

Case Report

Postmortem Hair Analysis Verifies Heroin Exposure and Leads to a Killer

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Abstract

This case-report concerns the determination of opiates in hair from a 28-year old female, who was missing and found dead after 18 months. After identification through DNA analysis, toxicological analysis of hair samples was carried out. A gas chromatography-mass spectrometric method was used for the determination of opiates in hair, after thorough decontamination, solid-phase extraction and derivatization with BSTFA with 1% TMCS. The concentrations of morphine, codeine and 6-acetyl-morphine in the hair sample of the victim were found to be 0.083, 0.050 and 0.033ng/mg hair, respectively. The detection of opiates in the hair of the victim essentially helped the police authorities to associate her with her killer.

Keywords: Forensic hair analysis; Homicide investigation; Opiates; GC/MS

Introduction

Forensic pathologists and toxicologists are often confronted with skeletonized remains. In these cases traditional specimens are no longer available but toxicological information may be needed to determine the cause of death. In such a case, the utility of analyzing whatever remains is available from the corpse, e.g. hair, bone, etc. is necessary to extract some information about the case [1]. Hair can be used as an alternative specimen in the absence of urine or blood samples and its analysis is routinely used in forensic science to document repetitive drug exposure [2]. The determination of drugs of abuse in hair is generating considerable toxicological and forensic interest during the last decades [3-5]. The main advantage of hair is that it retains drugs or chemical substances trapped for prolonged periods of time (weeks to months, depending on the length of the hair shaft) as drugs incorporated in it remain unaltered in the keratin matrix for a long time, since they do not participate in any biotransformation reactions or diffusion procedures [6]. By providing information on exposure to drugs over time, hair analysis may be useful in verifying past rather than recent drug use. In addition, hair analysis may be especially useful when a history of drug use is difficult or impossible to obtain [7]. In many fatal cases, the results of this analysis can contribute significantly to the establishment of the cause of death or the manner of death [8]. Although there is reasonable agreement that the qualitative results from hair analysis are valid, the interpretation of the results is still under debate owing to unresolved questions such as the influences of external contamination or cosmetic treatment, and possible genetic differences [9-11].

The aim of this report is to present a case where the application of hair analysis during the investigation of a forensic case revealed the exposure of a female victim of homicide to heroin leading to the killer and clarifying the manner and the conditions of death. Heroin is among the most commonly abused drugs in the world and it is the most frequently encountered drug of abuse in fatal cases of drug addicts. Morphine, codeine and 6-acetyl-morphine are usually identified in biological fluids obtained from opiate addicts. Only the

identification of 6-acetyl-morphine in biological samples provides unequivocal proof of heroin use [12,13].

Case Presentation

This case report concerns a 28-year-old woman, who disappeared from a small town of the Greek countryside where she had gone for summer holidays with her new boyfriend. Eighteen months after her disappearance, skeletal remains with skull and some hair were found in a field of olive-trees (Figure 1), not far from the town where the woman had disappeared. The autopsy and the forensic investigation was carried out by the Forensic Department of Patras. The specimens were sent to the Department of Forensic Medicine and Toxicology of Athens for toxicological investigation. The deceased was identified from her jewels and clothes that she was wearing, as well as from DNA analysis. Toxicological analysis of hair sample was carried out by using solid-phase extraction and a gas chromatography-mass spectrometric method.

Within the frame of the toxicological investigation of the case, a hair extract was screened for all groups of drugs of abuse (opiates, cannabinoids, cocaine, amphetamine type stimulants and benzodiazepines) by immunoassay (VIVA-E, Siemens, Germany)



Figure 1: Skeletal remains with skull and some hair were found in a field of olive-trees.

and it was positive only for opiates. The confirmation of opiates in hair sample was carried out using the analytical method that is used by our Laboratory in routine work. The method was based on the method developed by Papoutsis et al. 2014 [14] for the determination of opiates in blood and it was properly modified and validated. The limits of detection (LOD) and quantification (LOQ) for all three analytes (morphine, codeine and 6-acetyl-morphine) were found to be 0.005 and 0.015ng/mg hair, respectively. The linear dynamic range was between 0.015-1.50ng/mg hair ($R^2 \geq 0.992$) and the intra-/inter-day precision was found to be less than 7.7 and 8.9%, respectively.

The concentrations of morphine, codeine and 6-acetyl-morphine in the hair sample of the victim were found to be 0.083, 0.050 and 0.033ng/mg hair, respectively.

Materials and Methods

Chemicals and reagents

Reference standard solutions of morphine, codeine, 6-acetyl-morphine, morphine-d₃, codeine-d₃ and 6-acetyl-morphine-d₆ at a concentration of 1.00mg/mL (>99.9% pure) were purchased from LGC Promochem (Molsheim, France). All standards were stored according to the instructions of their commercial certificates. The solvents used (methanol, acetonitrile, dichloromethane, isopropanol and acetone) were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Analytical reagents were purchased as follows: N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) from Sigma-Aldrich (Steinheim, Germany), analytical grade ammonium hydroxide (NH₄OH), hydrochloric acid (HCl), glacial acetic acid (CH₃COOH), sodium hydroxide (NaOH) and sodium-dihydrogen phosphate dihydrate (NaH₂PO₄·2H₂O) were obtained from Merck (Darmstadt, Germany). Bond Elut LRC Certify (Sorbent Mass 130mg, Column Volume 10mL) columns were used for solid-phase extraction (SPE) that were obtained from Agilent Technologies (Lake Forest, CA, USA).

