The analysis of the keratin matrix as a new tool to evaluate the epidemiology of drug use in Perugia (Italy): a cross sectional study

Isabella Mercurio [1], Gianmarco Troiano [2], Paola Melai [3], Veronica Agostinelli [3], Rosaria Simone [4], Nicola Nante [5], Mauro Bacci [6]

(1) Forensic Medicine, Department of Medical Sciences, Surgical and Neurosciences, Hospital Santa Maria alle Scotte, Siena
(2) Post Graduate School of Public Health University of Siena
(3) University of Perugia
(4) Department of Politic Sciences - University of Naples Federico II
(5) Post Graduate School of Public Health University of Siena
(6) Coordinator of the Section of Legal Medicine, Forensic Science and Sports Medicine, University of Perugia

CORRESPONDING AUTHOR: *Gianmarco Troiano, MD - University of Siena, Department of Molecular and Developmental Medicine Area of Public Health, Via A. Moro 2 - Siena, 53100. Italy - Mobile: (+39) 347-9512771; Mail: gianmarco.troiano@student.unisi.it or gianmarco-89@hotmail.it; Fax: (+39) 0577-234090*

DOI: ***10.2427/11732***
Accepted on April 5, 2016

ABSTRACT

Background: Drugs and their metabolites can be incorporated into hair, so hair drug testing has become an alternative and complementary method to assess the extent of drug abuse. The aim of our study was to use hair samples to reconstruct the epidemiology of illicit substances used in Perugia.
Methods: We conducted a cross-sectional study from May to July 2012 asking hairdressers in Perugia to collect hair samples and to compile a worksheet for each one. The samples were analysed in laboratory: the extraction of basic substances was performed adding methanol; the extraction of acid substances was obtained adding NAOH. After derivatisation, 1µl of each solution was analysed through gas chromatography/mass spectrometry. The data were organised in a database and processed using R version 3.2.2.
Results: We collected 238 samples. The most detected drugs were: THC-TMS identified in 15 samples, MDMA in 9, Beg-TMS in 8. There was a statistically significant difference in drug use between the city centre (23,36%) and the suburbs (5,34%). Age and sex did not represent influencing factors. The substance with the highest concentration in the keratin matrix was ketamine (9834,86 ng/100 mg of hair).
Conclusion: The use of keratin matrix offered significant advantages in the toxicological analysis compared to conventional biological matrices and permitted us to investigate the situation in Perugia where the increased market for illicit drugs has caused a constant rise in drug addiction. However, because this method presented certain limitations only the simultaneous use of keratin matrix and other traditional indicators, could furnish more precise information.

Key words: epidemiology, drug abuse testing, keratin matrix, Italy

INTRODUCTION

The use of a drug to modify a person's behaviour is not a recent phenomenon. However, the recent increase in reports of drug-facilitated crimes (sexual assault, robbery) has caused alarm in the general public. Drugs involved can be pharmaceuticals such as benzodiazepines (flunitrazepam, lorazepam, etc), hypnotics (zopiclone, zolpidem), sedatives (neuroleptics, some histamine H antagonists), or anaesthetics (gamma-hydroxybutyrate, ketamine), drugs of abuse such as cannabis, ecstasy or lysergide or more often ethanol. Drugs used to facilitate crimes can be difficult to detect because they can be rapidly cleared from the body (short half-life). In these situations, blood or even urine samples can be utilised for the detection of drugs [1].

A lot of studies have shown that drugs and their metabolites can be incorporated into hair during the formation of the hair shaft (via diffusion from blood to the actively growing follicle), after formation (via secretions of the apocrine and sebaceous glands) and after the hair has emerged from the skin (from the external environment). Moreover, drugs can be transferred to hair from multiple body compartments or pools located in tissues surrounding the hair follicle. These mechanisms could also be drug-specific [2].

Hair testing for drugs of abuse offers the potential for detection of drug exposure over an extended period of time. Because hair grows at an average rate of 1-1.5 cm/month [3], it may be possible to test hair lengths that represent months to years of potential drug exposure. With the epidemic spread of cocaine and "crack" use in the United States in the 1980s, attempts have been made to take advantage of this ostensibly longer detection time of hair testing compared to urine testing as a tool to identify cocaine users [4].

