and 306 for deuterated cocaine; 182, 303 and 272 for cocaine; 424 and 303 for deuterated benzoylecgonine; 421, 316 and 300 for benzoylecgonine; 272, 212 and 196 for cocaethylene; 194, 313 and 214 for norcocaine.

A sample was not considered positive, unless it contained at least 500 pg/mg of cocaine, and also contained benzoylecgonine at a level greater than 5% of the cocaine value. The presence of either norcocaine or cocaethylene at a concentration greater than 50 pg/mg as well as 500 pg/mg of cocaine was also considered a positive result.

Oral Fluid

The oral fluid specimens were collected using a Quantisal collection device (Immunoanalysis, Pomona CA). When the absorbent collection pad had absorbed 1 mL of neat oral fluid (+/-10%), a blue dye was visible in the indicator window on the plastic stem of the collection pad. The pad was placed in a polypropylene transport tube containing 3 mL of extraction buffer solution, capped and taken to the laboratory.

Screening Assay

The Cocaine and Benzoylecgonine Direct ELISA Kit (Immunoanalysis Corporation, Pomona, CA) was used for screening the oral fluid specimens. In order to determine the limit of detection (LOD) of the assay, 16 replicates of negative and low level cocaine oral fluid specimens were analyzed using the ELISA procedure described. The minimum detectable concentration of benzoylecgonine was determined to be 1.25 ng/mL of neat oral fluid equivalents. The intra-day precision of the assay was determined by spiking 16 replicates of drug free oral fluid at 50%, 100%, 150% and 200% of the recommended screening cut-off concentration. At 20 ng/mL, the coefficient of variation (CV) of the assay was 9.27%. Inter-day precision was determined by the analysis of eight replicates at each of the five concentrations on three different days. The coefficient of variation at 20 ng/mL was 5.15%. The specificity of the kit was determined by analyzing structurally similar drugs in the ELISA format. The percentage cross-reactivity with respect to benzoylecgonine at 20 ng/mL was 80% for cocaine and cocaethylene. Other benzoylecgonine related compounds, e.g. norcocaine, ecgonine, ecgonine methyl ester were less than 10% cross-reactive to the antibody.

An aliquot of the diluted oral fluid specimen (40 µL) obtained from the Quantisal collection system was added to the individual micro-plate wells, and the assay was performed as previously described according to the manufacturer's package insert. Presumptive positive specimens were analyzed for cocaine and benzoylecgonine.

Confirmation Assay

Oral fluid + buffer (1 mL) was removed from the Quantisal collector. Deuterated internal standard was added to the calibrator and controls giving a concentration of 16 ng/mL, or an equivalent concentration to 4 ng/mL of diluted sample. Controls at concentrations of 4 ng/mL and 20 ng/mL were included in each confirmation batch. 0.1 M potassium phosphate buffer (pH 6.0, 1 mL) was added and the sample was mixed. Solid phase extraction columns (Clin-II, SPEWare) were conditioned with methanol (2 mL) and 0.1 M phosphate buffer (pH 6.0; 2mL). The samples were added and the columns washed with deionized water (2 mL); 0.1M hydrochloric acid (2 mL); methanol (3 mL) and ethyl acetate (3 mL). Glass collection tubes were placed into the sample rack and the drugs eluted with methylene chloride: isopropanol: ammonium hydroxide (78:20:2 v/v, 3 mL). The samples were evaporated to dryness under nitrogen.

Methylene chloride (40 µL), trifluoroethanol (20 µL), and heptafluorobutyric anhydride (HFBA, 20 µL) were added to the dried extracts, capped and allowed to equilibrate for 10 minutes. The samples were again evaporated to dryness in a vacuum oven and reconstituted in ethyl acetate (50 µL) for analysis using GC/MS.

Analytical Conditions (GC/MS)

A Shimadzu QP2010 instrument was used for analysis. The column was a RTX-XLB, Ultra low bleed, (Shimadzu Corporation, proprietary phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness. The injection volume was 2 µL in splitless mode and the injection temperature was 260°C. The column flow was 1.3 mL/min and the linear velocity 43.3 cm/sec. The oven was programmed from 130°C for 1 min, ramped at 25°C/min to 250°C, held for 3 min, ramped at 30°C/min to 310°C. The ion source temperature was 230°C and the transfer interface temperature was 250°C. The instrument was operated in Standard CI mode (positive ion) using methane as the reagent gas. *Note: using Shimadzu instrumentation, standard chemical ionization (SCI) is used as terminology for positive chemical ionization mode.*

The ions monitored were 307.1, 185.1 for deuterated cocaine (d3); 304.1, 182.1 for cocaine; 375.1, 253.1 for deuterated benzoylecgonine (d3); 372.1, 250.1 for benzoylecgonine. The linearity of the procedure was 0 - 32 ng/mL, and the limit of quantitation was 2 ng/mL for both cocaine and benzoylecgonine. A sample was not considered positive unless it contained at least 8 ng/mL of either cocaine or benzoylecgonine. A cumulative positive was not considered valid.

