Journal of Forensic and Legal Medicine 22 (2014) 62–67

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Determination of clozapine in hair and nail: The role of keratinous biological materials in the identification of a bloated cadaver case

Hang Chen, MD, Medical Student [a,b], Ping Xiang, PhD, Analytical Chemist [b], Min Shen, PhD, Professor, Forensic Medicine [b,*]

[a] Department of Forensic Medicine of Shanghai Medical College, Fudan University, 138 Medical College Road, Shanghai 200032, China
[b] Department of Forensic Toxicology, Institute of Forensic Science, Ministry of Justice, 1347 Guangfu Xi Road, Shanghai 200063, China

ARTICLE INFO

Article history:
Received 10 July 2013
Received in revised form
9 October 2013
Accepted 7 December 2013
Available online 18 December 2013

Keywords:
Hair
Nail
Clozapine
Cadaver identification
Keratinous biological material

ABSTRACT

Keratinous biological materials, such as hair and nails, offer a substantially longer retrospective window of detection compared to other body fluids. Little research on drug analysis in nails is currently being conducted. In this study, the hair and nails from a bloated cadaver was analyzed. The study showed that the forensic toxicology results of keratinous biological materials could provide valuable clues for solving cases.

In this study, a method was developed for the extraction and analysis of clozapine from hair and nails. The keratinous bio-samples were washed and then pulverized using a freeze mill. After ultrasonic bath extraction, the supernatants were analyzed by ultra-performance liquid chromatography tandem mass spectrometer (UPLC–MS/MS).

The method presented in this study proved to be reliable, specific, selective and sensitive with high precision and accuracy. Clozapine was found in both hair and nails from a long term user's remains, even after serious decomposition. The mean concentration of clozapine in the hair was 322.9 pg/mg and 138.3 pg/mg in the nails. Toxicological results helped police narrow the scope of the investigation and improved the efficiency of the breaking of the case.

The findings of the present study demonstrated that the method can be used in forensic investigation. Toxicological results increased the efficiency of cadaver identification and the solving of the case. The study demonstrated that hair and nail analysis could provide vital clues for solving cases and showed the value of keratinous biological materials in the forensics field.

1. Introduction

Hair and nails are keratinous biological materials that have a similar likelihood of accumulating drugs. The major advantage of keratinous biological materials in drug analysis compared to body fluids is that they have a larger surveillance window. This makes keratinous biological materials an important detection tool for toxicologists.[1] There are many applications in the literature in which hair analysis was used to document historic drug use or exposure, such as suspicious death, discrimination between single and chronic exposure, and crimes committed under the influence of drugs.[2] Hair testing for drugs has been successfully performed several months after death even following exhumation.[3] Balabanova et al. used hair to identify drugs in Egyptian mummies.[4] Nicotine was recently reported to have been detected in hair samples of pre-Columbian mummies.[5] Similar to hair, nails provide a stable material for detecting drug exposure[6] and can be a complement to hair in the detection of drugs.

Clozapine is an atypical antipsychotic medication with high toxicity. It is a prescribed drug in China, and few people can come into contact with this drug. Cirimele et al. detected clozapine in hair using gas chromatography coupled to mass spectrometry,[7] Weinmann et al. used liquid chromatography-tandem mass spectrometry to analyze hair samples of psychiatric patients,[8] and Thieme et al. studied hair segments.[9] Some reports shown that clozapine could be detected in hair even it was collected from a grave.[10] The determination of clozapine in hair and nail from schizophrenic patients was researched in our previous study.[11,12]

This paper presents a simple and sensitive UPLC–MS/MS method to detect clozapine in hair and nails. The method was validated, and the hair and nail samples from a bloated, immersed cadaver in a case were analyzed.

* Corresponding author. Tel.: +86 21 52360148.
E-mail addresses: Dio.chen.h@gmail.com (H. Chen), minshensfjd@hotmail.com (M. Shen).

1752-928X/$ – see front matter
http://dx.doi.org/10.1016/j.jflm.2013.12.009

2. Case

A bloated cadaver was found at the floodgate of a reservoir in a town that is located in the estuary of the Yangtse River, China, on 27 January 2013. The investigated remains were from a male of unknown name. His wrists, ankles and neck were tied, and no other obviously lethal wounds were present. Clozapine was detected in the blood at 470 ng/ml, which was within the therapeutic concentration range (the therapeutic concentration in blood of clozapine is 102–771 ng/ml[13]).

Identification of this anonymous deceased was difficult. The town is located in the estuary, where the local hydrographic net is complicated (drainage density = 3.25 km/km^2),[14] and the remains floated in the river for a long time. This delay period made it even harder for police to identify the location where the body was placed in the water. The population of this area is very high (the average population density in 2011 was 978 people/km^2).[15] DNA testing and fingerprint matching were unable to confirm the deceased in this case. To narrow the search and to determine whether the deceased was a psychiatric patient with a long history of clozapine use, hair and nail samples were collected for extensive testing.