The role of hair testing as an alternative or complementary matrix has expanded across the spectrum of toxicological investigations and, consequently, hair samples are routinely collected during criminal investigations [5]. However, there are limitations in the interpretation of hair analyses due to differences between individuals, variable growth rates of hair and uncertainty over the mechanism of entry of the drug into hair [6-8].

Nevertheless, hair tests offer some advantages over urine assays [9, 10]; the collection of hair specimens is less embarrassing and less intrusive for study subjects. Hair samples can be stored at room temperature and do not need to be quickly analysed after collection. Moreover, the window of detection of drug abuse in hair tests is considerably wider than that of urine assays and it is only limited by the length of the hair. It typically ranges from a week to several months compared with 2–3 days with urine analysis [11, 12].

In a study conducted in 2013 on Norway, Lund et al. used hair sample as an analytical matrix for epidemiological studies [13]. Another study investigated patterns of drug use in the UK in a large population between 2001 and 2005 and the authors concluded that the chances of identifying people using drugs by testing hair samples were twice as high as by using urine samples [14].

The aim of our study has been to analyse hair samples collected from hair salons to estimate the use of illegal and psychoactive drugs in the general population in the city of Perugia, where the phenomenon of drug dealing has become widespread, in order to assess the extent of the phenomenon and its spread.

MATERIALS AND METHODS

Study design

We conducted a cross-sectional study in the Forensic Toxicology Laboratory of the Department of Forensic Medicine of the University of Perugia from May to July 2012. As the population resident in Perugia amounts to 162986 persons [15], we calculated the adequate sample size for the study with a 2% of margin of error (i.e. 238 samples). We asked 20 hairdressers working in thirteen hair salons (ten situated in the centre and three in the suburbs) of Perugia to collect 250 hair samples. Each hair sample needed to weigh at least 250 mg and to be cut according to the Guidelines [5]; however, it was not possible to follow the protocol, because it was unavoidable the obtaining of samples from the extremity of the hair in lieu of root and some samples fell to the ground after being cut. Each sample was then inserted in a plastic bag for collection and simultaneously the hairdresser compiled a worksheet. In this worksheet the data were reported, in a totally anonymous way: the date on which the sample was collected, gender, age (asked directly to each participant and divided into three groups: scholastic 13-18 years old, working 19-65 years old, non-working over 65 years old), an eventual treatment the hair sustained, location, hair characteristics, length, type of hairdresser (for man, woman or unisex). Only one sample from each client was collected and no information about the participant was recorded. Each participant signed a consent form before participating in the study.

Sample preparation

We removed from each sample 100 mg of hair, which was washed twice with dichloromethane, dried under a stream of nitrogen and then cut with disinfected scissors to the size of 1 mm. Before the analysis we prepared two hair samples of the net weight of 50 mg, definitely negative for the presence of drugs; two samples containing only the reagents used in the entire analytical process; two positive control solutions containing a known amount (10 ng/ml) of each searched drug: in particular,

we searched for Ketamine, Oxazepam-TMS, clozapine, Flunitrazepam, Orphenadrine, Alprazolam, Methadone, Sertraline, Citalopram, Methaqualone, Cocaine, Diazepam, LSD-TMS, Mirtazapine, Chlordiazepoxide, Beg-TMS, Lorazepam, Quietapine, Methamphetamine, Clotiapine, THC-COOH Morphine, Pethidine, Mdma, Olanzapine, THC-TMS, 6-mam, Mde, Mescaline-TMS. Finally we added scopolamine, as an internal standard to all solutions.

Analysis

The extraction of basic substances was performed adding 2 ml of methanol RPE to each sample, which was then placed at 56°C for almost 16 hours.

After cooling, the solutions were centrifuged at 3500 rpm for five minutes, filtered and subjected to evaporation under a stream of nitrogen. Samples were reconstituted with 0,5 ml of methylene chloride RPE and dried using nitrogen.

Finally, the solution was derivatised at 70°C for 60 minutes with 50 µl of reagent BSTFA containing 1% TMS.

