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Hair analysis in order to evaluate drug abuse in driver's license regranting procedures

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ABSTRACT

In Italy, driving under the influence of drugs determines the suspension of the offender's driver's license. To regain the license the person must be drug free during an observation period. People whose license has been revoked or suspended can obtain, or re-obtain their driver's license subject to the judgment of a medical commission. The exclusion of illicit drug use is determined by means of toxicological analysis, mainly on urine or hair matrices. We reported the results of several years of experience of the forensic toxicology laboratory of the University of Macerata in the use of hair analysis for the assessment of past exposure to drugs in people suspected of driving under the influence of drugs. From 2004 to 2013, 8612 hair samples, were analyzed for opiates, cocaine and delta-9-tetrahydrocannabinol (Δ^9-THC) using gas chromatography/mass spectrometry (GC/MS) method. We used a cutoff (SoHT or national guidelines) to determine the positive data, regardless of the hair sample concentrations. 1213 samples resulted positive, 71.7% were positive for cocaine and metabolites, 19.8% for morphine and metabolites, 8.5% for Δ^9-THC. We also studied the timeframe of the abuse, as well as gender and age distribution of positive subjects. Moreover, we analyzed the possible deterrent effect of the hair analysis on driving under the influence of psychoactive substances.

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1. Introduction

Many of the accidents and deaths that occur on Italian roads are caused by drivers whose performance is impaired by the use of psychoactive substances. Therefore, over the last few years there has been an impressive increase in police activity aimed at identifying people driving under the influence of drugs. According to Italian law (art. 187 of the Code of Road Law [1]) the use of drugs is a valid reason for disqualification from driving, or revocation of the offender's driver's license. People whose license has been revoked or who have had their license suspended for driving under the influence of drugs, or people addicted to psychoactive drugs can obtain, or re-obtain their driver's license subject to the judgment of a medical commission (Local Medical Commission (CML) art. 119 of the Code of Road Law [1], DPR 495/92 [2]). One of the physical requirements involved is the exclusion of illicit drug use by means of toxicological analysis, mainly on urine or hair matrices. Urinalysis informs us of recent or current exposure, while hair analysis provides information on long-term use of illicit drugs. Moreover, hair analysis allows us to identify drug use more often than urinalysis, and may represent a better means by which to control drug abstinence as required by the law [3–6]. For these reasons, hair analysis has become an important part of forensic and clinical toxicology, also in our country (i.e. doping, work-place drug testing [4,7]).

In Italy, no national laws or guidelines exist to regulate toxicological analysis in assessing driving performance, so each provincial CML may use a different type of analysis. Moreover, four of the five provinces (Ascoli Piceno, Fermo, Macerata, Ancona) of the Marche region have adopted the same procedures and all involve the forensic toxicology laboratory of the University of Macerata for hair analysis within a panel of clinical and laboratory tests. Therefore, the hair data collected at this toxicology laboratory may be considered consistent and representative in order to evaluate the entity of the phenomena. The driver's license is revoked for a 6-month period in the positive presence of hair and/or urine data, and then the person is recalled for a new toxicological analysis and controlled for at least 1 year if negative.

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This paper presents the casework of hair analysis carried out by the forensic laboratory of the University of Macerata. We have focused on data pertaining to opiates, cocaine, cannabis and their metabolites, as commonly required by the protocols of the Commissions. These are, in fact, the most abused illicit substances in Italy and in our region [8,9], and a major cause of death [8]. The role of stimulant drugs like amphetamines or ecstasy appears less relevant in both phenomena.

2. Materials and methods

2.1. Chemicals and reagents

Nalorphine and proadifen (SKF-525A) (internal standards for opiates and cocaine respectively) were purchased from Sigma, while Δ^9-THC-D_3 (internal standard for THC), cocaine, benzoylecgonine and morphine were purchased from LCG standards. N-Methyl,N-trimethylsilyl trifluoroacetamide (MSTFA) was purchased from Sigma. Methanol, dichloromethane, prop-2-ol, ammonium hydroxide, hexane, ethyl acetate and cyclohexane (purchased by Panreac) were reagent grade. Isolute HCX cartridges (10 ml capacity, 130 mg) were obtained from International Sorbent Technology.

2.2. Sample preparation

Hair samples were collected from the posterior vertex of individuals for testing for use of drugs, within a complete amnestic and clinical data panel. The hair samples (4 cm length), after the washing step, were manually cut into small fragments (50 mg minimum quantity) for the detection of drugs.

