TECHNICAL NOTE

Gail A. A. Cooper,[1] Ph.D.; Desiree L. Allen,[1] Ph.D.; Karen S. Scott,[1] Ph.D.; John S. Oliver,[1] Ph.D.; Jason Ditton,[2] Ph.D.; and Iain D. Smith,[3] M.R.C.Psych.

Hair Analysis: Self-Reported Use of "Speed" and "Ecstasy" Compared with Laboratory Findings*

REFERENCE: Cooper GAA, Allen DL, Scott KS, Oliver JS, Ditton J, Smith ID. Hair analysis: self-reported use of "speed" and "ecstasy" compared with laboratory findings. J Forensic Sci 2000;45 (2):400–406.

ABSTRACT: Drug use histories were collected from 100 subjects recruited from the "dance scene" in and around Glasgow, Scotland. In addition, each subject donated a hair sample which was analyzed by gas chromatography/mass spectrometry (GC/MS) for amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MD MA) and 3,4-methylenedioxyethylamphetamine (MDEA).

The hair samples were analyzed in two 6 cm segments or in full, ranging from 1.5 to 12 cm depending on the length of the hair. Approximately 10 mg of hair was ground to a fine powder before treatment with β-glucuronidase/aryl sulfatase. A solid-phase extraction procedure was carried out followed by derivatization with pentafluoropropionic anhydride (PFPA). All extracts were analyzed by gas chromatography/mass spectrometry (GC/MS).

Of the 139 segments analyzed, 77 (52.5%) were positive for at least one of the five amphetamines. The drug concentrations found in the hair were compared with the self-reported drug histories. A concordance of greater than 50% was found between the self-report data and levels detected in hair. However, no correlation was found between the reported number of "ecstasy" tablets consumed and the drug levels detected in hair.

An increase in the average drug levels measured was observed from low to high use (number of "ecstasy" tablets/month). A large number of false negatives and a low number of false positives were observed.

KEYWORDS: forensic science, hair analysis, self-report, "speed," "ecstasy," solid-phase extraction

The analysis of hair is now accepted as an alternative method for determining drug use. It has several possible advantages over other biological matrices (e.g., blood and urine), including collection of information regarding long-term drug use and determination of compliance with treatment programs (1–3). Over the past few years there has been a substantial increase in the number of scientific papers detailing methods for the analysis of a variety of both prescription and non-prescription drugs in hair.

Several independent laboratories in Europe, Japan and the United States have confirmed the accumulation of drugs such as cocaine (4–6), heroin and other opiates (4,7–9), amphetamines (10–13) and phencyclidine (14,15) in the hair of drug users. Other studies published have reported a dose-response relationship in hair for a variety of drugs in controlled animal studies (16–19). Most of the studies involving human subjects compared self-reported drug use to hair or urinalysis. A linear relationship was observed between hair cotinine levels and daily nicotine intake (20); however, this is an exception to what is generally reported. A relationship between low and high use and the levels of drugs detected in hair has been observed for cocaine (21), heroin (22) and buprenorphine (2). The lack of a linear dose-response relationship is not surprising due to the number of unknown variables, such as the exact dose or purity of drug consumed, the accuracy of the self-report data, individual metabolism variations and the types and frequency of hair treatments.

The work presented here is part of a larger study investigating the use of "speed" and "ecstasy" in Glasgow (23). Subjects claiming use of "speed" and "ecstasy" were recruited from the "dance scene" by chain referral (i.e., subjects nominated other subjects). They were asked to complete a detailed questionnaire of their drug use over the previous 12 months and to donate a hair sample for analysis. Subjects were compensated for samples.

The aims of the project were to investigate the accuracy of hair analysis in confirming self-reported use of speed and ecstasy and to determine whether or not a correlation exists between the amount of drug ingested and the levels detected in hair. AP and MA were identified to confirm speed use and MDA, MDMA and MDEA for ecstasy use.

Materials and Methods

Sample Collection

One hundred subjects were interviewed, 51 of whom were male. Ages ranged from 15 to 44 years, with a mean age of 24 years. Ninety-five percent of the participants were Caucasian. Ecstasy use was recorded as the number of tablets consumed per month for

[1] Department of Forensic Medicine and Science, University of Glasgow, Glasgow, Scotland.

[2] Faculty of Law, The University of Sheffield and The Scottish Centre for Criminology, Charing Cross Clinic, Glasgow, Scotland.

[3] Consultant psychiatrist, Gartnavel Royal Hospital, Glasgow, Scotland.

* This study was funded by the chief scientist at the Scottish Office (Grant Ref.: K/OPR/2/2/D125).

Received 10 Feb. 1999; and in revised form 4 June 1999; accepted 7 July 1999.

each of the previous 12 months. Speed use was recorded as "ever consumed" and then broken down into how often it had been consumed in the previous week, month and year.