The extraction of acid substances was obtained adding 1 ml of NAOH 1M and placing each solution in a dry bath at the temperature of 70°C for 30 minutes. Each alkaline extract was then neutralised with 200 µl of concentrated acetic acid and then diluted with 2 ml of ammonium acetate buffer at pH 7.0. The extraction of the substance was performed using "IsoluteTM" SPE Column type HAX 200 mg/3 ml. The sample was pre-treated adding ammonium acetate buffer to reach a pH between 6 and 7. Meanwhile, the column was solvated with 2 ml of methanol and 2 ml of ammonium acetate buffer (pH 6-7) without air intake. After solvation of the column, the sample was added maintaining a flow which did not exceed 2 ml/min. We then proceeded to the elimination of interference through the washing of the column with 6 ml of deionized water. The column was dried for 10 minutes under high vacuum; then, through a second washing with 2 ml of acetonitrile the column was dried again for 5 minutes under vacuum (10-15" Hg). Finally we proceed to the eluation of 3 ml of a mixture 75/25 v/v of hexane/acidified ethyl acetate with glacial acetic acid at 1%. The eluate was completely dried under a stream of nitrogen and then reconstituted with 0,5 ml of methylene chloride, and subsequently dried again using nitrogen. The solution was finally derivatised at 70 °C for 20 minutes using 50 µl of BSTFA reagent, containing 1% TMS.

After derivatisation, 1µl of each solution was analysed through gas chromatography/mass spectrometry using a GC/MSD system 6850/5973 Network. The ion source was connected to a capillary column HP-5ms (5% phenylmethyl silicone-fused-silicacapillary column) of the length of 30 meters x 0.25 mm.

The system was placed in the following operating conditions:

- Column temperature: programmed from 120 to 300 °C with an increase of 10 °C/min.;
- Injector and detector temperature: 280 °C;
- Helium flow: 1 ml/min.;
- Type of injection: Split-splitless, 50 sec.;
- Scanning masses from 50 to 600 a.m.u.;
- The cut-off of the method is set to 10 ng of analyte per 100 milligrams of hair.

Data processing was controlled by a computer processor HED - HP Vectra PC with a Pentium III MS Drug Library: G 1039C - PMW-TOX3 for research or comparison with 6300 mass spectra of drugs and their metabolites. The identification of the derivatives was carried out first in FULL SCAN and then in SIM (Selected Ion Mode) selecting specific groups of ions [12, 16-18].

Data analysis

The data were organised in a database and processed using R (a free software environment) version 3.2.2. The Statistical analysis that was performed consisted mainly in contingency table analysis. Contingency table analysis was considered by cross-classifying the data according to the variables of interest: posivity to at least one compound, gender of each participant, location of sample collection. Pearson chi-squared test for independence was run to evaluate the independence of being positive to at least one compound from location and gender. Because of the low cell counts, Fisher's exact test was performed to calculate the odds ratio of the observed difference between the samples collected in the city centre and in the suburbs and between samples collected from men and from women. Logistic regression was also considered to explain posivity to a given compound, coming from a specific location. We then performed Fisher's exact test and computed the Cohen's kappa index to evaluate if the multidrug consumption had a statistically significant association. The significance level was set at alpha=5%.

RESULTS

We collected 238 samples because 6 samples were not received and 8 were not suitable for the analysis. 131 (55%) came from hair salons situated in the suburbs, 107 (45%) in the city centre; 89 (37,3%) underwent treatment such as colouring, bleaching, permanent hair straightening. The 48,4% of hair samples were cut from men; 51,6% from women. From the toxicological analysis, 206 samples (86%) resulted negative, 15 (6,3%) resulted positive for one drug; 17 (7,1%) for two or more substances. The following compounds were not detected in the hair samples: Methaqualone, THC-COOH, Lorazepam, Clozapine, Alprazolam, LSD, Quietapine, Citalopram, Mirtazapine, Petidine, Mescaline, Clotiapine, Olanzapine.

The most detected drugs were: THC-TMS which was identified in 15 samples (6,3%), followed by MDMA in 9 samples (3,7%) and Beg-TMS in 8 samples (3,3%) (Tab.1).

