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Analysis of Phencyclidine and Cocaine in Human Hair by Tandem Mass Spectrometry

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ABSTRACT: A confirmation procedure for the analysis of cocaine, benzoylecgonine, ecgonine, and phencyclidine (PCP) in human hair using tandem mass spectrometry has been developed. This procedure requires no solvent extraction and thus can examine the metabolites of drugs such as cocaine. Hairs from six cocaine users were examined and the amount of cocaine and its ratio to the metabolites was not correlated to the reported use pattern. Only PCP was detected in the hair from a PCP user, no metabolites were found. In passive exposure experiments, hair was found to tightly absorb PCP from aqueous solutions, which mimics the incorporation of PCP by the body. These results indicate that drugs may be absorbed onto the hair of nonusers and become tightly bound.

KEYWORDS: toxicology, cocaine, tandem mass spectrometry, hair

The analysis of hair for drugs of abuse has several advantages over the testing of urine. Some drugs, like morphine, have been shown to be stable in hair and not leached for long periods of time [*1*]. In addition, because drugs are often not entirely removed from hair by normal treatments, hair analysis can be repeated with another sample if the analytical results are questioned. In contrast, most drugs are rapidly cleared from urine within a day or two [*2*] and therefore consecutive urine specimens would not have the same concentrations of drugs due to metabolism and clearance. Because hair grows at a relatively constant rate of about 1 cm/month, [*3*] hair analysis also provides a long-term profile of drug use, which may aid as an independent check on an individual's veracity, if urinalysis results are called into question.

Hair analysis for cocaine and phencyclidine (PCP) has been performed by extraction and detection of the drug in the extract by radioimmunoassay [*1,4–8*]. Recent work on cocaine has concentrated on GC/MS confirmation of the drug of abuse in an extract of the hair [*6–8*]. Extraction is time consuming and requires 30 to 40 pieces of hair the size of a thin pencil (about 250 mg). Moreover, for cocaine, the strong acids necessary for extraction may degrade the cocaine. These degradation products are the same as the metabolites. Thus the metabolites cannot be determined directly, which may be useful in distinguishing passive from active exposure.

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Cocaine and benzoylecgonine ratios have been measured in hair after digestion of the hair with enzymes and extraction of the digest [*9,10*]. However, the digestion conditions used [*9*] were derived from those used to extract DNA from hair for forensic purposes [*11*] and produce a solution from which it is extremely difficult to extract benzoylecgonine. Likewise, none of the other metabolites of cocaine have been measured due to the difficulty of their extraction.

This research had three main objectives: 1) determine which metabolites were present in hair from cocaine and PCP users; 2) explore the use of thermal desorption tandem mass spectrometry to eliminate the problems associated with digestion of hair; 3) demonstrate that analysis can be performed on single hairs using tandem mass spectrometry and that the metabolites as well as the parent drug can be examined.

Experimental

Hairs were obtained from people entering a drug rehabilitation clinic in Los Angeles, CA. The hairs were cut from the apex of the back of the head at the scalp. A questionnaire was also obtained with some of the samples, listing the individual's self-reported drug history and hair-care habits.

Prior to analysis, hairs were washed in pentane by placing the hair in a test tube, filling with pentane, letting the hair soak for several minutes, removing the hairs with tweezers and allowing them to air dry. Pentane was chosen to mostly remove surface oils without extracting or removing the drug. The sample weight was estimated by weighing several hairs. An aluminum solids-probe cup, for mass spectrometry analysis, was cleaned by heating to 350°C in vacuum. After cleaning, the cup was removed from the vacuum and loaded with the hair sample. The amount of time that the cup was exposed to the atmosphere was minimized to reduce contamination. For preparation of the hair sample, one or two hairs were cut into 0.5 cm pieces, the sections were placed in the solids-probe cup, and then the solids-probe was heated at 110°C for 1 min in air to remove bound water. If all the moisture was not removed from the hair, the hair tended to be ejected from the cup into the ion source of the mass spectrometer by the expanding water vapor during the heating of the sample. For sectioning studies where the drug history of the user was obtained, five strands of hair were cut into 0.5 cm sections.

Instrumental conditions were:

Instrument: Finnigan-MAT TSQ 70, daughter mode or parent modes, 100 ms dwell time
Isobutane CI gas at 1.6 torr
Argon collision gas at 1 mtorr
Collisional energy: −20V (cocaine)
−15V (PCP)
Solids-Probe Heating: Initial temperature at 50°C for 0.5 min
Ramp temperature to 250°C in 0.5 min
Hold temperature at 250°C for 2 min
Ions monitored: *m/z* 244 ⇒ *m/z* 159 (PCP, daughter mode)
m/z 258 ⇒ *m/z* 173 (p-methylPCP, daughter mode)
m/z 304 ⇒ *m/z* 182 (cocaine, parent mode)
m/z 290 ⇒ *m/z* 168 (benzoylecgonine, parent mode)
m/z 186 ⇒ *m/z* 82 (ecgonine, parent mode)

The PCP concentration was determined by comparing the peak area to that of the internal standard. The internal standard for PCP was introduced by pipetting into a clean, solids-probe cup an aqueous solution containing 200 pg of 1-p-methylphenyl-1-cyclohexylpiperdine (p-methylPCP) and dried by gentle heating. Then the hair was introduced and the solids-probe heated to dry the hair, as above.