The hair samples were cut close to the scalp in the vertex region, wrapped in aluminum foil and sealed in labeled plastic bags. The self-report ecstasy data was recorded for each month prior to interview; therefore it was possible to do segmental analysis on a month-by-month or cm-by-cm basis. Ideally, 10 mg of hair is required for analysis; however, some of the hair samples were very small and thus had to be analyzed in full. Where possible, two 6 cm segments were analyzed. The proximal 6 cm was labeled the "root" sample and the distal 6 cm the "tip" sample. In total, 139 hair segments were analyzed without prior knowledge of the self-report data.

A summary of the segments analyzed is given in Table 1. The hair lengths ranged from 1.5 to 12 cm. A total of 56 samples were analyzed in full.

Summary of Drug Use History

Of the 100 subjects questioned, 90 admitted taking ecstasy during the year prior to interview. Consumption of ecstasy was primarily at one of four locations (nightclubs, licensed raves, illegal parties, and private parties). Not all users consumed "ecstasy" on a regular basis and were classed as stable or erratic users. In contrast to stable users ($n = 43$) who consistently used the same amount of ecstasy each month, erratic users ($n = 48$) tended to have an irregular pattern of use which varied from month to month.

The total number of ecstasy tablets consumed within these two groups ranged from 1 to 144 (mean = 19, median = 10) over the previous year. In this study, the heaviest user consumed 144 tablets in the previous year, which translates to more than weekly but less than daily consumption of ecstasy. This is in sharp contrast to heavy heroin users, who use heroin at least once a day. The control group ($n = 9$) consisted of individuals who participated in the dance scene, but denied ever using speed or ecstasy. This number is low due to the difficulty in recruiting individuals who satisfied this criteria.

Questions regarding use of other drugs were also answered by each subject. The drugs used by the respondents over the previous year are summarized in Table 2 in descending order of frequency of use. The majority of respondents consumed alcohol (96%), cannabis (95%), speed (84%), and tobacco (84%).

TABLE 1—*Lengths of hair segments analyzed.*

Sample Type	Length (cm)	Total
Roots	<3 cm	4
	3 cm <6 cm	10
	6 cm	40
Tips	6 cm	29
Full	6.1 cm <12 cm	10
	12 cm	46
Total		139

TABLE 2—*Drugs consumed by 100 respondents over the year prior to interview.*

Drug	Yes	No	No Response
Alcohol	96	4	…
Cannabis	95	5	…
"Speed"	84	16	…
Tobacco	84	16	…
LSD	67	33	…
Cocaine	53	42	5
Poppers*	46	50	4
Mushrooms†	30	67	3
Temazepam	29	68	3
Other Benzos‡	19	75	6
Other Opiates§	13	82	5
Glue	7	88	5
Heroin	6	86	8
Temgesic	6	84	10
Ketamine	5	86	9

* Alkyl nitrites.
† Hallucinogenic mushrooms.
‡ All benzodiazepines with the exception of temazepam.
§ All opiates with the exception of heroin.

Experimental

Standards and Reagents

Amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethaphetamine (MDMA), d_3-amphetamine (d_3-AP), lauryl sulfate (sodium dodecyl sulfate (SDS)) and β-glucuronidase (activity = 300 000 to 400 000 units/g solid, sulfatase activity = 15 000 to 40 000 units/g solid) were obtained from Sigma® Chemical Co., (Dorset, U.K.). 3,4-methylenedioxyethylamphetamine (MDEA) was manufactured by Radian International and supplied by Promochem, (Herts., U.K.). Pentafluoropropionic anhydride (PFPA) was supplied by Fluka Chemicals (Dorset, U.K.). HPLC-grade methanol, acetone, chloroform, deionized water, and ethyl acetate chromatographic (HPLC) grade were obtained from Lab-Scan Analytical Sciences (Dublin, Ireland). Analytical-grade ammonium hydroxide and glacial acetic acid and HiPerSolv grade potassium-dihydrogen phosphate (KH_2PO_4) were obtained from Merck (Poole, U.K.).

Stock Solutions

A combined stock standard solution (0.1 mg/mL) of AP, MA, MDA, MDMA, and MDEA was prepared in methanol. Working solutions were also prepared in methanol (10, 1 μg/mL) by appropriate dilution of the stock standard. The internal standard (I.S.), d_3-AP, was prepared in methanol to give a stock standard solution of 0.1 mg/mL. Working solutions of d_3-AP (10, 1 μg/mL) were prepared by dilution of the stock standard with methanol. All stock and working solutions were stored in a freezer at −20°C.

Gas Chromatography/Mass Spectrometry

A Fisons model GC8000 series was used, fitted with a HP-1 capillary column. The initial column temperature of 55°C was held for 2 min and then increased to 280°C at a rate of 20°C/min and held for a further 5 min. The carrier gas used was helium. Injections were made in the splitless mode.

The GC8000 was interfaced to a Fisons MD800 mass spectrometer operated in the electron-ionization mode at 70 eV. The ions monitored were m/z 118, 190* (AP), m/z 118, 160, 204* (MA), m/z 135, 162, 190, 325* (MDA), m/z 135, 162, 204, 339* (MDMA), m/z 135, 162, 190, 218, 353* (MDEA), and m/z 193* (d_3-AP). The ions labeled "*" were used for quantitation.