In the 15 samples that resulted positive for one drug, we detected Metadone in 2 samples (13,33%), Ketamine in 3 samples (20%), THC-TMS in 7 samples (46,66%), and MDMA in 3 samples (20%) .

TABLE 1. Drugs detected in hair samples.

COMPOUND	ABSOLUTE FREQUENCY	% OF ALL SAMPLES
Ketamine	4	1.68%
Metadone	3	1.26%
THC-TMS	15	6.30%
MDMA	9	3.78%
Orphenadrine	1	0.42%
MDE - Metamphetamine	5	2.10%
Psychiatric Drugs	7	2.94%
Morphine, 6-mam	4	1.68%
Cocaine/Beg-TMS	8	3.36%

Analysis of location and multidrug consumption:

78,1% of hair samples came from hair salons situated in the centre of Perugia and 21,8% from hair salons in the suburbs. The difference between the rate of positive to at least one compound for units sampled in the city centre (23,36%) and the suburbs (5,34%) was statistically significant (p<0,05) (Fig.1).

In particular, statistically significant different proportions are observed between city centre and the suburbs for THC-TMS, BZP, 6-MAM, morphine, MDMA and the group "psychiatric drugs", including BZP and sertraline (all p<0.05).

Substances detected most in the city centre were THC-TMS identified in 13 samples (52%) and MDMA in 8 (32%).

As to multidrug consumption, a statistically significant association for the Fisher's exact test (p<0,05) was observed for pairs of substances in the following list:

1. Beg-TMS (Cocaine and Beg-TMS)
2. Morphine and 6-MAM
3. BZP
4. Mde – Methamphetamine
5. THC-TMS

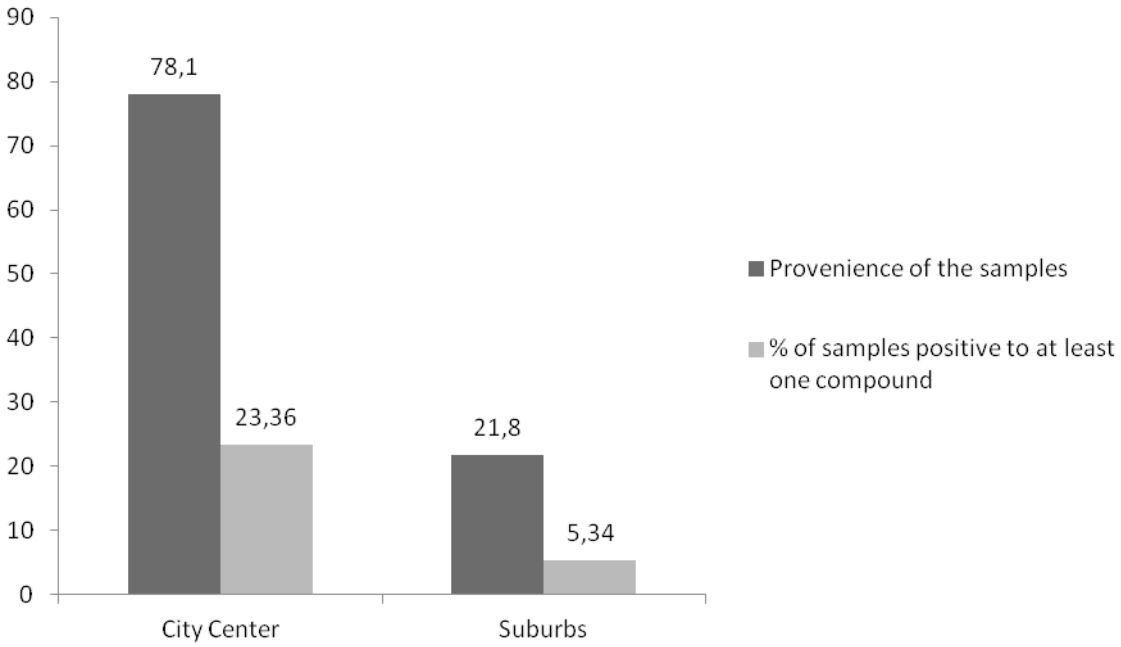
Analysis of gender and age:

The observed sample consisted of 48,7% of samples referred to female units and 51,3% to male units. The rate of positive samples (for at least one compound) in women was 13,91%, while for men was 12,4%. The difference of proportion was not statistically significant (p>0,05).

