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ANNALES DE TOXICOLOGIE ANALYTIQUE

Letter to the Editor – Lettre à la Rédaction

Assault under the influence of methylphenidate documented by hair analysis

Une agression sous influence de methylphénidate mise en évidence par analyse des cheveux

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1 Introduction

Attention-deficit hyperactivity disorder (ADHD) is the most common neuro-behavioral disorder of childhood affecting school-aged children, with a prevalence generally estimated to be 5–10% of general population [1]. Methylphenidate (MPH), a phenethylamine derivative, is the available medication the most extensively studied and widely prescribed. MPH is reported to be absorbed quickly and completely from the gut after oral administration and it is rapidly hydrolyzed in the methyl ester linkage to its metabolite, ritalinic acid [2].

Although the drug is generally well tolerated, side effects of MPH may cause agitation, delirium or hallucinations. Given the drug is a stimulant, it is acceptable to consider that impairment can occur in case of ingestion [3]. This compound is listed as a drug of abuse in France and has no indication for adults, according to the European Pharmacopoeia.

Hair sampling is a useful complement to blood and urine analyses to increase the window of detection and to permit differentiation of a single exposure from chronic use of a drug by segmentation. Moreover, due to the long delays that are frequently encountered between the event and the matter being reported to the police, hair can often be the only matrix capable of providing corroborative evidence of a committed event.

The literature is very poor in papers dealing with the detection of MPH in hair. Marchei et al. [2] and Sticht et al. [4] have published procedures using liquid chromatography and gas chromatography coupled to mass spectrometry, respectively.

Given that the measured concentrations are low, less than 1 ng/mg, even after chronic daily exposure, any method devoted to testing for MPH in hair after a single ingestion must be of very high sensitivity.

We present here an original method to test for MPH in hair by LC-MS/MS and its application to an assault case.

2 Case history

A 26 year-old woman was accused of assaulting another person at a party on late November 2008. She states she was very drunk and was asked to leave the party. She became aggressive and damaged doors and cars and finally assaulted a female. She stated her drink was spiked with MPH, which would account for her bizarre behavior. No urine nor blood were sampled as she did not mention exposure to a drug in her first interview with the police. Several days after the incident, examination of blood or urine was felt to offer little assistance in determining if drugs had been employed. Consequently it was decided that a hair sample should be obtained from the woman for analysis. Hair was taken (as close as possible from the skin, in the vertex area) on late February 2009 and sent to the laboratory for MPH testing. The reasons to wait for 3 months before a hair sample was collected are unclear, but probably due to administrative problems.

The laboratory received a lock of hair of >20 cm length, of brown color with the request to analyze for MPH by segmentation.

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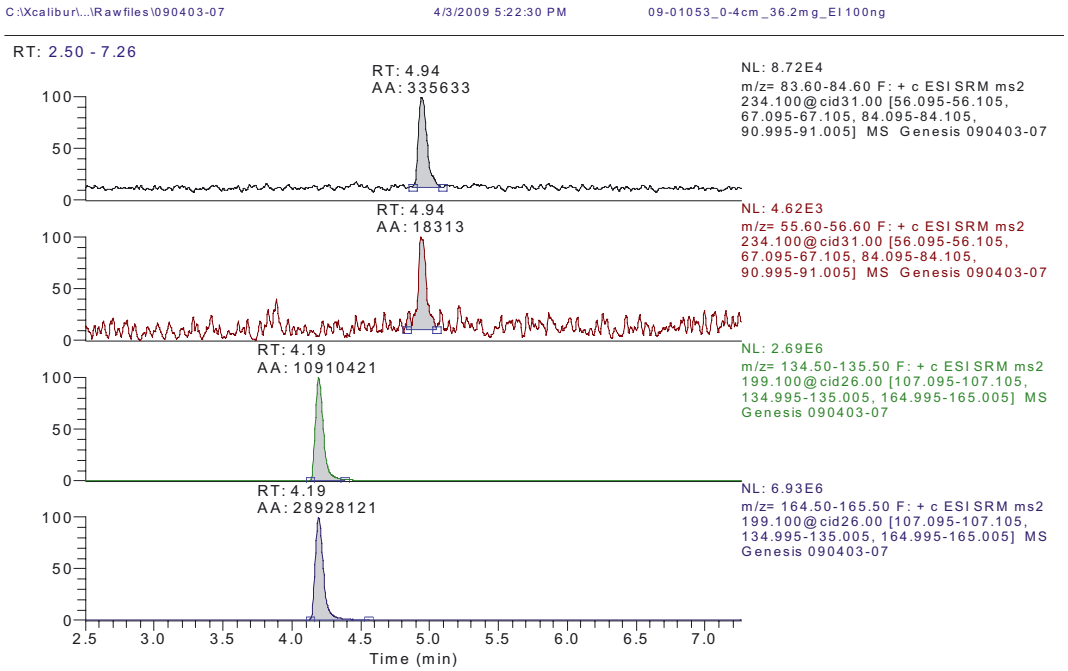