Sample Pretreatment

Hair samples were washed once with 0.1% SDS in deionized water and then in triplicate with deionized water. Each wash step was carried out by sonicating for 15 min. The hair was then rinsed with methanol and allowed to dry overnight in a dessicator. The dried hair was measured and cut to the appropriate lengths, before being ground to a fine powder under liquid nitrogen using a mortar and pestle. The powdered hair was placed in a clean vial and weighed accurately.

Each hair sample was treated with 50 μL of β-glucuronidase [0.1 mg/mL in phosphate buffer (pH 7.4, 0.1 M)] and 2 mL of phosphate buffer (pH 7.4, 0.1 M). One hundred μL of the internal standard, d_3- AP (10 ng/mg based on a 10 mg hair sample) was added and the samples were incubated at 40°C for two hours. The samples were allowed to cool to room temperature, and then the supernatant was removed following centrifugation (2000 rpm, 5 min). A further 2 mL of phosphate buffer was then added, centrifuged as before and the two supernatants combined for further analysis.

Solid-Phase Extraction

To separate the drugs from the hair matrix, solid-phase extraction was carried out on Isolute® Confirm HCX-3 (130 mg) SPE columns [manufactured by International Sorbent Technology LTD (Hengoed, U.K.) and supplied by Crawford Scientific (Strathaven, U.K.)].

The method used was adapted from a method previously reported for the analysis of methadone (24) and differs only by the pH of the phosphate buffer used. The SPE columns were conditioned with methanol and phosphate buffer (pH 7.4, 0.1 M). The supernatant was applied onto the column which was then washed with deionized water. The pH was adjusted with 0.01 M acetic acid pH 3.3 and the column subjected to two drying steps before elution of the drugs. The first fraction (A) was eluted with acetone:chloroform (1:1 v/v) and was discarded as no quantifiable levels of the drugs were detected. The analytes of interest were eluted into the second fraction (B) using 2 mL of 2% ammoniated ethyl acetate.

Derivatization and GC/MS Analysis

Fraction B was evaporated to dryness under a stream of nitrogen at room temperature. The drugs were then reconstituted using 50 μL of PFPA:ethyl acetate (1:1) sealed and allowed to derivatize at 50°C for 15 min. After derivatization was complete, the samples were evaporated to dryness as before and reconstituted in 50 μL of ethyl acetate. One μL was injected for analysis by GC/MS.

Method Validation

Hair samples were spiked by adding a set volume of the working solution to 100 mg of ground blank hair (washed prior to milling). The methanol was allowed to evaporate to dryness overnight at room temperature. The hair was then mixed thoroughly to ensure homogeneity of the sample.

The developed method was validated using blank hair spiked with the five amphetamines at three different concentrations (5, 10 and 20 ng/mg), using approximately 10 mg of hair.

The recoveries from the spiked hair were greater than 70% for all five amphetamines and the method was linear in the concentration range 0 to 100 ng/mg. The limit of detection (L.O.D.) for AP and MA was 0.5 ng/mg and 0.1 ng/mg for MDA, MDMA and MDEA. The results are summarized in Table 3.

Spiking hair results in surface bound drugs and does not accurately represent how drugs are bound in the inner layers of drug users' hair. This should be taken into account when interpreting the validation results.

Analysis of "Ecstasy" Preparations

Substances (n = 15) which were sold as ecstasy were obtained by the research group. A Home Office licence (Ref. No.: 93/MM/254) was obtained that authorized the possession of drugs specified in Schedule I and II to the Misuse of Drugs Regulation 1985 (As amended). The 15 ecstasy preparations were analyzed by gas chromatography-flame ionization detection (GC-FID) and GC/MS. GC-FID analysis was carried out using an HP 5890 GC fitted with a CPSil 5 0.5 i.d. × 10 m column. The initial temperature was set at 80°C and then ramped to a final temperature of 200°C at a rate of 10°C/min and held for 5 min. The injector and detector temperatures were set at 290°C. Confirmation was carried out by GC/MS using a Fisons GC8000/MD 800. The initial temperature of 60°C was ramped to 300°C at 10°C/min and held for 10 min.

Results and Discussion

Due to the limited sample size, each hair segment (n = 139) could only be analyzed once by the described method. The reliability of this single analysis should be taken into account when interpreting the results.

Of the 139 hair segments, 73 (52.5%) tested positive for at least one of the five amphetamines, the remaining 66 segments were negative for all five. In general, the levels of AP, MA, MDA,

TABLE 3—*Recovery and linearity results for the extraction of amphetamines from hair.*

Analyte*	Recovery (%) ± Std. Dev.†	L.O.D.‡ (ng/mg)	Linearity (r)§
AP	70.6 ± 7.92	0.5	0.990
MA	79.4 ± 8.68	0.5	0.993
MDA	82.2 ± 6.26	0.1	0.993
MDMA	87.1 ± 4.62	0.1	0.995
MDEA	85.4 ± 2.12	0.1	0.996

* AP = amphetamine, MA = methamphetamine, MDA = 3,4-methylenedioxyamphetamine, MDMA = 3,4-methylenedioxymethamphetamine, MDEA = 3,4-methylenedioxyethylamphetamine.
† Standard deviation.
‡ Limit of detection.
§ Correlation coefficient.