Women tended to use especially THC-TMS (8 of all female samples, 6,96%); MDMA (4 samples, 3,48%); BZP and beg-TMS (3 samples, 2,6 %). Men instead, tended to use principally THC-TMS (6 samples, 4,96%); Beg-TMS and MDMA (5 samples, 4,1%) and Mde-Methamphetamine (4 samples, 3,3%).

Age did not represent a factor influencing the presence of drugs in hair samples (p=0,29). In the scholastic age

FIGURE 1. Percentage of samples positive to at least one compound compared with the origin of the samples.



group 11 (4,6% of all samples) were positive; 20 (8,4%) in the working age; no one in the non-working age.

The substances with the higher concentration in the keratin matrix are ketamine (9834,86 ng/100 mg of hair), THC (5572,44 ng/100 mg) and diazepam (5467,89 ng/100 mg of hair).

DISCUSSION

In our study we collected hair samples from hair salons to investigate what is the spread of drug abuse in Perugia. The most detected drugs were: THC, cocaine, heroine and MDMA.

Hair samples are suitable for the detection of drug consumed during the last several months, as demonstrated in studies about drug use in the general population [19], among students [20, 21], patients [11, 22] or drug addicts [23, 24]. However the analysis is time-consuming and the analytical costs are very high. It is therefore not very well suited for mass screening or studies of large cohorts [25]; hair samples are best suited for studying cohorts with high proportion of drug users.

In general, hair samples are very well suited for the detection of cocaine use, fairly well suited for detection of amphetamines and benzodiazepines and not very well suited for detection of cannabis use. If hair segments are analysed, variations in hair growth between individuals may lead to inaccurate interpretation of the timeframe in which the drug was taken [25].

In a study of 100 users of amphetamines, only 56% had positive hair sample [26], indicating that a negative finding does not exclude the possibility of drug use. Baeck et al. investigated whether repeated hair washing and a single hair dyeing reduced the concentration of methamphetamine and amphetamine in human hair and found that the concentrations in methamphetamine addicts decreased by about one-third in comparison to untreated hair [27].

For cannabis a positive result is not guaranteed even after daily use [28]. THC concentrations in hair samples are low, which makes detection and quantification challenging due to limitations in the detection capabilities of the analytical methods [28, 29]. Huestis et al. have shown that even daily cannabis smoking may not be detected in hair samples, partly because THC is an UV-unstable compound [28]. These data continue to support previous research, suggesting that the feasibility of hair testing as a detector of marijuana prevalence has not been established [30-32].

A semi-quantitative relationship between dose and hair concentration has been found for cocaine [33] and the prevalence of this compound may be interpreted as true without false positive or negative interferences, if the samples are being washed prior to analysis as a decontamination step. The drug concentration does not accurately reflect the taken dose [34], however a semi-quantitative relationship between dose and hair concentration has been studied for codeine [33] and the interpretation of the use of medicinal codeine is therefore straightforward if heroin abuse can be excluded. Hair was clearly more useful in detecting under-reporting of cocaine than either urine or oral fluid. Oral fluid was the most useful method for detecting under-reporting of marijuana. Urine testing was most useful in detecting under-reporting of heroin. These findings suggest that multiple tests have considerably more utility in epidemiological research than any single procedure. Thus, while all tests were relatively poor at detecting amphetamine, hair testing was particularly poor at detecting marijuana use [35].

The measured prevalence of amphetamine and methamphetamine, as the case of THC as well, is therefore probably not totally representative for the population of Perugia.

An important finding of our study has been the higher prevalence of positive samples in the center of the city. This result is in line with another study which showed that heavy drug trafficking was much greater in the inner city areas than in the suburbs [36]. A report from Police Headquarter in Perugia, showed the enormous traffic in illicit substances in the centre of the city which led to the arrest of drug dealers who operated principally in this area [37].