GC/MS analysis

The analysis for opiates was performed on an Agilent GC/MSD model 6890N/5975, equipped with a DB-5MS column (30m x 0.25mm i.d. x 0.25µm film thickness). Helium was used as the carrier gas at a flow rate of 1.0mL/min. 1µL was injected in the splitless mode using an Agilent 7683B Series auto-sampler system. The optimized GC conditions were as follows: initial column temperature 150°C, held for 1min, increased at a rate of 40°C/min to the final column temperature of 300°C and held for 5min. Injector, ion source and interface temperatures were set at 260, 230 and 280°C, respectively. The mass spectrometer was operated in electron impact (EI) ionization mode with selective ion monitoring (SIM) mode. The mass fragments used for the identification of the analytes were: *m/z* **429**, 401, 414 and 146 for silylated morphine, *m/z* **371**, 356, 343 and 234 for silylated codeine and *m/z* **399**, 340, 384 and 324 for silylated 6-acetyl-morphine, whereas the mass fragments used for the silylated morphine-d₃, codeine-d₃ and 6-acetyl-morphine-d₆ (internal standards) were **432**, **374** and **405**, respectively (the bold marked ions were used for the quantification).

Sample preparation

Hair sections of 1 cm from the hair root should be analyzed, but it was not possible, since the hair was a messy mass, mixed with mad.

So, sample of hair (300mg) was selected, although the region of the posterior vertex was unknown. The sample was placed in a collection paper packet at room temperature. The hair was decontaminated by successive washing with shampoo, dichloromethane, isopropanol and acetone for 5min at room temperature and the hair was dried under nitrogen flow. Then, the hair sample was manually cut with scissors into short segments of 1-2mm. The hair sample was incubated for 24h in 2mL 0.1N HCl solution at 50°C after addition of 50µL of the working internal standard solution (1.00µg/mL). The resulting mixture was cooled at room temperature, neutralized with 2.0mL of phosphate buffer solution pH 6.0 (0.1M), added with 130µL NaOH 2.0M at pH 6-7 and centrifuged at 2000rpm for 10min. Bond Elut LRC Certify SPE columns were conditioned sequentially with 3mL of methanol, 3mL of deionized water and 1mL of 0.1M phosphate buffer (pH=6.0) prior to sample loading. The samples were applied to the columns at a flow rate of approximately 1.0mL/min. Then, the columns were washed successively with 3mL of deionized water, 1mL of 0.1M acetate buffer (pH 4.0) and 3mL of methanol and after that they were dried under high vacuum (≥ 10 mmHg) for 5min. The analytes were eluted twice with 1.5mL of a freshly prepared mixture of dichloromethane: isopropanol: ammonium hydroxide (85:15:2, v/v/v). The eluates were collected in clean tubes and evaporated to dryness under a gentle stream of N₂ at 40°C. The extracted samples were derivatized by silylation. Silylation was achieved by adding subsequently 50µL of acetonitrile and 50µL of BSTFA with 1% TMCS to each tube and after vortex mixing the tubes were tightly capped and heated at 70°C for 30min. After cooling the tubes, the solution was transferred to auto-sampler vials and 1µL was injected into the GC/MSD system (splitless mode).

Discussion and Conclusion

Hair is one of the most common forms of forensic trace evidence, which explains why crime scene investigators are extremely interested in developing methods using hair for identifying and distinguishing people. The measurement of a unique drug (either illicit or therapeutic) in hair samples could help associate a person with a crime scene, especially if corroborated with another tissue sample (e.g. blood). Since hair is among the most common forensic evidence collected at crime scenes, the techniques used for associating/excluding evidential hair samples from hair of known origins are extremely important tools for law enforcement [15-17].

The identification of opiates in the hair of the victim in the presented case indicated that the deceased had a history of exposure (and possibly use) to heroin, but could not answer what the cause or the manner of death was. These results led the police authorities to investigate her acquaintances and this investigation led to the brother of her boyfriend who was a known heroin-addict. After thorough interrogation he confessed that he had an affair with the victim and they were using heroin together for some months. After an argument the perpetrator strangled her and threw her into a nearby river. The dead body was carried away by the waters of the river several kilometres away to the agricultural site, where the skeletal remains were found.

The critical issue in hair analysis is that potential contamination of hair from external sources can lead to false-positive results [18]. Despite this issue of passive contamination, hair can offer,

under certain circumstances, extremely useful information for the investigation of specific forensic cases. In this case-report, the detection of opiates in the hair of the victim essentially helped the police authorities to associate her with her killer.

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