TABLE 4—*Drug concentrations detected in hair segments.*

Drug	No. (Range) ng/mg	Median ng/mg	Mean ng/mg
AP	11 (0.7–97.7)	2.5	12.5
MA	27 (0.6–32.3)	2.6	5.2
MDA	20 (0.1–8.4)	1.0	1.9
MDMA	56 (0.1–82.9)	0.7	4.6
MDEA	23 (0.1–12.0)	0.5	2.8

MDMA, and MDEA detected in the hair samples are in the same range as those reported elsewhere (10,13,25–27). Some exceptions to this were found where levels detected were higher than those previously reported. These cases were few and the median of the results was found to lie within the reported ranges. The results are summarized in Table 4.

The ratio of metabolite to parent drug levels in hair has been used to determine whether a positive result is due to contamination or from ingestion (28,29). This ratio is expected to be lower than 1.0 due to the preferential incorporation of the parent drug to the metabolite. Therefore, a value greater than 1 is thought to result from contamination. The Society of Hair Testing recognizes set ratios for cocaine and heroin and their metabolites, but not as yet for amphetamines (29).

The metabolite to parent drug ratios are summarized in Table 5. With the exception of the ratio MDA:MDMA, the ranges of the others were higher than those previously reported. This can be explained in the case of AP and MA. "Speed" can contain both AP and MA as the parent drug, which will affect the metabolite to parent drug ratio. Combining the levels of MDMA and MDEA found in individual hair samples was carried out to determine if a relationship exists between the combined levels of these parent drugs in hair and the amount of drug consumed. Results obtained for the ratio MDA:(MDMA+MDEA) ranged from 0.03 to 6.5 in contrast to those found by Rothe et al. (25) (0.03 to 0.2, $n = 67$). However, they too reported cases with ratios >1. A possible explanation for this would be the consumption of MDA as a parent drug as well as its metabolic contribution from MDMA and MDEA.

TABLE 5—*Metabolite to parent drug ratios.*

Ratio	No. (Range)	Median	Mean
AP : MA	9 (0.15–44.40)	0.85	7.26
MDA : MDMA	12 (0.01–6.28)	0.40	0.54
MDA : MDEA	11 (0.10–6.50)	0.67	1.59
MDA : (MDMA + MDEA)	14 (0.03–6.50)	0.33	0.95

TABLE 6—*Content of ecstasy preparations analyzed by GC-FID and GC/MS.*

Street Name	Form	MDMA	Other
Emerald E	capsule	MDMA (tr.)*	AP(46%), Caff. †(41%)
Tornado Powder	powder	MDMA(13%)	
Rhubarb + Custard	capsule	MDMA(17%)	
Lemon and Lime	capsule	MDMA(19%)	MDEA (1%)
Clog	tablet	MDMA(20%)	
Pink Snowball	tablet	MDMA(20%)	
Madman	tablet	MDMA(21%)	
Turbo	tablet	MDMA(21%)	
Disco Biscuit	tablet	MDMA(31%)	MDEA (tr.)
Unnamed	tablet	MDMA(43%)	
Blaster	tablet	MDMA(46%)	
White/Love Dove	tablet	MDMA(58%)	
Madwoman Powder	powder	MDMA(65%)	
Super Dove/Robin	powder	MDMA(68%)	MDEA (tr.)
Madwoman	tablet	MDMA(70%)	

* Trace levels detected.
† Caffeine.

To investigate this, the contents of 15 illegal ecstasy preparations collected at the approximate time of sampling were analyzed. The results are summarized in Table 6. The amount of MDMA present was found to range from trace levels in "Emerald E" (which consisted mainly of AP and caffeine) to 70% in "Madwoman" ($n = 15$, range: trace–239 mg, median = 90). MDEA was detected in only three samples (range: trace–3.5 mg). MDA was not detected in any of the 15 preparations. However, this is not a representative sample and does not eliminate the possibility that ecstasy consumed by individuals in this study contained MDA. Ecstasy tablets seized within the U.K. have been reported to contain MDA (30,31).

Concordance between the self-reported data and the levels detected in hair was investigated for all 139 segments. A total of 73 segments tested positive for at least one of the five amphetamines with 71 of these agreeing with the self-report data. Sixty-six were were found to be negative, with only six agreeing with the self-report data. Overall, agreement or concordance was greater than 50% with a low number of false positives, consistent with other studies. The results are summarized in Fig. 1.

A large number of false negatives were found. There are a number of possible explanations for this, including the method lacking sensitivity, overreporting of drug use by the individual and the influence of cosmetic treatment on the hair (32–34). Unfortunately, no information was available on previous hair treatments (e.g., perms, bleaching) and so the contribution of this to the results could not be assessed.

Comparing the different types of sample analyzed, it was expected that root samples would be more accurate than tip samples as they have been subject to fewer environmental and cosmetic influences, which have been shown to affect the drug stability in hair. In addition, the self-report data would be expected to be more reliable due to better recollection of drug use in recent months as opposed to 12 months prior to interview.