These findings are very interesting: it is necessary to allot greater resources from public health and local police, in order to reduce the phenomenon, by monitoring areas with a high risk and a high traffic of illicit substances.

The strengths of our study have been: I) the ease in finding the samples; II) the anonymity that ensured a high participation rate; III) the procedures of washing, decontamination and quantitative and qualitative analysis which ensured a wide and secure screening of the compounds; IV) information about age and gender of each participant which let us make interesting comparisons. An important limitation of our study was represented by the technique of hair cutting which was not in line with the international guidelines: for this reason it was not possible to identify the period of drug consumption. The small sample size was also a limitation, especially for comparing the results with other epidemiological studies. This limitation was due to the scarcity of funds allocated by the Department. Cosmetic hair treatment can reduce drug concentrations and lead to an underestimation of prevalence. Drug concentrations may also be related to hair colour which introduce ethnical bias [29, 38, 39].

CONCLUSIONS

The use of keratin matrix offers compelling advantages in the toxicological analysis compared to conventional biological matrices. In this study this matrix was used to evaluate the epidemiology of substances and drugs of

abuse in the general population of Perugia, where the phenomenon of drug addiction is becoming a significant problem. The study was based on analysis of 238 hair samples collected in different hair salons and tested for the most common drugs.

The most frequently detected substances were the derivatives of cannabis (THC-TMS), MDMA, cocaine and heroine. In our study there was not a significant difference due to gender or age; instead the localization of the hair salon represented an important influencing factor: a high percentage of positive samples came from the centre of the city, where the phenomenon of drug dealing is more prominent.

This study showed the validity of the keratin matrix analysis in evaluating the spread of drug abuse in the general population. However, because of the limitations of this method, only the simultaneous use of keratin matrix and other traditional indicators, could furnish precise information about this phenomenon in Perugia, where an increased illicit drug market has caused a constant rise of drug addiction.

ACKNOWLEDGMENTS

The authors thank Dr. George Rettagliata for his valuable contribution in the final revision of this article.