The cocaine, benzoylecgonine, and ecgonine concentrations were determined from the peak areas of external standards. Known amounts of the materials were introduced into the instrument and analyzed. Then the hair samples were analyzed. Finally, more standards were introduced and analyzed. This procedure is necessary for external standards because the amounts of daughter ions produced are very sensitive to instrumental tuning and can vary substantially with time as the ion source becomes dirty.

Results and Discussion

Tandem mass spectrometry has been used before in the analysis of morphine in hair [*12*]. However, the hair was dissolved in strong base and the morphine extracted before the analysis. Although this technology could be applied to the analysis of PCP, it would not be applicable to the analysis of cocaine because cocaine and its metabolites would be degraded to ecgonine by the strong base needed to dissolve the hair. As stated in the introduction, more gentle conditions using enzymes produce solutions from which it is very difficult to extract benzoylecgonine and methylecgonine and impossible to extract ecgonine. Also, until this study, the distribution of the metabolites were unknown, therefore the extraction conditions may vary depending upon the metabolite targeted.

The principles behind tandem mass spectrometry have been reviewed [*13*]. Briefly, tandem mass spectrometry consists of two mass spectrometers connected in series (see Fig. 1). A sample is introduced into the first mass spectrometer by any number of methods. For hair analysis a solids-probe is used and the sample is heated to vaporize the volatile components. The volatile components are ionized, usually by chemical ionization, to generate mostly protonated molecular ions of the many species present. If possible, the reagent gas used in chemical ionization is chosen to give some selectivity to the ionization process, that is, the least aggressive reagent gas is used. Once the molecular ions are generated, one mass is selected and transmitted out of the first mass spectrometer into a collision region where energy is imparted to the ion by a collision with a neutral gas. This energy causes the ion to fragment in a specific manner and the fragments (daughters) of the ion are detected by the second mass spectrometer. During the analysis, various molecular ions may be passed sequentially by the first mass spectrometer. In this manner, several compounds can be analyzed simultaneously.

A tandem mass spectrometer may be scanned in a number of different modes. The most common are daughter and parent scans. As used in these analyses with selected ion monitoring, both scan modes give equivalent spectra. However, they can produce

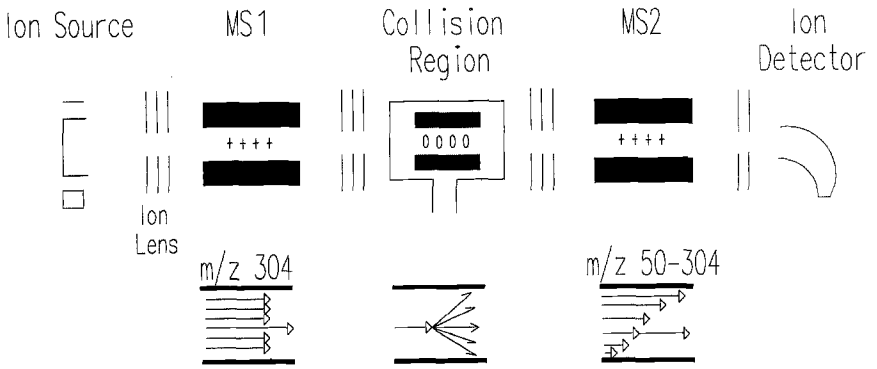

FIG. 1—*Principle of tandem mass spectrometry.*

different sensitivities depending on which quadrupole is of a higher quality due to manufacturing and cleanliness. Both modes were tested and the one that produced the greater sensitivity, at a given time, was chosen for the analysis.

Selectivity is gained in three areas with tandem mass spectrometry. First, not all the volatile components are ionized with the reagent gas employed. Second, the first mass spectrometer transmits only selected ions into the collision region. Third, the fragmentation of these selected ions produces unique daughter ions that are detected by the second mass spectrometer.

The daughter spectra of PCP and the internal standard p-methyl PCP are shown in Fig. 2 and the daughter spectra of cocaine, benzoylecgonine, and ecgonine are shown in Fig. 3. The starred peaks were monitored by selected ion monitoring.

Typical desorption profiles for cocaine and its metabolites are shown in the upper portion of Fig. 4. A typical desorption profile for blank hair is shown in the lower portion of Fig. 4. The signal level for the blank hair is about 100 times lower than the intensity of the ions recorded for the drug containing hair.