3. Methods

3.1. Chemicals and reagent

Clozapine and clozapine-d_4, 1 mg/ml and 100 μg/ml ampoules, respectively, were purchased from Cerilliant (Round Rock, TX). High performance liquid chromatography (HPLC) grade acetonitrile was purchased from Sigma–Aldrich (St.Louis, MO, USA). Ammonium acetate (HPLC grade) and formic acid (HPLC grade, 50%) were purchased from Fluka Chemical Co. (Buchs, Switzerland). The deionized water used in experiments was produced by a Millipore milli-Q water purification system (Millipore, MA, USA). All other referred organic reagents were of analytical-reagent grade.

3.2. UPLC–MS/MS conditions

An Acquity™ Ultra Performance LC system (Waters Corporation, Milford, MA, USA) equipped with a quaternary pump, vacuum degasser, auto sampler, and column over compartment was used. Chromatographic separations were performed on a Restek Ultra IBD column (50 mm × 2.1 mm, i.d., 5 μm, Bellefonte, PA). The column temperature was set to 25 °C and the total flow rate was set to 200 μl/min. The mobile phase consisted of 70% acetonitrile and 30% ammonium acetate buffer (20 mM ammonium acetate buffer with 0.1% formic acid, pH 4.0), and run time was 7.0 min.

A 4000 QTRAP (Applied Biosystems/MDS Sciex, Concord, ON, Canada) hybrid triple quadruple-linear ion trap mass spectrometer equipped with electro spray ionization (ESI) source was used to analyze the samples. Samples were analyzed in the Multiple-reaction monitoring (MRM), positive mode. The clozapine (m/z 327.3 > 296.2, 327.3 > 270.1) and clozapine-d4 (m/z 331.2 > 299.2) ion transitions were monitored. The ESI source temperature was 500 °C. The curtain gas flow was 30 psi; collision gas was 7 psi; ion spray voltage was 5000 V; and entrance potential was 10 V.

The Analyst 1.5 software (Applied Biosystems, USA) was used for instrument control and data acquisition.

3.3. Sample collection and preparation

Hair strands (6 cm long) were cut as close as possible to the scalp. The ends corresponding to the cut were marked. The original color of the hair strands was black, but they looked blanched from the long soaking time. Extraordinarily, the nail samples were collected from the free edge of each finger or toe. Unfortunately, the fingernail on the middle finger of left hand, the toe nail on the middle toe of the right foot, the little toe of the right foot, and the little toe of the left foot were lost from the rotting. All samples were separated and stored in clean paper packs at room temperature.

The blank hairs and nails were obtained from healthy volunteers with no drug history from our laboratory and used for selectivity studies and to create spiked samples for the validation of the analytical procedure.

The method of sample preparation was optimized in previous studies.[11]

Hair strands were divided into 6 segments of 1 cm long from root to end. The hair segments or nail clippings from each finger or toe (15 mg) were washed independently with 2 ml deionized water twice by vigorous shaking for 3 min; then the water residue from the two washes was combined and moved to a separate tube for later analysis. Next, 2 ml of ethyl acetate were added to the hair or nail samples in the tube and shaken vigorously for 3 min and repeated once. After that, all organic residues were combined and saved in another tube for later analysis.

After drying at room temperature, the hair or nail samples were pulverized using a freeze mill (6770 Freezer/Mill, SPEX CertiPrep, Metuchen, NJ). A stainless steel impactor with an agitation rate at 5cps for 3 min at liquid nitrogen temperature was used, followed by a 2 min cool-down time, and then grounding again for 3 min. Hair or nail powder (5 mg) was then sonicated in an ultrasonic bath (KQ-100B, Kunshan, PRC) for an hour with 900 μl mobile phase and 100 μl 5 ng/ml clozapine-d_4. The ultrasound frequency was 40 kHz, and the power output of ultrasonic bath was 100 W. The water in the ultrasonic bath was changed periodically to avoid a temperature increase. The mixture was then centrifuged at 12 000 rpm for 5 min; the supernatant was injected into the UPLC–MS/MS system for analysis.

The preparation methods for the aqueous and organic wash residues were referred to as Tsanaclis L[16] and Hill V,[17] respectively. The aqueous or organic wash residues dried unaided and were reconstituted in 1000 μl of the mobile phase when the drying was complete. The reconstituted wash residues were analyzed as biological samples using UPLC–MS/MS. The analyte concentrations in the wash residue (W) and in the keratinous sample (K) were determined, and the results used to calculate the W/K ratio for each analyte in the samples.

3.4. Validation

Validations of the method were performed on blank hair and nail samples. According to EURACHEM guidelines,[18] the selectivity, linearity, accuracy and precision of the method was validated. Quality control (QC) samples (50, 200, and 750 pg/mg for clozapine) were included in each analytical batch to check calibration, accuracy and precision. All QC and calibration samples were prepared by spiking drug-free hair and nail samples with methanol working standard solutions and stored at −4 °C.

The selectivity of the method was confirmed by analyzing 6 different samples of blank hair and 6 different samples of blank nail. Detection and quantitation limits meet the requirements for practical examination.

Calibration curves were prepared and analyzed on four consecutive days. Linearity was determined from the calculation of a linear regression fit from the peak area vs. concentration plot for seven standard solutions spiked in blank matrices (10, 50, 100, 200, 500, 750 and 1000 pg/mg) using linear least squares methodology and analysis of the respective response factors.

The accuracy and precision were based on three different concentrations of QC samples. The accuracy was calculated as the