References

1. Kintz P, Villain M, Ludes B. Testing for the undetectable in drug-facilitated sexual assault using hair analyzed by tandem mass spectrometry as evidence. Ther Drug Monit 2004; 26(2):211-4.
2. Henderson GL. Mechanisms of drug incorporation into hair. Forensic Sci Int 1993;63(1-3): 19-29.
3. Saitoh M, Sakamoto M. Rate of hair growth. Advances in Biology of Skin, Vol IX Hair Growth, 1969:183-201.
4. Cone EJ, et al. Testing human hair for drugs of abuse. II. Identification of unique cocaine metabolites in hair of drug abusers and evaluation of decontamination procedures. J Anal Toxicol 1991;15(5):250-5.
5. Cooper GA, Kronstrand R, Kintz P. Society of Hair Testing guidelines for drug testing in hair. Forensic Sci Int 2012;218(1-3):20-4.
6. Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 2006;370(1-2):17-49.
7. Barroso M, et al. Hair: a complementary source of bioanalytical information in forensic toxicology. Bioanalysis 2011;3(1):67-79.
8. Barbosa J, et al. Hair as an alternative matrix in bioanalysis. Bioanalysis 2013;5(8):895-914.
9. Kintz P. Drug Testing in Hair1996, FL: CRC Press.
10. Madea B, Haaranalytik FM. Technik und Interpretation in Medizin und Recht 2004, Cologne: Deutscher Arzteverlag.
11. Musshoff F, et al. Results of hair analyses for drugs of abuse and comparison with self-reports and urine tests. Forensic Sci Int 2006;156(2-3):118-23.
12. Simona Pichini RP. LINEE GUIDA PER LA DETERMINAZIONE DELLE SOSTANZE D'ABUSO NELLA MATRICE PILIFERA. 2010.
13. Lund HM, et al. A Norwegian study of the suitability of hair samples in epidemiological research of alcohol, nicotine and drug use. J Anal Toxicol 2013;37(6):362-8.
14. Tsanaclis L, Wicks JF. Patterns in drug use in the United Kingdom as revealed through analysis of hair in a large population sample. Forensic Sci Int 2007;170(2-3):121-8.
15. Perugia, C.d., La popolazione residente nel comune di Perugia. 2013.
16. Committee LG. FORENSIC TOXICOLOGY LABORATORY GUIDELINES 2012.
17. Gambelunghe C, et al. Hair analysis by GC/MS/MS to verify abuse of drugs. J Appl Toxicol 2005;25(3):205-11.
18. Tassoni G, et al. Detection of Cannabinoids by ELISA and GC-MS Methods in a Hair Sample Previously Used to Detect Other Drugs of Abuse. J Anal Toxicol, 2015.
19. Colon HM, Robles RR, Sahai H. The validity of drug use responses in a household survey in Puerto Rico: comparison of survey responses of cocaine and heroin use with hair tests. Int J Epidemiol 2001;30(5):1042-9.
20. Kidwell DA, Blanco MA, Smith FP. Cocaine detection in a university population by hair analysis and skin swab testing. Forensic Sci Int 1997;84(1-3):75-86.
21. Quintela O, et al. Evaluation of cocaine, amphetamines and cannabis use in university students through hair analysis: preliminary results. Forensic Science International 2000;107(1-3):273-9.
22. Swartz MS, Swanson JW, Hannon MJ. Detection of illicit substance use among persons with schizophrenia by radioimmunoassay of hair. Psychiatr Serv 2003;54(6):891-5.
23. Magura S, Kang SY. Validity of self-reported drug use in high risk populations: a meta-analytical review. Subst Use Misuse 1996;31(9):1131-53.
24. Welp EA, et al. Amount of self-reported illicit drug use compared to quantitative hair test results in community-recruited young drug users in Amsterdam. Addiction 2003;98(7):987-94.
25. Hallvard Gjerde, E.L.Ø.a.A.S.C. Using biological samples in epidemiological research on drugs of abuse. Norsk Epidemiologi 2011;21(1):5-14.
26. Moore C, Giorgi N, Ross W, et al. Dosage des amphétamines dans les cheveux, la salive et les urines. Ann Toxicol Anal 2005;17(4):229-35.
27. Baeck S, et al. Effects of repeated hair washing and a single hair dyeing on concentrations of methamphetamine and amphetamine in human hairs. Forensic Sci Int 2011;206(1-3):77-80.
28. Huestis MA, et al. Cannabinoid concentrations in hair from documented cannabis users. Forensic Sci Int 2007;169(2-3):129-36.
29. Musshoff F, Madea B. Analytical pitfalls in hair testing. Anal Bioanal Chem 2007;388(7):1475-94.
30. Nakahara YK, Takahashi, Kikura R. Hair analysis for drugs of abuse. X. Effect of physicochemical properties of drugs on the incorporation rates into hair. Biol Pharm Bull 1995;18(9):1223-7.
31. Kauert G, Rohrich J. Concentrations of delta 9-tetrahydrocannabinol, cocaine and 6-monoacetylmorphine in hair of drug abusers. Int J Legal Med 1996;108(6):294-9.
32. Cone EJ. New developments in biological measures of drug prevalence. NIDA Res Monogr 1997;167:108-29.

33. Scheidweiler KB, et al. Dose-related distribution of codeine, cocaine, and metabolites into human hair following controlled oral codeine and subcutaneous cocaine administration. J Pharmacol Exp Ther 2005;313(2):909-15.
34. Harkey MR. Technical issues concerning hair analysis for drugs of abuse. NIDA Res Monogr 1995;154:218-34.
35. Fendrich M, et al. The utility of drug testing in epidemiological research: results from a general population survey. Addiction 2004;99(2):197-208.
36. Ensminger ME, Anthony JC, McCord J. The inner city and drug use: initial findings from an epidemiological study. Drug Alcohol Depend 1997;48(3):175-84.
37. Carlone U. Criminalità e sicurezza in Umbria: i dati della Questura di Perugia. Regione Umbria - Consiglio Regionale, Rapporto 2008.
38. Stout PR. Hair Testing for Drugs - Challenges for Interpretation. Forensic Sci Rev 2007;19(1-2):69-84.
39. Apelberg BJ, et al. Racial differences in hair nicotine concentrations among smokers. Nicotine Tob Res 2012;14(8):933-41.

*