The levels of cocaine and its metabolites from the hair of different users are given in Table 1 along with some use histories. Only one hair sample from a PCP user was available. It showed a PCP level of 16 ng/mg hair. Although PCP is known to be highly metabolized before excretion in urine, [*14,15*] no hydroxy metabolites of PCP could be detected in hair by tandem mass spectrometry. The hydroxy metabolites were searched for by admitting the MH^+ of the expected hydroxy metabolites at *m/z* 260 and scanning for the characteristic loss of 85 from the protonated molecular ion due to the piperidine ring. This loss is observed in most PCP derivatives by chemical ionization [*16,17*]. The production of the protonated piperidine ion at *m/z* 86 was also examined. However, most PCP metabolites are conjugated to either sulfate or glucuronic acid. It is not known if these metabolites would desorb under the conditions employed, although other glucuronides, such as morphine-3-glucuronide, have been observed from a solids probe as the aglycone portion [*18*].

What is most noticeable from Table 1 is the wide variation in the ratio of cocaine to its metabolites. For example, the ratio of cocaine/benzoylecgonine varies from 1 to 14, a factor of 14, whereas the ratio of the two metabolites varies from 0.3 to 1.7, a factor of 5.6. The wider variation in ratio of cocaine/benzoylecgonine could be explained by passive exposure to cocaine during its use, which is then incorporated into the hair or by biologically variability.

Section Analysis

Hair grows at an approximate rate of 1 cm/month [*3*]. As it grows, hair records the history of drug use by the individual [*1*]. To confirm this observation, several hairs were sectioned in 1 cm pieces from a cocaine user with a constant history of cocaine use. The concentration of cocaine and its metabolites found in this hair are shown in Fig. 5. As can be seen, the concentration of cocaine and benzoylecgonine remains relatively constant with time (length), whereas the concentration of ecgonine increases with time. Although morphine has been shown to be stable in hair for long periods of time, [*7*] cocaine has not. The increase in ecgonine levels may reflect degradation of cocaine, benzoylecgonine, or methylecgonine (which was not quantitated) to ecgonine with time or environmental exposure. The noticeable depression in Fig. 5 at 3 cm may be due to an instrumental artifact related to instrumental detuning, caused by contaminants from the hair. However, the relative ratio of cocaine/benzoylecgonine/ecgonine within a section should be unaffected. Deuterated internal standards would have produced more accurate quantitation, but these were unavailable at the start of this work. No cocaine or metabolites were

