

Hair analysis by using radioimmunoassay, high-performance liquid chromatography and capillary electrophoresis to investigate chronic exposure to heroin, cocaine and/or ecstasy in applicants for driving licences

F. Tagliaro^{a,b,*}, R. Valentini^b, G. Manetto^b, F. Crivellente^b, G. Carli^b,
M. Marigo^b

^a*Institute of Forensic Medicine, Catholic University of the Sacred Heart, Largo Francesco Vito 1,
00168 Rome, Italy*

^b*Institute of Forensic Medicine, University of Verona, Policlinico, 37134 Verona, Italy*

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Abstract

The present paper describes an integrated diagnostic strategy to check the physical fitness of subjects, formerly users of illicit drugs, to obtain a driving licence, after having quit their addiction. According to the Italian law, applicants for a driving licence with a history of drug abuse must give evidence to have quit this behaviour and to show no risk of relapse in the future. To prove this, at our institute, they undergo medical examination, hair analysis and a urinalysis program on eight serial samples, collected over about 40 days. About 700 subjects per year are investigated with this strategy. The hair samples are screened for opiates (morphine), cocaine and ecstasy, the most abused illicit substances in our region, by using commercial radioimmunoassays adopting cut-off levels of 0.1 ng/mg. All positive samples and about 10% of negatives are confirmed by high-performance liquid chromatography. Further confirmation of results can be carried out by capillary electrophoresis (and/or GC/MS or MS/MS). In 1998, the prevalence of positives for morphine, cocaine and ecstasy was 4.8, 11.3 and 2.6%, respectively. In this year, for the first time, the percentage of hair samples positive for cocaine was greater than that for opiates. The results of this integrated diagnostic strategy are presented and discussed, with particular

*Corresponding author. Fax: +39-45-505-259.

E-mail address: ftmedl@borgoroma.univr.it (F. Tagliaro)

emphasis on the comparison between hair analysis on a single sample and seriate urinalyses (on eight samples). © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

According to the law presently in force in Italy (DPR 495/92), a driving licence cannot be issued or re-issued to persons addicted to psychoactive drugs nor to chronic users of substances impairing driving ability. Persons, whose driving licence has been refused, revoked or suspended for addiction to psychoactive drugs or for driving 'under the influence', claiming to have quit any drug abuse, can obtain (or re-obtain) a licence after a medical committee has confirmed the actual and complete abstinence from illicit drugs and excluded any additional risk of future relapse of drug abuse.

To provide objective evidence of abstinence from drugs with an acceptable chronological window in order to support the clinical decision of this medical committee, since 1990 we have included hair analysis in a panel of clinical and laboratory tests, aimed at investigating retrospectively the toxicological behaviour of subjects.

The present paper is an update of a previous article [1] presenting the casework of hair analysis of the Institute of Forensic Medicine of the University of Verona for the years 1988–1995 and includes also the methodology adopted for the investigation of ecstasy use.

2. The diagnostic strategy and methodology

The diagnostic strategy has not been changed substantially in comparison to what was published previously [1], but hair analysis has been extended to determine the major constituents of ecstasy, which in Italy is a popular recreational drug in the younger generations. Furthermore, capillary electrophoresis (CE) has been improved to enhance its sensitivity in order to meet the most strict cut-offs adopted for hair analysis in this context (i.e., 0.1 ng/mg).

2.1. Sample collection and general strategy

In brief, applicants for a driving licence undergo medical examination, hair sampling (4–5 cm in length from the vertex posterior) and a urinalysis program carried out on eight seriate urine samples, collected at random under direct supervision over a period of about 40 days. Urinalyses consist of immunochemical assays (Synchro[®], Beckman, Fullerton, CA, USA) for opiates, methadone, cocaine, barbiturates, amphetamines, cannabinoids and benzodiazepines with GC/MS confirmation of the positive results, according to the NIDA cut-offs.

The hair samples are screened for opiates (morphine), cocaine and ecstasy constituents

using commercial radioimmunoassays (RIA) (DPC, Palo Alto, CA, USA) adopting a cut-off level of 0.1 ng/mg. All positive samples and about 10% of the negative are then confirmed by high-performance liquid chromatography (HPLC) with electrochemical (for morphine) or fluorimetric (for cocaine and ecstasy) detection [2–4]. In case of positive results for the presence in the hair sample of any drug, the subject is informed and is allowed to provide a second hair sample, which is analysed in parallel with the hair remaining from the first sample collection. In case of confirmation and persisting denial of previous use or claim of unadverted passive exposure, further confirmation of results (with additional costs for the subject) can be carried out by capillary zone electrophoresis (CZE) [5,6], tandem mass spectrometry (MS/MS) [7,8] and/or gas chromatography/mass spectrometry (GC/MS) [9] aimed to detect also drug metabolites such as benzoylecgonine or monoacetylmorphine.

External contamination issues, in the present context, are not extremely relevant because an increased risk of relapse of drug abuse (which is to be specifically excluded according to the law's provisions) can be clearly foreseen for those subjects, formerly addicts although not presently users, who are chronically in contact with illicit drugs.

