Testing for Atropine and Scopolamine in Hair by LC–MS–MS after Datura inoxia Abuse

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Abstract

Datura inoxia belongs to the family of Solanaceae. This is a very common plant in New Caledonia that contains two main toxic alkaloids, l-atropine and l-scopolamine. In this study, we report the case of a 20-year-old male admitted to an Emergency Unit after consumption of 6 dried flowers in hot water for hallucinations, mydriasis, and agitation associated with tachycardia and increase of systolic blood pressure to 180. Full recovery was observed after one week. Three weeks later, a lack of about 80 hairs (200 mg) was collected from the subject in vertex posterior with scissors to be tested for both atropine and scopolamine. After decontamination with dichloromethane, a strand of hair was segmented into three parts, cut into small segments (< 1 mm), incubated overnight in 1 mL pH 8.4 phosphate buffer in the presence of 2.5 ng atropine-d3, the internal standard, then extracted with 5 mL dichloromethane/isopropanol/n-heptane (1:1:1) and eluted with a gradient of acetonitrile and formate buffer delivered at a flow rate of 0.2 mL/min. A Quattro Micro triple-quadrupole mass spectrometer (MS) was used for analyses. Ionization was achieved using electrospray in the positive ionization mode. For each compound, detection was related to two daughter ions m/z 290.2 to 124.0 and 92.9; atropine-d3: m/z 293.1 to 127.0 and 92.9; scopolamine: m/z 304.1 to 138.0 and 156.0).

Introduction

The Datura plants are members of the botanical family Solanaceae, which also contains such common foods as tomatoes, potatoes, eggplants, peppers, and tobacco. The daturas are herbaceous plants with fragrant, trumpet-shaped flowers. They grow most often in disturbed soil and waste areas such as abandoned fields, ditches, trash heaps, and roadsides throughout the Americas, Eurasia, and Africa.

There is archaeological evidence in the form of botanical remains and petroglyphs that Datura has been used in the American southwest since at least 4000 years ago, often being associated with other hallucinogenic plants, including peyote. Datura has been also used by sorceresses to cause illness or death or to cast a spell of love through manipulation of the spirit world.

Though the exact taxonomy of Datura is poorly understood; there are at least 15 different species, including Datura inoxia. It is an upright annual (60 to 120 cm tall) plant from perennial rootstock. The entire plant is covered with small, spreading hairs. The dark green or purplish leaves are 20 to 30 cm long and have an asymmetric base. The large white flowers are 20 to 30 cm long and bloom at dusk. It has a bushy growth habit with up to 200 kidney-shaped seeds borne in pods with closely spaced thorns.

The chemistry of Datura is primarily composed of active tropane alkaloids including L-scopolamine (L-hyoscine), L-atropine (L-hyoscyamine), tropine, meteloidine, and over 20 others (1). Tropane alkaloids are muscarinic antagonists that block neurotransmission across muscarinic cholinergic receptors. Characteristics of muscarinic antagonism, depending on dosage, include dry mouth, flushing or rashes, hypertension, tachycardia, bronchodilatation, blurred vision, dizziness or vertigo, sedation, and amnesia (2). Atropine and scopolamine are physiologically active at doses of 0.5–1 mg. Alkaloids content is known to vary significantly among species, within a species depending on season or time of day, and even within a particular plant. It appears that the immature plants mainly contain scopolamine. Often, roots, stems, leaves, flowers, and seeds have differing suites of alkaloids in differing concentrations. For this reason, preparation for recreational versus divinatory use is often accomplished using different species or different parts of the plant. Traditional preparations include adding roots, leaves, or seeds to a fermented drink; drinking an infusion of the leaves or other parts; smoking the...
leaves; or chewing the fruit. When the plant material is taken orally, the effects last longer than when smoked and will also be more narcotic and hallucinogenic.

The toxicity of *Datura* species is well known and has been linked to poisonings and deaths, usually due to heart or respiratory failure, for centuries. *Datura* intoxication can last from a few hours to many days, depending on dosage and method of ingestion. Exposure manifests as a classic anticholinergic syndrome comprising central and peripheral signs and symptoms. Central toxic effects include confusion, agitation, anxiety, hallucinations, seizures, and coma. Peripheral toxic effects include dry mucous membranes, thirst, flushed face, hyperthermia, urinary retention, and decreased gut motility (3).

*Datura* poisoning can be observed in various situations, including criminal activities (4), accidental cases (5–8), intentional suicide (9), and recreational abuse (10,11). Because daturas, *Datura stramonium* and *Datura inoxia* in particular, are used as ornamental plants, gardening practices in a community might provide novel opportunities for experimenting with intoxicating substances.