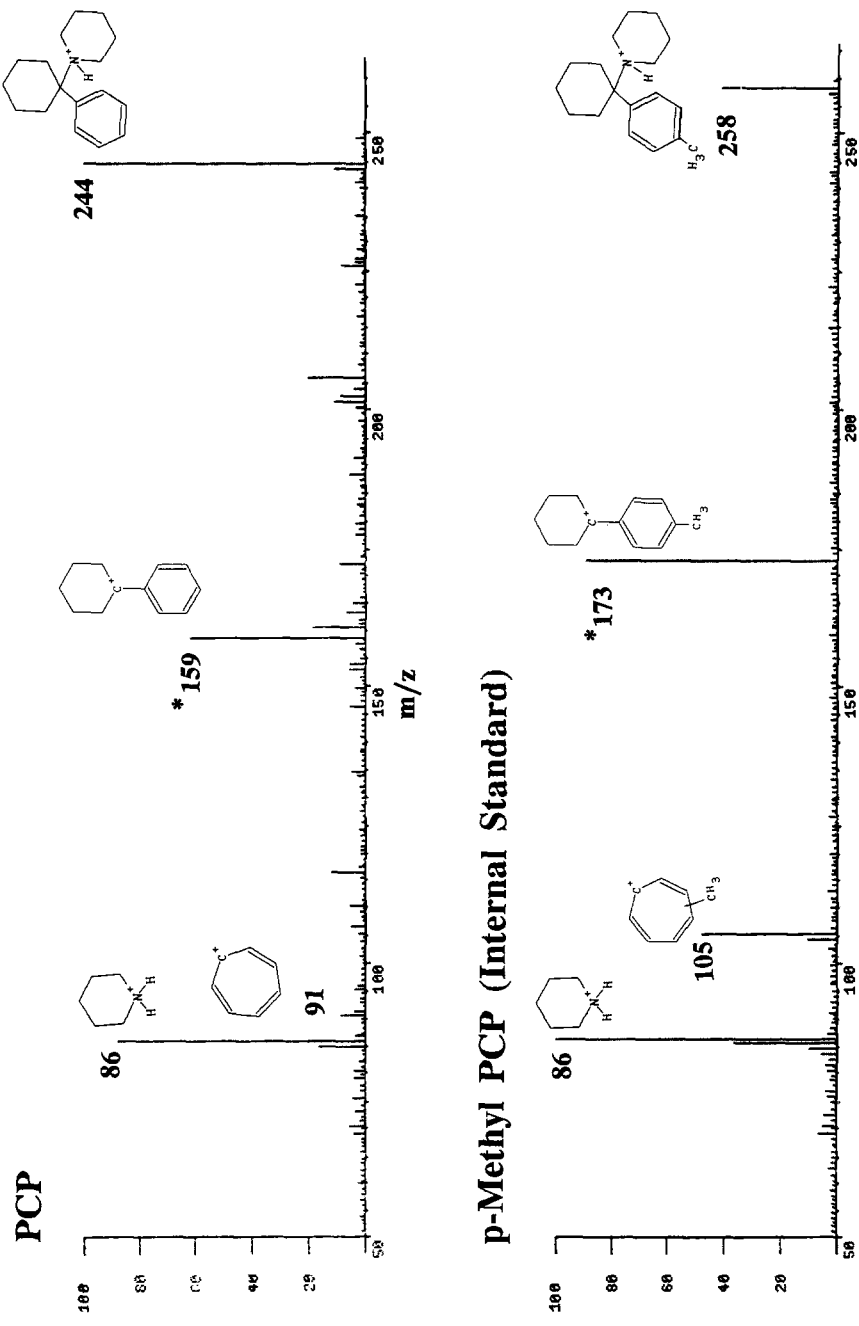

FIG. 2—*Daughter spectra of PCP and the internal standard p-methylPCP.*

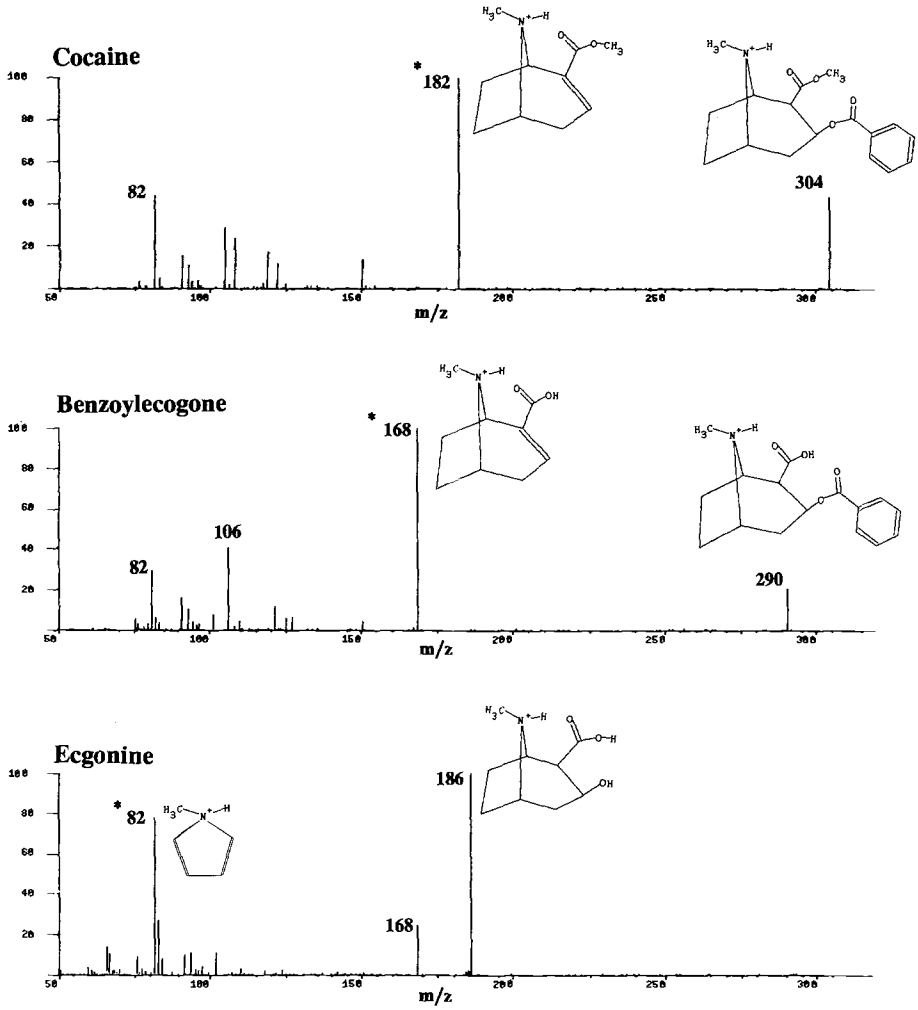

FIG. 3—*Daughter spectra of cocaine, benzoylecgonine, and ecgonine.*

observed by section analysis of another specimen from a user that reported a single use of cocaine several months prior to obtaining the hair. This negative result may be due to lack of sensitivity or removal of cocaine with time.

Experimental Precautions

Although an excellent filter, a tandem mass spectrometer can be overwhelmed with ion signals in certain cases. In early work, the hair was heated to 350°C. As shown in the upper portion of Fig. 6, high blanks resulted in several cases, and it was difficult to distinguish the cocaine. Not all blank hair produced these interferences. Interferences also were observed in some scans for benzoylecgonine, ecgonine, and PCP. A lower, final probe temperature resulted in much improved signal/noise ratios (Fig. 6, lower). In addition, cleaning of the ion source must be performed less frequently with the lower heating temperatures.