Urine

The DRI Enzyme Immunoassay Kit for Cocaine Metabolite, Benzoylecgonine (Diagnostic Reagents

Inc., Sunnyvale, CA) was used to screen the urine specimens at a cut-off value of 300 ng/mL according to the manufacturer's package insert. Presumptive positives were re-tested using the Cocaine Metabolite Direct Radioimmunoassay Kit (RIA, Immunalysis, Pomona CA). Since both immunoassay kits were specific to benzoylecgonine, a positive result on both was considered valid for the purposes of this study, to report a urine specimen as positive.

Results and discussion

The hair and oral fluid specimens were considered positive when they were confirmed using GC/MS procedures as described. In the self-reported drug using population, 17 individuals were not positive by any specimen type. Conversely, 7% of self-reported non-users were positive in all specimen types. The results are shown in Table I. In previously reported household surveys, under-reporting of recent drug use was noted and was particularly problematic for cocaine and heroin (16). Where possible, in those studies, all three specimens were collected from the subjects. Where multiple samples were collected, oral fluid collection had lower refusal rates than the other matrices (17).

In our study, there were no subjects detected by an oral fluid positive only, from the self-reported users; all positive oral fluid samples had a corresponding urine and/or hair positive specimen. In the self-reported non-drug using subjects, there were no individuals with both positive urine and positive oral fluid who did not have a corresponding positive hair. In fact, there were only two subjects who were positive for drug use (one oral fluid and one urine) who did not have a positive hair test in the non-drug using population. The individual who produced the positive urine had insufficient hair to confirm; the individual with the oral fluid positive (DT0197) also had a hair concentration of 390 pg/mg of cocaine 70 pg/mg for benzoylecgonine.

These data indicate that hair, when sufficient specimen is collected for analysis, provides substantially more

Table I : Analytical results from a population admitting cocaine use, and from subjects denying cocaine use.

	Self-reported Cocaine use	Self-reported No cocaine use	Overall
All matrices negative	17	73	90
All matrices positive	52	7	59
+ Hair only	13	12	25
+ Hair and oral fluid	5	5	10
+ Hair and urine	8	1	9
+ Urine only	2	1	3
+ Oral fluid and urine	3	0	3
+ Oral fluid only	0	1	1
Total	100	100	200

information on cocaine use than any other matrix collected in this study. This is in agreement with other publications, indicating hair identifies more positive drug users than the other specimens tested (18).

In total, 90 of the 200 subjects were negative in all sample types, but of the 110 individuals who were positive in at least one matrix, 103 (93.6%) had a positive hair assay, while 67.2% had positive urinalysis, and 66.3% a positive oral fluid test. Two hair specimens had insufficient specimen remaining for confirmation. Hair identified 78% of admitted users. Overall, in the admitted drug using population, the urine detected 65% of users, oral fluid 60%, although there were a further 11 samples which screened positively but there was insufficient volume to confirm, due to multiple positive screen results.

Among the self-reported non-drug using individuals, the urine was able to identify 9% of subjects as positive. Oral fluid was better with a 13% positive detection rate, but hair identified 25% of individuals who did not admit drug use (Figure 1). Including both populations, the hair identified a higher percentage of cocaine users than oral fluid or urine. Urine and oral fluid gave similar identification rates in the admitted drug using population, with oral fluid higher in those not admitting drug use.

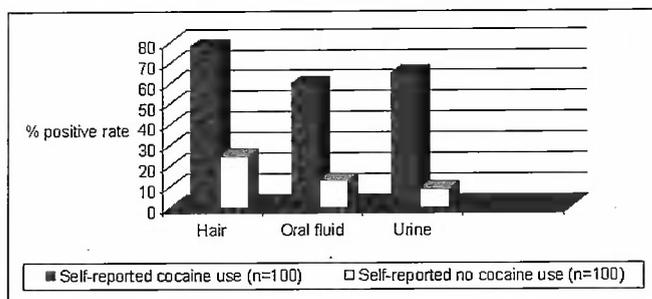


Figure 1 : Percentage of cocaine users identified by each specimen type.