The root samples have slightly better concordance (59.3%) than the tips (51.7%) and full (53.6%) samples. Figure 2 summarizes the concordance between self-report and hair analysis for each of the segment groups. These consist of roots (proximal sample ≤6 cm), tips (distal 6 cm) and full samples (>6 cm).

When comparing the self-report data for the use of speed only or ecstasy only, the concordance is very low for speed (28.3%) but higher for ecstasy (57.5%). The results are illustrated in Fig. 3. Unlike the ecstasy data, the speed data were not recorded for each

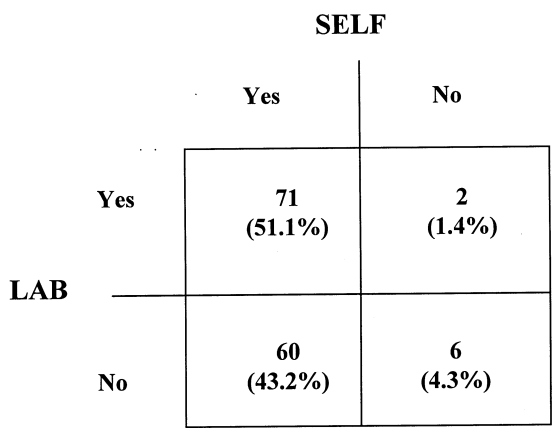

FIG. 1—*Overall concordance between self-report and hair analysis (*n *= 139).*

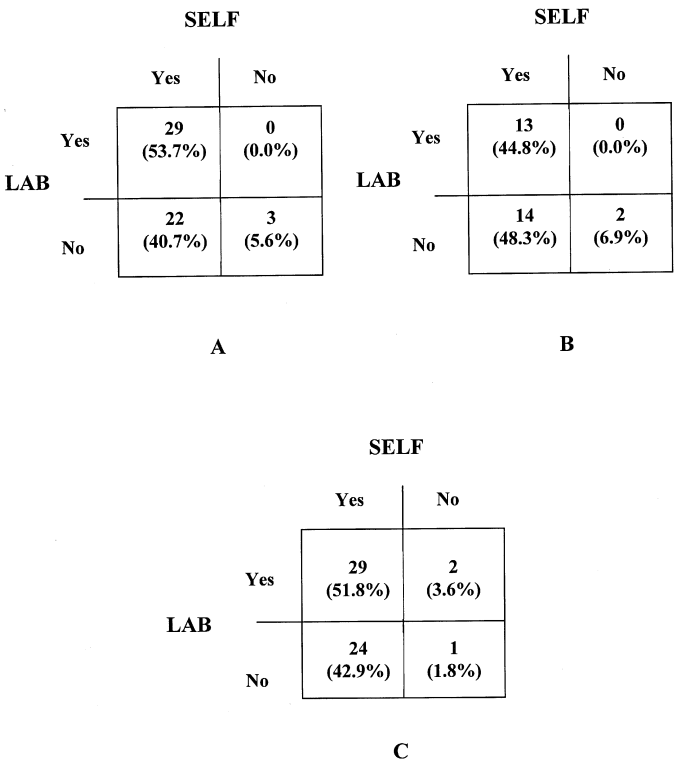

FIG. 2—*Concordance between self-report and hair analysis for each segment type;* (A) *Roots (*n = *54),* (B) *Tips (*n = *29), and* (C) *Full (*n = *56).*

month of the previous year and, in addition, higher limits of detection were employed. The combination of these factors will decrease the number of potentially positive results.

Of the six false positive segments where individuals denied speed use but tested positive for amphetamine and/or methamphetamine, five admitted ecstasy use and were also positive for MDA, MDMA or MDEA. Six of the seven false positives for ecstasy admitted taking speed but only one case was positive for amphetamine. It is certainly plausible that the subjects were unaware whether they were consuming speed or ecstasy and this would account for some of the false positive or negatives. For example, one of the 15 "ecstasy" preparations consisted of mainly amphetamine and caffeine, with only trace levels of MDMA.

Figure 4 illustrates the correlation between the total number of ecstasy tablets consumed and the total concentration of ecstasy (MDMA + MDEA) detected in each hair segment. There is a large scatter of results with the majority congregated along the axes. No linear dose-response relationship was observed (r = 0.0484, p = 0.572). This was also the case when comparing the amount of ecstasy consumed and the total concentrations of MDMA or (MDA + MDMA + MDEA) measured.

The results for each hair segment were separated into five groups depending on the average number of ecstasy tablets consumed each month. Each group consisted of approximately 20% of the segments analyzed. The mean, median and range are noted for each group and summarized in Table 7.

An increase in the mean and the median values for the total concentration of MDMA + MDEA is observed from low to high frequency of use. The one exception to this is the group where between 0.5 to 1.25 tablets were consumed each month. The mean value is higher due to one case that had a total concentration of 93.4 ng/mg. Another important point here is the high number of false negatives that are prevalent throughout each of the four groups claiming ecstasy use. However, the number of false negatives did decrease from low to high use.