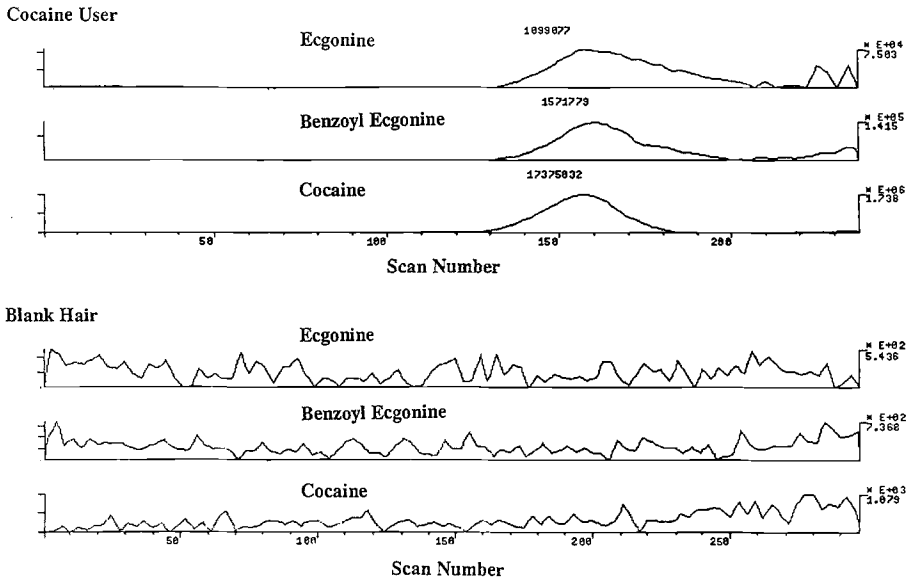

FIG. 4—*Typical tandem mass spectrometer results for a heavy cocaine user and a typical blank hair sample. The spectral intensity for the hair containing cocaine is at least 100 times that for the blank hair. Integrated areas are shown above the peaks. Each scan was approximately 1 s.*

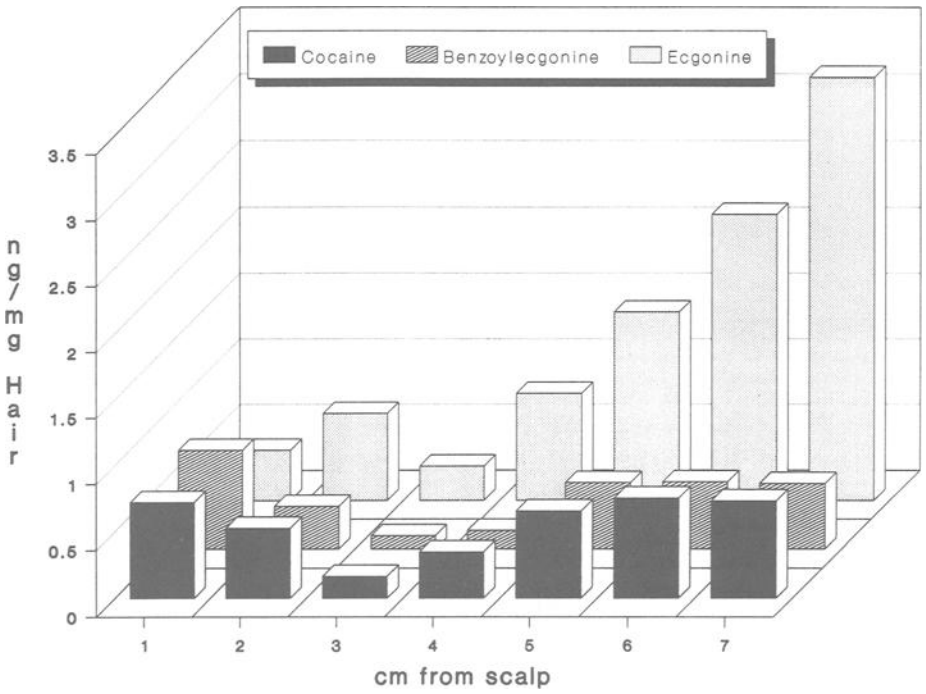

FIG. 5—*Concentration of cocaine, benzoylecgonine and ecgonine in a user's hair. The intensity decrease at the third section was probably due to problems in the quantitation. The relative amounts of cocaine and the metabolites at this section would not be affected.*

TABLE 1—*Amount of cocaine and its metabolites found in various user's hair.*

User number	Use type	Cocaine ng/mg hair	Benzoylecgonine ng/mg hair	Ecgonine ng/mg hair	Ratio Coc/BE	Ratio BE/EG
1	Unknown	2.0	1.9	1.1	1	1.7
2	Unknown	98	8.9	6.2	11	1.4
3	I.V. 0.5 g/day	40	2.9	9.2	14	0.3
4	Smoking 1–2 g/day	12	2.8	5.6	4.3	0.5
5	Smoking	6.8	5.1	5.9	1.3	0.9
6	Single use		Negative by whole hair and section analysis			