Correlation

It is difficult to determine whether a correlation exists between the frequency of dose reported by the subjects and the amounts of drug and metabolites detected in the specimens. Variation in the amount of drug, purity of drug, time after sampling, as well as the truthfulness of subjects all affect the outcome. However, within this study group, which was predominantly of chronic users in the self-reported group, it is clear that hair is the best specimen to test in order to identify the highest number of drug users, whether or not self-reported.

Hair Color and Ethnicity

The hair color and ethnicity of the subjects was recorded at the time of the interview. The state of the art in hair testing is that it is most likely the melanin content of hair which is critical in determining the cocaine

content in hair, and not ethnicity. In our subjects, the predominant hair color was black across all ethnic types (56.1%). Dark brown and grey accounted for approximately 20% each of the subjects, with only one subject having blonde hair, and one light brown. Since there was little variation in hair color and melanin content, it was difficult to form any conclusions on the effect of pigmentation on cocaine incorporation. The ethnicity of the subjects also had little effect on the identification of cocaine users. Overall, 40.5% of all subjects were black, 38% were white, 11.5% were Hispanic and 10% described themselves as "other". Regardless of the ethnic group, hair identified the highest number of positive subjects, followed by similar rates for oral fluid and urine. Blacks accounted for the highest cocaine positivity rates in all specimen types. 63/103 (61.1%) of the total hair positives identified were from black subjects; 51/73 (69.8%) of the oral fluid specimens and 50/74 (67.5%) of the urine specimens. Whites accounted for 17.4% of the hair positives, 16.4% of the positive oral fluid specimens, and 17.5% of the positive urine samples. This suggests that positive rates, in this particular geographical area, may be related more to drug use patterns, rather than any kind of ethnic or method bias.

Concentrations detected in hair

The range of concentrations detected in hair was extremely wide (Table II), with both the mean and median values being substantially higher than the proposed cut-offs for regulatory samples. The mean cocaine level is over 100 times higher than the proposed cut-off of 500 pg/mg. Hair should be less affected by the timing of drug taking, than the other matrices, since the process of incorporation of the drug into the hair occurs over a longer time period than incorporation into urine or oral fluid. The highest cocaine level recorded was over 300,000 pg/mg. The subject, a black female, was 53 years old, and had gray hair. She stated that she used cocaine only once a week. Her hair contained 305,130 pg/mg of cocaine, 22,549 pg/mg of benzoylecgonine and 8,833 pg/mg of cocaethylene, but did not show the presence of norcocaine. The lowest positive level detected was from the black hair of a white male.

Table II : Concentration of cocaine and its metabolites detected in hair samples (n=103).

	COC (ng/mg)	BZE (ng/mg)	NC (ng/mg)	CE (ng/mg)
Low	0.51	0.054	0.059	0.058
High	305.1	33.7	8.0	17.6
Median	46.6	4.5	1.4	2.0
Mean	72.7	6.9	2.0	3.8
% of cocaine				
Median		9.6%	3.0%	4.3%
Mean		9.5%	2.7%	5.2%

His hair contained 54 pg/mg of benzoylecgonine and 509 pg/mg of cocaine. He did not admit to using cocaine. It is problematic to try to predict any kind of dose-response correlation due to the difficulties with self-reported drug use behavior.

Overall, benzoylecgonine was present at a median value of 9.6% of the cocaine concentration. Norcocaine, if present approximated 3% of the total cocaine, and cocaethylene, if present, accounted for 4.3% of the cocaine level (Table II).

A chromatogram from a hair specimen testing positive for cocaine, benzoylecgonine, norcocaine and cocaethylene is shown in Figure 2 (DT0011). The system would only allow 6 extracted ion chromatograms to be shown on one page, but the other ions were collected for each sample and used in the determination of positivity according to accepted GC/MS validation protocols.

The hair contained 170 pg/mg of benzoylecgonine, 1,400 pg/mg of cocaine, 71 pg/mg of norcocaine and 58 pg/mg of cocaethylene. This particular chromatogram was chosen, since it provided analysis of all four drugs at levels which could be seen on one scale. In many subjects, the cocaine level detected was so high that the norcocaine and cocaethylene present would need to be viewed on a separate mathematical scale.