The data were evaluated with respect to sex and hair color. There was no significant difference between the sexes. Ninety-five percent of the participants were Caucasian with the vast majority hav-

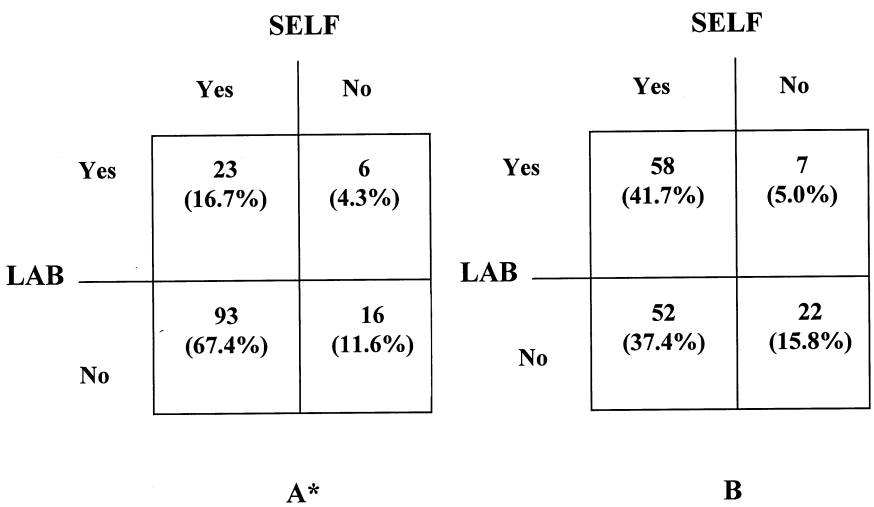

FIG. 3—*Concordance between self-report data an hair analysis for speed only* (A) *and ecstasy only* (B). *(*One subject did not answer question on speed use.)*

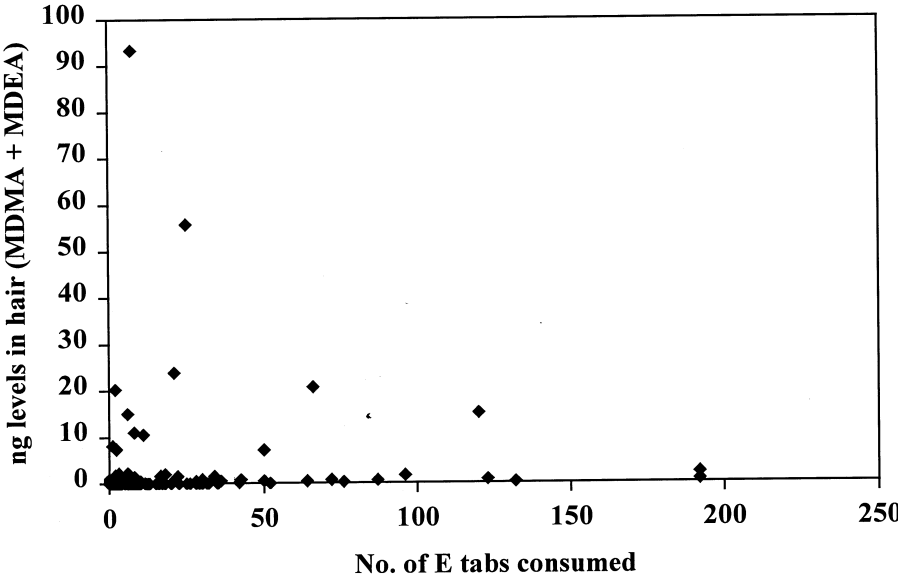

FIG. 4—*Correlation between amount of ecstasy consumed and total concentration of (MDMA + MDEA) in hair (*n *= 139).*

ing hair of various shades of brown. The lack of a relationship with hair color is probably indicative of this.

Conclusion

The described method combining enzyme extraction, SPE, derivatization and GC/MS successfully detected all five amphetamines (AP, MA, MDA, MDMA, and MDEA) in hair samples donated by participants in the Glasgow dance scene, thus confirming the use of speed and ecstasy.

Although concordance between self-reported data and laboratory findings was approximately 50%, there were a high number of false negatives (43.2%) which may highlight the method's lack of sensitivity in confirming low use of speed or ecstasy or both. No correlation was observed between the amount of ecstasy consumed and the levels detected in hair. However, there was evidence of a relationship existing between the level of use (measured in average ecstasy tablets consumed per month) and the levels detected in hair from low to high use.

The results of this study have given rise to a number of key questions that need to be answered to realize fully the potential of hair analysis, in particular, for the analysis of ecstasy in hair. A combination of low concordance between self-report data and hair analysis results, and the high number of false negatives, raises questions regarding both the method sensitivity and the reliability of the self-report data.

The method of collection of hair samples is also of utmost importance. As highlighted earlier in this paper, the samples obtained for this study were too small to be analyzed more than once. In some cases, this one analysis yielded a false negative result as the sample size was too small to give a significant level by GC/MS. It is acknowledged that obtaining larger samples on a voluntary basis could be problematic due to the sampling area (vertex region). However, it is practical to be able to analyze samples in triplicate in order to decrease experimental errors and thus increase the validity of analytical results.