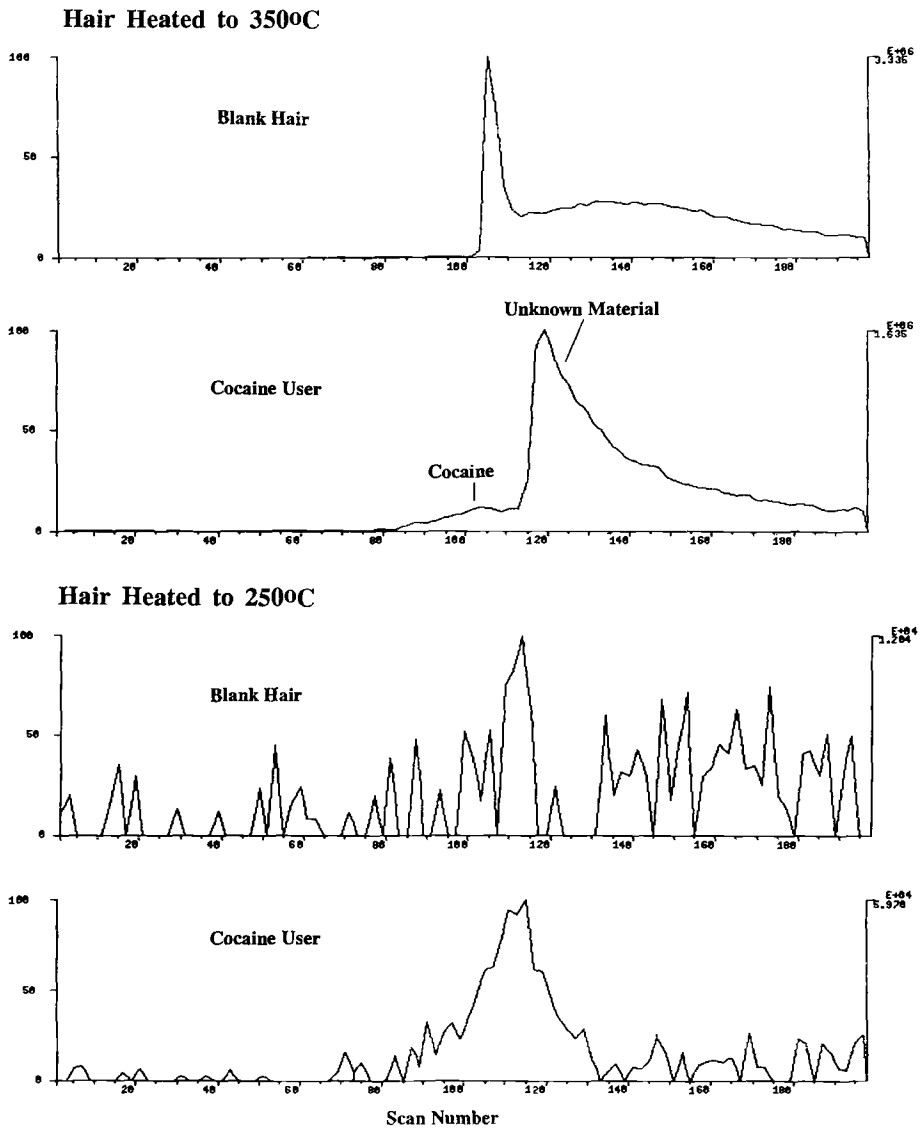

FIG. 6—*Interferences observed with inappropriate heating temperature of hair from a cocaine user. The hair in the upper graph was heated to 350°C, the lower to only 250°C. Note the very high background due to breakdown of the hair matrix. Each scan was approximately 1 s.*