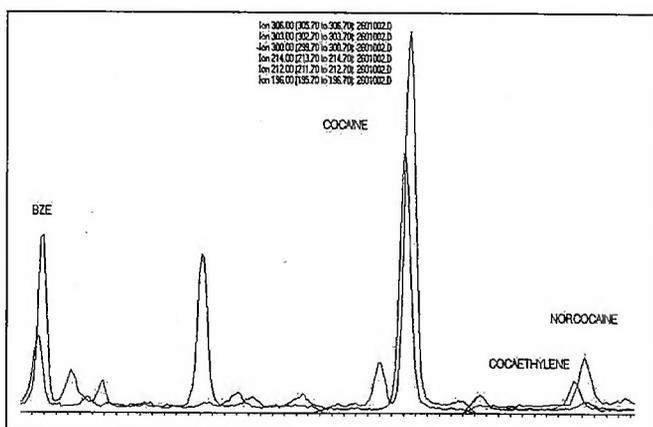


Figure 2 : Ion chromatogram of a positive hair specimen (DT0011).

Concentrations detected in oral fluid

In 2001, a report comparing saliva testing with urine testing among a criminal justice population was published, concluding that when using urine as the reference standard, the saliva test results indicated a sensitivity of 100% and a specificity of 99% for cocaine use (19). Other publications have concluded that urine and oral fluid provide similar positive rates (20) and prevalence information reported in 2005 concluded that in workplace populations, oral fluid and urine gave similar confirmation rates for cocaine metabolites: 1.31% and 0.93% respectively. When the standard five drug classes were considered, the confirmation rates for oral fluid

and urine were 4.91 % and 4.88% respectively (21).

In our study, the positive rates for oral fluid and urine were similar in both the admitted drug users and the non-drug using subjects. The range of concentrations measured in oral fluid was from low nanograms per milliliter to over 1000 ng/mL of both cocaine and benzoylecgonine. Obviously the timing of collection following recent cocaine use is a factor in the level detected. As expected, the highest levels of cocaine were detected in subjects admitting to very recent cocaine use.

The highest recorded oral fluid values for cocaine were over 1000 ng/mL in three individuals. Two of these subjects admitted using cocaine daily, and one admitted twice a week. As expected, all had used within hours of sample collection and the corresponding hair specimens were also positive.

Figure 3 shows an ion chromatogram of an oral fluid specimen positive for both benzoylecgonine (13 ng/mL) and cocaine (4 ng/mL). The subject admitted to using cocaine three times a week and his last intake was at 2 am on the day of collection. The samples were collected in the morning (between 8 and noon), therefore the subject, if truthful in his admission still had parent drug remaining in the oral fluid sample 6-8 hours after ingestion. The urine was also positive, as was his hair, with a cocaine concentration of 162,042 pg/mg and BZE present at 10,933 pg/mg (6.7%). No norcocaine or cocaethylene was detected.

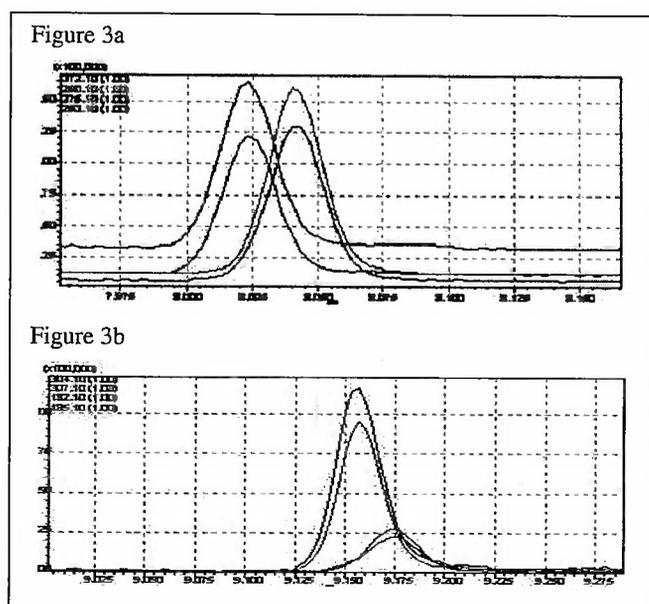


Figure 3 : Extracted ion chromatogram of a positive oral fluid specimen (DT0167)

3a. Benzoylecgonine 13 ng/mL (Internal standard concentration 16 ng/mL) d3-benzoylecgonine: Retention time: 8.02; Ions monitored: 375.1, 253.1

Benzoylecgonine: Retention time: 8.04 min; Ions monitored: 372.1, 250.1

3b. Cocaine 4 ng/mL (Internal standard concentration 16 ng/mL) d3-cocaine: Retention time: 9.15 min; Ions monitored: 307.1, 185.1

Cocaine: Retention time: 9.17 min; Ions monitored: 304.1, 182.1

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