TABLE 7—*Correlation between number of ecstasy tablets consumed/month and total concentration of (MDMA + MDEA) detected in hair.*

No. E Tablets per month*	Frequency	ng/mg in Hair		
		Mean	Median	Range
None	29	0.086	0.00	0–1.00
<0.5	29	1.493	0.10	0–20.3
0.5–1.25	28	4.425	0.00	0–93.4
>1.25–3.5	27	1.615	0.00	0–23.9
>3.5	26	4.285	0.55	0–55.8
Total	139	1.942	2.00	0–93.4

* Average number of ecstasy tablets consumed each month.

The large number of false negatives and low drug levels detected is likely to be a reflection on low drug consumption, with the heaviest users consuming approximately one ecstasy tablet per week of unknown purity. Comparing this with the consumption by heavy heroin or cocaine users (>1 per day), approximately 80% of ecstasy users in this study consumed less than one tablet per week.

The parent drug to metabolite ratio is unusual in comparison with the studies carried out on heroin and cocaine, where ratios of less than one are expected. The ecstasy preparations analyzed during this project were not a representative sample. Tablets seized from within the U.K. prior to the hair samples being collected had high quantities of MDA present. With this in mind, the ratios are not unusual due to the unknown contribution of MDA as either a parent drug or a metabolite or both.

Large discrepancies between cases are reflected in the lack of a dose-response relationship for the segments analyzed. Two examples are given which clearly illustrate this.

One subject reported use of 52 ecstasy tablets over a six-month period; however, the hair was negative for all five amphetamines.

Conversely, another subject denied use of ecstasy but 0.6 ng/mg of MDMA were found in the corresponding 2 cm hair segment.

Determining the smallest dose detectable in hair, in combination with dose-response studies, would certainly help explain why in one case, where only five ecstasy tablets were consumed, a level of 1.1 ng/mg of MDMA was detected. However, when 50 tablets were consumed by another subject, this gave rise to a level of only 0.5 ng/mg of MDMA.

The influence of various factors, i.e., cosmetic treatments, individual metabolism variations, and the uncertainty of the content and dose of the "ecstasy" tablets, is currently being investigated further. A wealth of information can be gained from studies involving consumption of known doses and subsequent hair analysis. One additional factor, highlighted by Rothe et al. (25), is the influence of drug incorporation via sweat. This is of particular relevance with ecstasy use, where participants in the dance scene are known to dance for several hours in hot, humid conditions.

The relationship observed between the average number of ecstasy tablets consumed per month and the total concentration of (MDMA + MDEA) detected in hair gives some indication of the potential of hair analysis as an indicator of ecstasy use. This, in addition to the previous points, will aid greatly with future interpretations of amphetamine levels in hair.

Acknowledgments

The authors offer their thanks Alasdair Forsyth, Domonic Gallagher, Sally Haw, Firzana Khan, Emma Short, and Asoon Suddle, who were all involved in the main project at different stages. This project was carried out under a grant from the Chief Scientist's Office of the Scottish Office, Reference K/OPR/2/2/D125.