However, to verify any hypothesis of contamination from the environment, when this possibility is alleged by the subject, analysis of drug metabolites, differential drug determination in non-washed and washed hair and hair sampling from multiple body sites (scalp, axillary, pubic hair) with different susceptibility to external contamination can be carried out.

Since the start, our laboratory follows the inter-laboratory exercises in hair analysis coordinated by Dr Michael J. Welch, National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA.

2.2. Sample preparation and RIA screening

Sample collection, preparation and screening has not been substantially changed in comparison to what was published previously [1]. In short, hair samples (50–200 mg) are washed with 20×2 ml of 0.3% Tween 20 (Sigma, St. Louis, MO) solution in water, thoroughly rinsed with water, dried at 37°C, manually cut into small fragments and incubated overnight in 1 ml of 0.12 M HCl at 45°C. The resulting mixtures are neutralised with equimolar amounts of 0.1 M NaOH and twice extracted into organic phase with ready-to-use Toxi-Tubes A[®] (Marion, Irvine, CA). The pooled organic layers are evaporated to dryness and the residues reconstituted with 1 ml of 0.05 M phosphate buffer, pH 5.0.

The samples are then screened for morphine, cocaine and ecstasy constituents using commercial RIA kits (Coat-a-Count[®]) furnished by DPC (Los Angeles, CA). The antiserum used for morphine determination is highly specific for morphine with only a ~0.3% cross-reactivity for morphine-3-glucuronide (only relevant cross-reactivities were with nalorphine, 27%, and normorphine 9%), the antiserum used for cocaine assay is specific for cocaine and cocaethylene, with a ~2% cross-reactivity with benzoylecgonine, whereas that for MDMA shows a broader cross-reactivity for methamphetamine and related compounds. Standard curves in 0.05 M phosphate buffer, pH 5.0, are prepared daily in the range of concentrations 1–500 ng/ml, from stock solutions of

morphine, cocaine and MDMA in methanol (1 mg/ml). The adopted cut-off is 0.1 ng/mg for cocaine, morphine and MDMA.

2.3. HPLC instrumentation and analytical conditions

The HPLC method for morphine analysis has been fully described elsewhere [2]. In short, it is based on an isocratic separation using a 150×4.6-mm polystyrene-divinylbenzene column with 5- μ m particle size (PLRP-S, Polymer Labs., Church Stretton, UK) and a mobile phase composed of 0.05 M potassium phosphate (pH 9.5):acetonitrile (80/20). Detection is amperometric at +0.45 V vs. a Ag/AgCl reference electrode. An increased concentration of acetonitrile in the mobile phase (about 40%) allows the determination of 6-monoacetylmorphine, but because of its poor stability under the extraction conditions this analyte is not routinely determined.

The HPLC method for cocaine and benzoylecgonine determination [3] uses the same column as the previous method but adopts fluorescence detection (excitation and emission wavelengths are 230 and 315 nm, respectively). The mobile phase is composed of 0.05 M potassium phosphate (pH 3):methanol:THF (72/25/3).

The HPLC method for the major ecstasy constituents, namely MDA, MDMA and MDE, [4] is again based on the same column type eluted with 0.05 M potassium phosphate (pH 3):acetonitrile (72/18) and uses fluorimetric detection with excitation and emission wavelengths of 285 and 320 nm, respectively.

Because until now suitable internal standards have been lacking, external standardisation was adopted for quantification of all analytes.

2.4. CE instrumentation and methods

An automated instrument P/ACE mod. 5500 (Beckman, Fullerton, CA) equipped with an 'on column' diode array detector is currently used.

The standard CZE method for the simultaneous analysis of morphine and cocaine is fully described in Ref. [5]. In short, it is based on high-voltage electrophoresis (15 kV) in tiny bare-fused silica capillaries (50 μ m inner diameter) filled with plain 50 mM borate buffer, pH 9.2, with UV detection (214 nm wavelength for morphine, 238 nm for cocaine). For increased sensitivity, a field amplified sample stacking technique can also be used, based on the dissolution of the hair extract in 0.1 mM phosphoric acid and electrokinetic injection [6]. Tetracaine (for cocaine and amphetamines) and nalorphine (for morphine) are the used internal standards for quantitative analysis.

3. Results and discussion

The analytical strategy here presented, based on RIA screening and HPLC confirmation, is particularly robust and highly productive to meet the needs of a heavy routine work (in our laboratory, a single part-time technician carries out the entire procedure).

The analytical flow-chart and the specific procedures have already been presented in our previous paper [1], and here we want to discuss only some specific points.

HPLC and RIA for ecstasy constituents is obviously poor. Therefore, the RIA results were regarded as merely qualitative data.

On the basis of the first three inter-laboratory exercises on hair testing for drugs of abuse, organised by the NIST, it was reported “(the laboratory using RIA and HPLC) . . . produced results that were generally accurate qualitatively and did not produce any outliers quantitatively. These results suggest that although GC/MS is the preferred approach for measurements for most laboratories, other approaches, such as MS/MS with extraction and HPLC, may be satisfactory with proper care” [10].

