Analytica Chimica Acta 948 (2016) 40–47

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Three-step drug extraction from a single sub-millimeter segment of hair and nail to determine the exact day of drug intake

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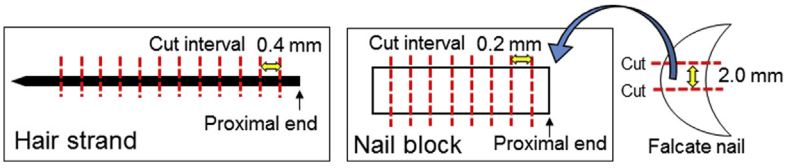
HIGHLIGHTS

- We prepared accurate 0.4-mm hair and 0.2-mm nail segments using a tissue slicer.
- Those correspond to their respective growth rates of 1–2 days.
- Sonication was an efficient drug extraction method from a sub-millimeter segment.
- Drug distributions in a single hair and a nail block were measured at day levels.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form
17 October 2016
Accepted 18 October 2016
Available online 30 October 2016

Keywords:
Hair
Nail
LC/MS
Drug
Distribution

ABSTRACT

Hair and nails are often used to prove drug intake over several months. However, it is impossible to determine the day of drug intake by conventional segmental analysis of bulk samples. To improve this segmental analysis, we prepared accurate 0.4-mm hair and 0.2-mm nail segments, which correspond to their respective growth rates of 1–2 days, using a tissue slicer. The aim of this study was to develop an efficient method to extract drugs from a single sub-millimeter segment of hair and nail. Hair and nails were collected from a subject who was administered a single dose of chlorpheniramine. Four drug extraction methods based on different principles such as sonication, microwaves, micropulverization, and alkaline dissolution were compared. Short-duration sonication followed by long-duration soaking served the aim. Drug extraction from a sub-millimeter segment was performed in three steps as follows: a segment was first washed, followed by sonication for 10 min soaking in the extraction solution for 24 h. The drug concentrations in the three extracts from each segment were quantified using high performance liquid chromatography-tandem mass spectrometry. Each concentration was displayed on a single hair strand and a single nail block so that the first, second, and third extracts corresponded to components on the surface, in the outer layer, and within the sample, respectively. The distribution of chlorpheniramine in a hair successfully reflected the intake history. This method can be used in the future to measure the detailed distribution of drugs in a single hair and nail.

1. Introduction

It is important to elucidate the drug intake history of suspects and victims in drug-related crimes. Hair and nails are often used to prove chronic drug intake because ingested drugs can stably remain in these specimens over several months [1–3]. In forensic drug testing, multiple pieces of hair and nail (normally several milligrams) are lumped together. The bulk sample is then analyzed to detect trace amounts of drugs with certainty, and to average the

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http://dx.doi.org/10.1016/j.aca.2016.10.029
0003-2670/

maldistribution of drugs in such solid specimens unlike that in urine and blood.

Although hair can also be used to predict the time of drug intake by segmental analysis [1,2,4,5], normally tens of hairs are cut into several centimeters and the pieces of hair from the same segment are pooled as one sample. Therefore, it is impossible to determine the day of drug intake by conventional segmental analysis of these bulk samples and from such long segments.

Recently, a single hair analysis using matrix-assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS) has been highlighted [4–7], and the distributions of drugs such as ketamine and cocaine, which can be targeted in forensic laboratories, in a single hair strand were investigated. However, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) is superior to MALDI-IMS in qualitative and quantitative analysis of drugs because of the chromatographic separation of components. In addition, remarkable improvement in detection sensitivity of HPLC-MS/MS instruments has enabled drugs to be detected at sub-femtogram levels on column. Therefore, it would be possible to detect drugs from shorter and smaller segments of hairs and nails than those used in conventional segmental analysis.

As a preliminary study to advance segmental analysis of hair and nails, we examined a method to cut a single hair strand and a single nail block at intervals of sub-millimeters (0.4 mm for hair and 0.2 mm for nail), which correspond to their respective growth rates of 1–2 days [8,9]. It was found that a tissue slicer with a micrometer, which enables to move the sample stage at a minimum scale of 10 μm, was useful to cut a hair strand and a nail block accurately at sub-millimeter levels.