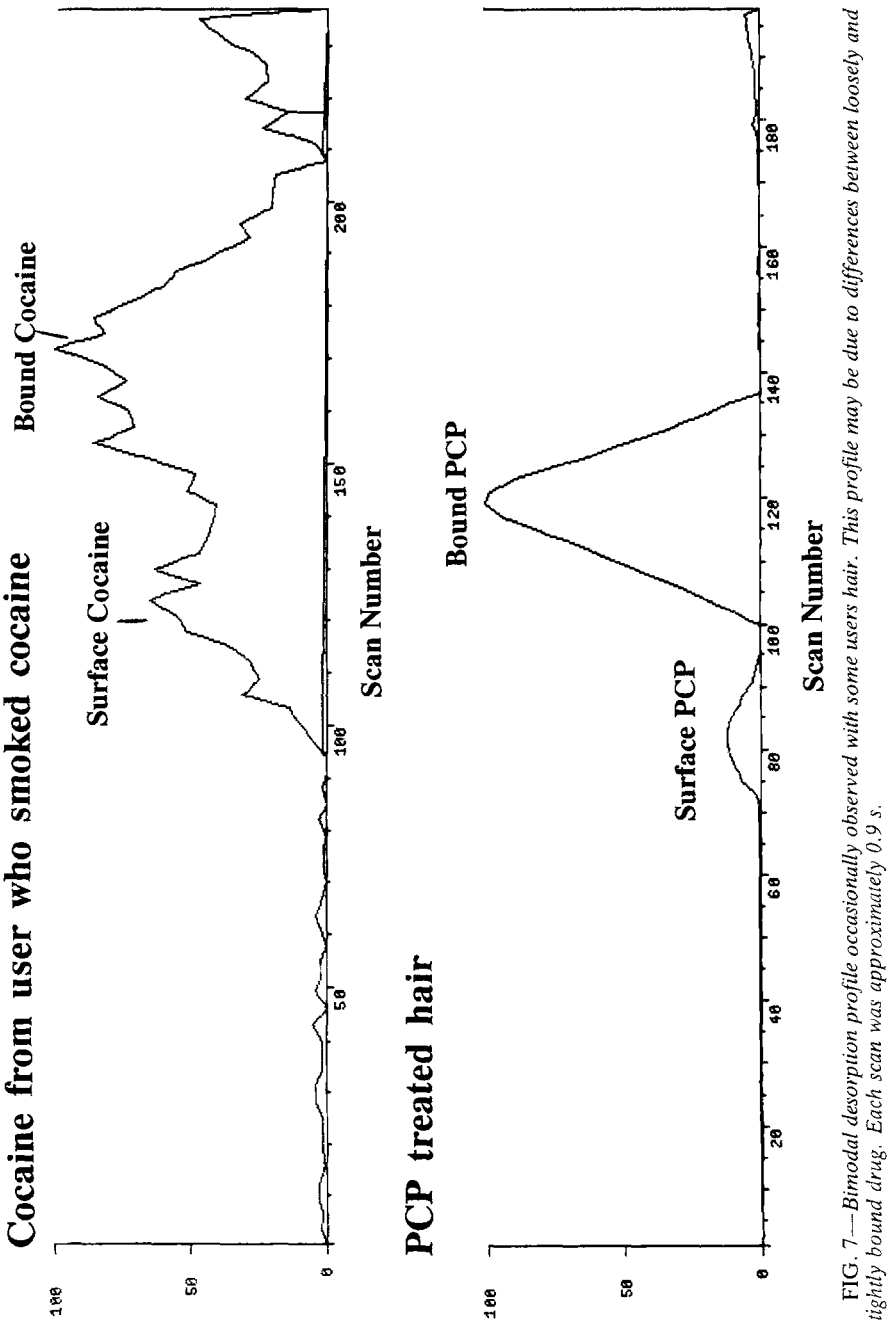

FIG. 7—*Bimodal desorption profile occasionally observed with some users hair. This profile may be due to differences between loosely and tightly bound drug. Each scan was approximately 0.9 s.*

Passive Incorporation

Extensive washing of the hair is done by a number of investigators to diminish the possibility of external contamination of the hair [*19*]. For example, entering a room where cocaine is smoked could contaminate a nonuser's hair with cocaine and falsely indicate use. Because hair is frequently washed in normal hygiene, it is difficult to understand why this is less effective than laboratory procedures in removing surface bound drugs. Therefore, these laboratory decontamination procedures were not used.

Occasionally a bimodal desorption profile is observed, as shown in Fig. 7. Loosely bound cocaine and PCP have a different desorption profile than that bound in the hair shaft. These desorption profiles result from slowly ramping the solids-probe. Because hair has some thermal resistance, the drug on the surface is desorbed before the drug in the center of the hair. The surface absorbed drug is assumed to be that resulting from passive exposure, however, because the loosely absorbed drug can be distinguished from that tightly bound, washing of the hair may be unnecessary using this technique.

To test if drugs could be passively incorporated into hair, two types of undamaged and unbleached hair, light brown hair and thick black hair, were soaked in a solution of PCP for 1 h, removed, and allowed to dry. As shown in Fig. 8, only strong acid or base