Fig. 1. Chromatogram of the proximal 0–4 cm segment of hair extract from the subject. methylphenidate concentration was 1 pg/mg. Top: 2 transitions of methylphenidate; bottom: 2 transitions of the internal standard

3 Method

3.1 Extraction

Hair strand was twice decontaminated using methylene chloride (5 mL, 2 min) and then segmented (3 segments of 4 cm). Each segment was cut into small pieces (<1 mm). About 30 mg were incubated for 3 hours in an ultrasonic bath in 1 mL of acetate buffer at pH 5.5, in the presence of 10 ng of MDMA-d_5 used as an internal standard (IS). After neutralization to pH 7.0 with 1N NaOH, a liquid-liquid extraction with 5 mL of a mixture of hexane/ethyl acetate (90/10, v/v) and evaporation to dryness, the residue was reconstituted in 200 µL of acetonitrile/2 mM formate buffer (5/95, v/v).

3.2 LC-MS/MS procedure

Hair extract was separated on an XTerra MS C18 column (100 × 2.1 mm, 3.5 µm) using a Thermo Fischer Scientific (Waltham, MA, USA) Accela system. Chromatography was achieved using a gradient of acetonitrile and formate buffer delivered at a flow rate of 0.2 mL/min (5% acetonitrile – 95% formate buffer adjusted to pH 3.0 with formic acid 0.1% to a ratio 80–20% at 9 min). An injection volume of 10 µL was used in all cases. A Quantum Ultra triple-quadrupole mass spectrometer (Thermo Fischer Scientific) was used for detection. Ionization was achieved using electrospray in the positive ionization mode (ES+). Total run time was 16 min.

The following conditions were found to be optimal for the analysis of MPH and the IS: a spray voltage of 4000 V, a capillary temperature at 350 °C, an offset at 5 V, tube lens near 90 V, sheath gas and auxiliary gas pressure at 40 and 30 (arbitrary units), respectively, and collision cell pressure at 1.5 m Torr of argon. To pseudo-molecular ion (M + H)+, two product ions were acquired at a scan time of 0.03 s and collision energies (eV) were optimized (table I).

For both MPH and MDMA-d_5, detection was related to two daughter ions (m/z 234 > 84 and 56 for MPH; m/z 199 > 135 and 165 for IS).

4 Result of the validation

Linearity was observed for MPH concentrations ranging from 0.5 to 500 pg/mg with a correlation coefficient of 0.9986. Within-batch precision at 50 pg/mg was 14.1%, and the extraction recovery was about 70%. The limit of detection was 0.3 pg/mg, with a limit of quantitation of 0.5 pg/mg. Ion suppression nor matrix effects higher than 10% were not observed.

The stability of MPH to the incubation / extraction conditions was verified.

5 Detection in hair

In accordance with the fact that acidic substances are incorporated to a much lesser extent than basic compounds in the hair matrix, preliminary observations [2,4] showed that ritalinic acid is absent in hair from treated subjects.

As hair collection occurred 3 months after the event, it was decided to analyze 3 sections of 4 cm. The hair washings were negative (same chromatographic signal as a blank).

Figure 1 is the chromatogram obtained after extraction of the proximal segment of hair. Methylphenidate concentration was 1.0 pg/mg. In comparison with the seldom-available data

Table I. MRM transitions and conditions for the measurement of methylphenidate and the internal standard.

Compound	Parent ion (m/z)	Product ion (m/z)	Tube lens voltage (V)	Collision energy (eV)
Methylphenidate	234	84	88	19
		56	88	31
MDMA-d_5	199	135	86	21
		165	86	14

from chronic exposed children [2, 4], the measured concentration can be considered as very low. Segmental analyses demonstrated that only the proximal segment tested positive for MPH. In the 2 consecutive segments, the concentrations were negative (no chromatographic response). These segmental results support a single exposure to MPH during the last 4 months (period including the party). It is not possible to interpret the measured concentration and to put any quantitative data about the dosage and the level of exposure, due to a lack of controlled studies involving MPH.

The major practical advantage of hair testing compared to urine or blood testing for drugs is that it has a larger surveillance window (weeks to months, depending on the length of the hair shaft, against 2–4 days). For practical purposes, the two tests complement each other. In addition, hair analysis may be especially useful when a history of drug use is difficult or impossible to obtain. The discrimination between a single exposure and long-term use can be documented by multisectional analysis [5].

6 Conclusion

Hair testing should be used to complement conventional blood and urine analysis as it increases the window of detection and permits differentiation, by segmentation, of long-term therapeutic use from a single exposure. Selectivity and sensitivity of MS/MS are a pre-requisite for testing a single exposure to methylphenidate, given the low concentrations to be measured.

Conflict of interest. The authors declare that there are no conflicts of interest.

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