In this study, chlorpheniramine (CP), an anti-allergic medicine, was selected as a model drug because CP was detected in hair and nails with high sensitivity by HPLC-MS/MS in our previous study [10]. We developed an efficient method to extract drugs from a single sub-millimeter segment of hair and nail by comparing four extraction methods namely sonication [11,12], microwave-assisted [13], micropulverization [10,14], and alkaline dissolution (AD) [11,12,14]. Furthermore, a new analytical procedure for measuring the detailed distribution of drugs in a single hair and a single nail block using a tissue slicer and a highly sensitive HPLC-MS/MS instrument is presented, and our interpretation on drug uptake into hair is also described.

2. Materials and methods

2.1. Materials

New Stac Eve Ace, a pharmaceutical product, was purchased from SS Pharmaceutical Co., Ltd. (Tokyo, Japan). CP-d_6 maleate was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). CP maleate and the other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetonitrile (MeCN) and water were of liquid chromatography/mass spectrometry grade. Ultrafree MC (polytetrafluoroethylene, pore size of 0.2 μm), a filtration device, was purchased from Merck KGaA (Darmstadt, Germany). A stainless-steel bullet with an 8-mm diameter (SK-100-DLC10) was purchased from Tokken, Inc. (Kashiwa, Japan). A 2-mL safe-lock tube was purchased from Eppendorf AG (Hamburg, Germany). RAPID EPS, an adhesive sheet for sealing a sample plate, was purchased from BioChromato, Inc. (Fujisawa, Japan).

2.2. Drug administration experiments

Tablets of the pharmaceutical product containing CP maleate were administered to a healthy volunteer at the single therapeutic dose for adults (2.5 mg). He had not taken any medication during the six-month period preceding the experiment. Hair and nails were collected 35 and 70 days after CP administration. Hairs were cut near the scalp with scissors. Free edges of toenails were cut with a nail clipper. The drug administration experiment was performed under the approval of the ethics committee at the National Research Institute of Police Science (Kashiwa, Japan).

2.3. Preparation of hair and nail samples

Some of the hairs and nails, collected 35 days after CP administration, were stored to measure drug distribution in a single hair strand and a single nail block, and the rest were used to optimize the drug extraction method.

Tens of hairs, collected 35 and 70 days after CP administration, were cut at 3-cm length from the proximal cut end and the 3-cm proximal hairs were placed in a 13.5-mL glass tube. After the hair's surfaces were washed by sonication in an aqueous solution containing 0.1% sodium dodecyl sulfate, water, and methanol as previously reported [15], the washed hairs were cut into pieces that were less than 1 mm long with clean scissors for homogeneity. Hair pieces were placed into a 2-mL safe-lock tube to weigh approximately 6 mg.

Tens of nails, collected 35 and 70 days after CP administration, were placed in a 25-mL plastic tube, washed, and cut into less than 1 mm in the same manner as the hairs. Nail pieces were placed into a 2-mL safe-lock tube to weigh approximately 15 mg.

2.4. Drug extraction

CP was extracted from the hairs and nails using four methods: ultrasonic extraction (USE), microwave-assisted extraction (MWE), micropulverized extraction (MPE) with a stainless steel bullet, liquid-liquid extraction (LLE) after AD (AD-LLE). The initial mobile phase as shown in Supplementary table 1 was used as the extraction solution. CP-d_6 maleate was dissolved in the initial mobile phase to provide an internal standard (IS) solution (100 pg mL^{-1}). The initial mobile phase (480 μL) and the IS solution (20 μL) were added to a tube containing the sample for USE, MWE, and MPE. Detailed procedures are described in the following sections.

2.4.1. USE procedure

The sample tube was inserted into a float, floated on water in an ultrasonic cleaner with two-frequency switching of 23 kHz and 43 kHz (ASU-10D; AS ONE Corporation, Osaka, Japan), and sonicated at 43 kHz for 5 min. After the sample tube was shaken for 30 s and centrifuged at 16000 × g for 1 min, the supernatant (75 μL) was filtered, the filtrate was diluted with the initial mobile phase (75 μL). The solution was analyzed as an extract from 5-min USE.

Next, the residue in the sample tube was treated in the same manner as described above to obtain an extract from 10-min USE. Moreover, the residue in the sample tube was kept at room temperature for 24 h to evaluate the effect of long-duration soaking in the extraction solution, and then treated in the same manner as described above to obtain an extract from 24-h soaking after 10-min USE.

2.4.2. MWE procedure

The sample tube was placed in a pressure-proof container and the sample was heated at 700 W for 5 min in a microwave oven (RE-T13; Sharp Corporation, Osaka, Japan). Extracts from 5-min MWE, 10-min MWE, and 24-h soaking after 10-min MWE were obtained using a similar procedure as USE.