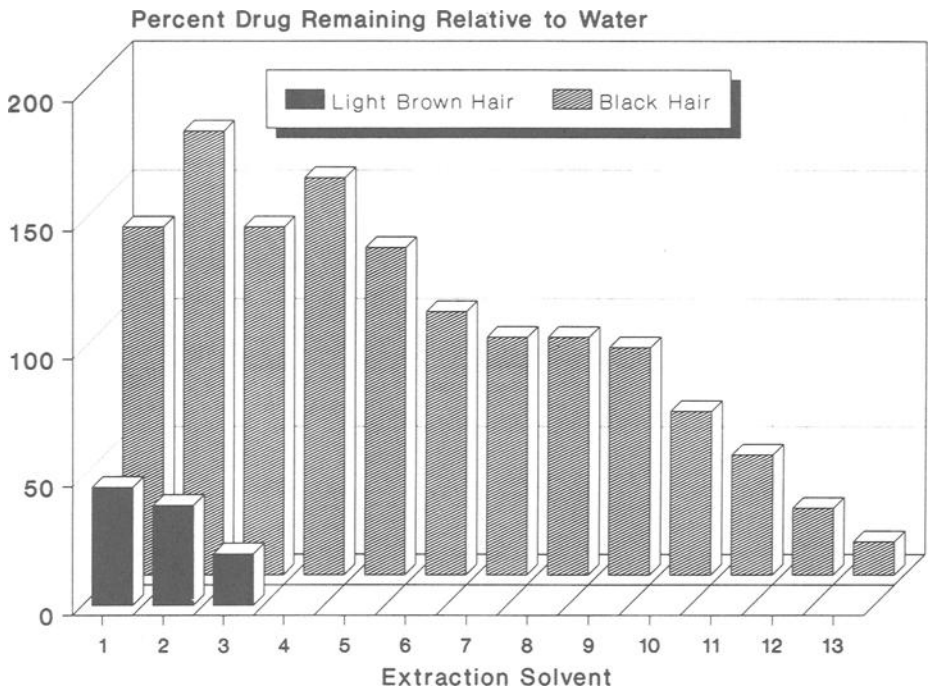

FIG. 8—*PCP remaining in blank hair soaked in PCP after various wash solutions. The amounts of PCP remaining in the hair are relative to water washing. Only the first three solutions were tested on the brown hair. 1. = Triton X100, 2. = sodium dodecyl sulfate, 3. = dodecyltrimethylammonium chloride, 4. = tris hydrochloride, 5. = calcium chloride, 6. = triethylammonium chloride, 7. = p-Bromo PCP, 8. = methylammonium chloride, 9. = magnesium sulfate, 10. = sodium chloride (1M), 11. = phosphate buffer pH 7, 12. is 0.1M HCl, 13. = sodium carbonate. Except as noted, all concentrations are 0.1M.*

removes appreciable amounts of PCP. Some solutions appear to be poorer extractants than distilled water, which may be due to the swelling of the hair varying with the soaking solution. As these data indicate, drugs were removed from the brown hair much more readily than from the black hair. In Fig. 8, the brown hair was only treated with the first three solutions since these effectively removed the drug. It is interesting to note that the PCP derivative, 1-p-bromophenyl-1-cyclohexylpiperdine (p-bromoPCP), did not displace significant amounts of PCP from the binding sites in the hair. Because hair can be exposed to an external source of PCP, such as a solution, and the drug bound tightly into the hair, external contamination appears to be a significant problem in correlating the amount of drug in hair with the amount ingested. Subsequent experiments have confirmed these results with cocaine and its metabolites. They have been reported elsewhere [*20,21*].

Often hair soaked in solutions of PCP did not show the bimodal distribution as depicted in Fig. 7. This may be due to hydration and swelling of the hair. Hair hydrates up to 20% of its weight when wet and swells [*22*]. Swelling may allow the drug solution to penetrate the interior of the hair, depositing the drug in the interior when the hair is dried. Alternatively, all loosely bound adsorbed PCP was removed by washing leaving only the interior absorbed PCP for analysis. Limited experiments suggest that the uptake and release of drugs in hair depend on the hair type and the environmental damage that the hair has received. Thick, black hair takes up drugs slower from solution and releases them slower. Hair damaged by exposure to sunlight has increased uptake of drugs. This makes relating the quantity of drug in hair to the amount ingested difficult to determine. These results and other data showing incorporation of fluorescently labeled drugs into the interior of hair have been presented elsewhere [*19*]. Other workers have also reported a variation in drug concentrations with damaged hair [*18*].

Another method to distinguish between passive exposure and active incorporation is by examining metabolites. For PCP, no hydroxy metabolites were found. Metabolites were present for cocaine, but these metabolites are the same species produced by degradation. A preliminary experiment was performed to determine the stability of cocaine in hair. Cocaine was incorporated by soaking the hair as for PCP above. The hair was then exposed to 0.1M sodium carbonate solution (pH ca. 12) for 1 h. Tandem mass spectrometric analysis of this treated hair showed only cocaine to be present. No appreciable concentrations of benzoylecgonine or ecgonine were found. However, hair may be subjected to such degradents as UV light, shampoo, bleach, or perming solution. Since the environmental conditions may be quite varied among individuals, these potential degradents were not examined.

Conclusions

A confirmation procedure for cocaine, its metabolites and PCP in human hair using tandem mass spectrometry has been developed. This procedure requires no extraction and can examine the metabolites of cocaine. Benzoylecgonine and ecgonine were observed as possible metabolites of cocaine, no metabolites of PCP were found. Hair was found to absorb and tightly bind PCP from aqueous solutions, which mimics the incorporation of PCP by the body. This raises questions about passive exposure as drugs may be absorbed into the moist hair of nonusers and become tightly bound. If this occurs, the screening of metabolites, and not the parent drug, may become mandatory to eliminate potential passive exposure problems. However, until more information is known about the fate of cocaine in hair and the chance for passive incorporation, interpretation behind the presence of a drug or its metabolites in an individual's hair should be viewed with caution. Certainly, the presence of a compound indicates exposure, but it may not indicate use.

Acknowledgment

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