References

1. Moeller MR, Fey P, Wennig R. Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program. FSI, 1993;63: 185–206.
2. Williams J. Sectional hair analysis as a potential index of therapeutic compliance in an epileptic population. Proceedings of the 1995 Hair Analysis Conference, Abudhabi, 443–66.
3. Wilkins D, Valdez A, Slawson M, Sison C, Huber A, Ling W, et al. Buprenorphine and norbuprenorphine in human hair of substance abuse treatment subjects. Presented at The SOFT/TIAFT Meeting in Albuquerque, NM 5–9 Oct. 1998.
4. Magura S, Freeman RC, Siddiqi Q, Lipton DS. The validity of hair analysis for detecting cocaine and heroin use among addicts. Int J Addictions 1992;27(1):51–69.
5. Moeller MR, Fey P, Rimbach S. Identification and quantitation of cocaine and its metabolites, benzoylecgonine and ecgonine methyl ester, in hair of Bolivian coca chewers by gas chromatography/mass spectrometry. J Anal Toxicol 1992;16:291–6.
6. Welch MJ, Sniegoski, LT, Asllgood CC. Interlaboratory comparison studies on the analysis of hair for drugs of abuse. FSI 1993;63:295–303.
7. Polettini A, Stramesi C, Vignali C, Montagna M. Determination of opiates in hair. Effects of extraction methods on recovery and on stability of analytes. FSI 1997;84:259–69.
8. George S, Braithwaite RA. The measurement of morphine in the hair of heroin abusers. Ann Clin Biochem 1997;34:375–83.
9. Tagliaro F, De Battisti Z, Smith FP, Marigo M. Death from heroin overdose: findings from hair analysis. The Lancet 1998;315(9120):1923–5.
10. Suzuki O, Hattori H, Asano M. Detection of methamphetamine and amphetamine in a single human hair by gas chromatography/chemical ionization mass spectrometry. J Forensic Sci 1984;26(2):611–7.
11. Kintz P, Cirimele V, Tracqui A, Mangin P. Simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylene-dioxymethamphetamine in human hair by gas chromatography-mass spectrometry. J Chromatogr B 1995;670:162–6.
12. Nakahara Y. Detection and diagnostic interpretation of amphetamines in hair. FSI 1995;70:135–53.
13. Rohrich J, Kauert G. Determination of amphetamine and methylenedioxy-amphetamine-derivatives in hair. FSI 1997;84:179–88.
14. Sakamoto T, Tanaka A, Nakahara Y. Hair analysis for drugs of abuse XII. Determination of PCP and its major metabolites, PCHP and PPC, in rat hair after administration of PCP. J Anal Toxicol 1996;20(2):124–30.
15. Nakahara Y, Kikura R, Sakamoto T, Mieczkowski T, Tagliaro F, Foltz RL. Findings in hair analysis for some hallucinogens (LSD, MDA/MDMA and PCP). Proceedings of the 1995 Hair Analysis Conference, Abudhabi, 161–84.
16. Nakahara Y, Takahashi K, Takeda Y, Konuma K, Fukui S, Tokui T. Hair analysis for drug abuse, part II. Hair analysis for monitoring of methamphetamine abuse by stable isotope dilution gas chromatography/mass spectrometry. FSI 1990;46:243–54.
17. Nakahara Y, Takahashi K, Shimamine M, Takeda Y. Hair analysis for drug abuse: I. Determination of methamphetamine and amphetamine in hair by stable isotope dilution gas chromatography/mass spectrometry method. J Forensic Sci 1991;36(1):70–8.
18. Nakahara Y, Toshiaki O, Kikura R. Hair analysis for drugs of abuse. V. The facility in incorporation of cocaine into hair over its major metabolites, benzoylecgonine and ecgonine methyl ester. Arch Toxicol 1992; 66:446–9.
19. Ferko AP, Barbieri EJ, DiGregorio GJ, Ruch EK. The appearance and disappearance of cocaine and benzoylecgonine in rat hair following prolonged administration of cocaine. Life Sci 1992;51:1823–32.
20. Eliopoulos C, Klein J, Koren G. Validation of self-reported smoking by analysis of hair for nicotine and cotinine. Therap Drug Monitor 1996; 18:532–6.
21. Pepin G, Gaillard Y. Concordance between self-reported drug use and findings in hair about cocaine and heroin. FSI 1997;84(1–3):37–41.
22. Kintz P, Bundeli P, Brenneisen R, Ludes B. Dose-concentration relationships in hair from subjects in a controlled heroin-maintenance program. J Anal Toxicol 1998;22(3):231–6.
23. Ditton J, Cooper GAA, Scott KS, Allen DL, Oliver JS, Smith ID. Forensic hair testing for "ecstasy" in a large volunteer sample of Scottish drug users. In press.
24. Cooper GAA, Oliver JS. Improved solid-phase extraction of methadone and its two major metabolites from whole blood. J Anal Toxicol 1998; 22(5):389–92.
25. Rothe M, Pragst F, Spiegel K, Harrach T, Fischer K, Kunkel J. Hair concentrations and self-reported abuse history of 20 amphetamine and "ecstasy" users. FSI 1997;89:111–28.
26. Ishiyama I, Nagai T, Toshida S. Detection of basic drugs (methamphetamine, antidepressants, and nicotine) from human hair. J Forensic Sci 1983;28(2):380–5.
27. Kikura R, Nakahara Y, Mieczkowski T, Tagliaro F. Hair analysis for drug abuse XV. Disposition of 3,4-methylenedioxymethamphetamine (MDMA) and its related compounds into rat hair and application to hair analysis for MDMA abuse. FSI 1997;84:165–77.
28. Sachs H. Quality control by the Society of hair testing. FSI 1997; 84(1–3):145–50.
29. Sachs H, Staub C, Kintz P, Uhl M. Society of Hair Testing. FSI 1997; 84(1–3):3–6.
30. Forrest ARW, Galloway JH, Marsh ID, Strachan GA, Clark JC. A fatal overdose with 3,4-methylenedioxyamphetamine derivatives. FSI 1994; 64:57–9.
31. Milroy CM, Clark JC, Forrest ARW. Pathology of deaths associated with "ecstasy" and "eve" misuse. J Clin Pathol 1996;49:149–53.
32. Skopp G, Potsch L, Moeller MR. On cosmetically treated hair—aspects and pitfalls of interpretation. FSI 1997;84:43–52.
33. Dawber R. Hair: its structure and response to cosmetic preparations. Clinics in Dermat 1996;14:105–12.
34. Jurado C, Kintz P, Menedez M, Repetto M. Influence of cosmetic treatment of hair on drug testing. Int J Leg Med 1997;110:159–63.

Additional information and reprint requests:
Gail A.A. Cooper
Department of Forensic Medicine and Science
University of Glasgow
Glasgow G12 8QQ
Scotland, U.K.