After RIA and HPLC almost all the subjects investigated accept the results, admitting repeated use of heroin, cocaine and/or ecstasy within 5–6 months before the hair sampling. In fact, we have an extremely low number (~1%) of disputed results which undergo further confirmation with CZE and/or GC/MS. We have also recently reported a substantial concordance between the results from HPLC and GC/MS (for ecstasy components) or CZE (for morphine and cocaine), as it has been discussed in specific papers [4,5].

A well-known limit of CE is sensitivity, and this is particularly relevant when UV spectra are to be recorded to enhance the identification power. Drug concentration usually present in hair are too low to allow spectral identification without a preconcentration technique. We have recently overcome this problem by applying head column field amplified sample stacking [6]. By this technique, head column concentration factors greater than 100 can be obtained, thus allowing UV spectral identification of analytes from most of hair extracts (down to about 0.5 ng/mg).

This strategy, in the near future will be tested for CE–mass spectrometry, the sensitivity of which is also hampered by the intrinsic limitations in sample loadability of CE.

The quantitative repeatability of field amplified sample stacking is relatively poor in terms of absolute peak areas (RSDs of about 20–40%), because of a great influence of sample matrix composition on analyte loading. However, with the use of an internal standard this variability can be controlled with resulting RSDs of about 5–10%, which are acceptable for the purposes of hair analysis [6].

A comparison between hair analysis and seriate urinalyses (eight samples) carried out on 2011 cases for opiates and cocaine and 429 for ecstasy is shown in Table 2.

In this table, hair analysis shows a diagnostic sensitivity much higher than seriate urinalyses (on eight samples collected over about 40 days). For opiates and cocaine, the frequency of positives from hair analysis is higher than that from urinalyses, but this difference is striking for ecstasy. For this compound, the only positive cases found come from hair analysis, while urine analysis has constantly been negative. This is in agreement with the generally recreational and irregular pattern of abuse of ecstasy which does not fit the limited chronological detection window of urinalysis.

However, a few cases in our casework were negative in hair (but in most instances with drug concentrations just below the cut-offs) but positive in urine. This reveals occasional relapses of drug use after a period of virtual abstinence and strongly points out the usefulness of combining hair analysis with seriate urine analysis for an effective monitoring of drug abuses.

Table 2

Comparison between positive cases identified by hair testing and/or seriate urinalyses (eight samples randomly collected over 40 days)

<i>Opiates (2011 subjects):</i>	
+Hair/ +urine (1 or more samples)	13 cases
+Hair/ –urine	74 cases
–Hair/ +urine (1 or more samples)	8 cases
–Hair/ –urine	1916 cases
<i>Cocaine (2011 subjects):</i>	
+Hair/ +urine (1 or more samples)	10 cases
+Hair/ –urine	67cases
–Hair/ +urine (1 or more samples)	25 cases
–Hair/ –urine	1909 cases
<i>Ecstasy (429 subjects):</i>	
+Hair/ +urine (1 or more samples)	0 cases
+Hair/ –urine	7 cases
–Hair/ +urine (1 or more samples)	0 cases
–Hair/ –urine	422 cases

4. Conclusion

The aim of the diagnostic strategy here presented is the identification not only of heavy addicts, but also of light users of illicit drugs, including subjects simply ‘exposed’ to these substances. This is justified by the provision of the law presently in force in Italy which prohibits the issuing of a driving licence not only to persons addicted to drugs, but also to subjects who, although abstinent, may show any risk of relapse to drug abuse in future. From this point of view, the simple exposure to controlled drugs of a person formerly addicted clearly represents an indication of increased risk of relapse and is per se incompatible with the fitness for obtaining a driving licence. So, in this context, the widely debated possibility of passive contamination of hair by drugs present in the environment, although fundamental elsewhere, is of minor relevance.

In our diagnostic strategy, the aim is to obtain the “admission of abuse of (or exposure to) illicit drugs” from the subjects at the end of the medical and laboratory investigation, and this goal is actually achieved in almost 100% of positive cases.

The subjects formerly positive can apply again for a driving licence after a few months and undergo a new series of hair and urine testing. On this second occasion, they generally observe a more complete abstinence from drugs and in the great majority of cases they test negative or substantially ‘less positive’, indirectly supporting the reliability of hair analysis.

As reported in our previous paper [1], we have not developed a hair testing method for cannabis and hashish abuses. Indeed, the slow clearance of cannabinoids allows a sufficiently wide diagnostic window also of seriate urine testing.

In conclusion, hair analysis integrated with seriate urinalyses is, in our experience, a highly effective and reliable tool for the investigation of drug abuse behaviours in the population. Particularly in a heavy routine environment, the proposed strategy based on

RIA screening and HPLC confirmation, with possibility of further confirmation in disputed cases by GC/MS and/or CZE looks particularly efficient and productive.

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