The samples were incubated overnight in 2 mL of 0.1 N HCl solution at 50 °C and added with internal standard (SKF-525A for cocaine, nalorphine for opiates) for the detection of cocaine, opiates and their metabolites. The resulting mixtures were cooled at room temperature, neutralized with 2 mL of phosphate buffer solution pH 6, added with 130 μl of NaOH 2 M at pH 6–7, and extracted by means of a SPE technique. The columns were conditioned sequentially with a methanol (2 mL) and phosphate buffer pH 6 (2 mL). Samples were then slowly drawn through the column under a low vacuum for at least 2 min. Then the columns were rinsed sequentially with water (2 mL), 0.1 N HCl (3 mL) and methanol (3 mL). After the columns were completely dried (5 min under full vacuum) the analytes were eluted with 2 mL of a dichloromethane/isopropyl alcohol solution (8:2) with 2% ammonium hydroxide. The eluate was completely evaporated and then derivatized with 50 μl of MSTFA at 60 °C for 20 min. One microliters of the derivatized eluate was injected in GC/MS.

The residual hair samples used in the previous analyses were used for Δ^9-THC detention. The samples, to which internal standard (Δ^9-THC-D_3) was added, were subjected to basic hydrolysis (NaOH 1 N solution, at 95 °C for 15 min), cooled at room temperature and subjected a liquid–liquid extraction method. 3 mL of an extraction solution of hexane/ethyl acetate (9:1) was added to the samples, and shaken for at least 15 min. The organic phases were separated. The eluates were completely evaporated, and then 20 μl of cyclohexane was added. One microliters was injected in GC/MS.

2.3. GC/MS instrumental and analytical condition

The GC/MS analytical conditions for drug analysis were: a well-coated open-tubular capillary column (fused silica 30 m × 0.32 mm). The carrier gas was helium at flow rate of 1.5 mL/min. The temperature program started at 100 °C for 1 min, increased first to 220 °C at 40 °C/min for 1 min and then to 310 °C at 10 °C/min. The injection volume was 1 μl in splitless mode. Electron ionization (70 eV) was used; mass spectra range was 50–500.

The analyses were performed monitoring ions *m/z* 82, 182, 303 for cocaine (COC); *m/z* 82, 240, 182 for the cocaine metabolite benzoylecgonine trimethylsilyl derivate (BZE-TMS); *m/z* 86, 99, 165, for SKF-525A (standard internal for cocaine analysis); *m/z* 429, 414, 324 for morphine-TMS (MOR-TMS); *m/z* 399, 340, 324 for 6-monoacetylmorphine; and *m/z* 455, 414, 440, 324 for nalorphine-TMS (internal standard for opiates analysis); *m/z* 299, 314, 231 for Δ^9-THC, *m/z* 234, 302, 317 for Δ^9-THC-D_3, and *m/z* 295, 296, 238 for cannabinol (CBN). The presence of drugs in hair analyzed behind the cut-off values (SOH until 2008 [10], national guideline after this year [7]), was considered as positive data regardless of the concentration. Table 1 presents validation data of the analytical procedures used.

3. Results and discussion

During the period 2004–2013, hair from 8612 samples was analyzed. The analysis of the frequency of the data for each year shows a progressive increase in the number of samples analyzed until 2009, the year in which it becomes almost stable (about 1200 samples every year from 2010to 2013). The progressive increase in the data analyzed over the years is also confirmed in literature [9], and could be explained by a larger increase in controls due to the perception of driving under the effect of drugs as a significant public health problem.

Of these 1213 samples at least one substance proved positive, i.e. 14.08% of the samples checked.

Considering the overall analysis, positive data involves mainly male individuals (94% of positive samples) compared to 6% of female individuals, as shown in literature [4,11]. Whereas, if the positive data is examined in relation to sex this becomes 12.6% for females and 15.8% for males, with no significant statistical differences ($\chi^2 = 2.64$, df = 1, $p = 0.105$), confirming data in literature [11]. Fig. 1 shows the frequency of positive data in relation to the years. In this case we have a progressive decrease from 2006 onwards in the total number of positive data.

Possible causes for the progressive fall in positive samples could be due to a phenomenon of migration of the subjects to other CMLs out of the region that only required examination of urine sample, and/or an effectiveness of hair analysis in discouraging the use of drugs, and to a progressive reduction of drug assumption in recent years [8].

Table 1
Results of validation procedures.

	Linearity range (ng/mg)	R^2	LOQ (LOD) (ng/mg)	Intra-day precision (%CV)	
				0.5 ng/mg	5 ng/mg
Morphine	0.2–10	0.9967	0.2 (0.08)	12.0	4.3
Cocaine	0.2–10	0.9987	0.2 (0.07)	9.0	5.8
				0.1 ng/mg	1 ng/mg
Δ^9-THC	0.05–2	0.9933	0.05 (0.02)	12.8	5.6

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