We present here an original method to test for both atropine and scopolamine in hair by liquid chromatography-tandem mass spectrometry (LC–MS–MS) and its application to a *Datura inoxia* poisoning.

### Materials and Methods

#### Case report

The subject, a 20-year-old male and known cannabis abuser, prepared an infusion of hot water with six dried *Datura inoxia* flowers. Ten minutes after ingestion, he lapsed into a coma. Upon waking, he experienced hallucinations, tachycardia, and an increase of systolic blood pressure up to 180. Once admitted to the Emergency Unit, the medical staff noticed agitation, mydriasis, and reduced salivary secretions. Treatment included gastric decontamination and IV perfusion of 50 mg of chlorazepate di-potassium. He was fully recovered one week later, after long-term agitation and mydriasis.

Neither blood nor urine was collected during hospitalization, but the psychiatrist sampled a strand of hair three weeks later.

Drug-free hair samples were obtained from laboratory personnel.

#### Chemicals and reagents

Acetonitrile, methanol, isopropanol, n-heptane, and methylene chloride were high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). Chemicals for the saturated phosphate buffer, (NH₄)₂HPO₄, adjusted to pH 8.4 with orthophosphoric acid, were purchased from Fluka (Saint-Quentin Fallavier, France). Scopolamine, atropine, and atropine-d₃ were purchased from Sigma (Saint-Quentin Fallavier, France).

#### Extraction

Hair strands were twice decontaminated using methylene chloride (immersion in 5 mL for 2 min) and then segmented (3 segments of 3 cm). Each segment was cut into small pieces (< 1 mm). About 50 mg was incubated overnight in 1 mL of phosphate buffer at pH 8.4, in the presence of 2.5 ng of atropine-d₃ used as internal standard (IS). After a liquid-liquid extraction with 5 mL of a mixture of methylene chloride/isopropanol/n-heptane (50:17:33, v/v/v) and evaporation to dryness, the residue was reconstituted in 100 μL of methanol.

#### LC–MS–MS procedure

LC was performed using a Waters Alliance 2695 system. Chromatography was achieved using an X Terra MS C18 column (100 × 2.1 mm, 3.5 μm) eluted with a gradient delivered at a flow rate of 0.2 mL/min (Table I). An injection volume of 10 μL was used in all cases. A Quattro Micro triple-quadrupole MS (Micromass-Waters) fitted with a Z-Spray ion interface was used for analyses. Ionization was achieved using electrospray in the positive ionization mode (ES+).

The following conditions were found to be optimal for the analysis of both atropine and scopolamine and the IS: capillary voltage, 1.0 kV; source block temperature, 120°C; and desolvation gas (nitrogen) heated to 350°C and delivered at a flow rate of 550 L/h. In order to establish appropriate multiple reaction monitoring conditions, the cone voltage was adjusted to maximize the intensity of the protonated molecular ion and collision-induced dissociation of both species was performed. Collision gas (argon) pressure was maintained at 3.0 mBarr, and the collision energy (eV) was adjusted to optimize the signal for the two most abundant product ions (Table II). Optimization for the atropine and scopolamine products and

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<table>
<thead>
<tr>
<th>Time  (min)</th>
<th>Acetonitrile (%)</th>
<th>Formate Buffer (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>20</td>
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<tr>
<td>10</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>10.5</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>95</td>
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</table>

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<thead>
<tr>
<th>Compound</th>
<th>Parent Ion (m/z)</th>
<th>Product Ion (m/z)</th>
<th>Cone Voltage (V)</th>
<th>Collision Energy (eV)</th>
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<tbody>
<tr>
<td>Atropine</td>
<td>290.2</td>
<td>124.0</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>92.9</td>
<td></td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>304.1</td>
<td>138.0</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>156.0</td>
<td></td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>Atropine-d₃</td>
<td>293.1</td>
<td>127.0</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>92.9</td>
<td></td>
<td>60</td>
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daughter ions was achieved by infusion through the MS of individual solutions at 10 mg/L in methanol. MassLynx 4.0 software was used for quantitation.

**Method validation**

A standard calibration curve was prepared in hair fortified with the drug and obtained by preparing spiked standards containing 2, 5, 10, 20, 50, and 100 pg/mg of atropine and scopolamine. Within-batch precision \((n = 6)\) was determined using blank hair spiked with atropine and scopolamine at 50 pg/mg. The limit of detection (LOD) was evaluated by decreasing concentrations of atropine and scopolamine until a response equivalent to three times the background noise was observed. Relative extraction recovery \((n = 3)\) was determined by comparing the representative peak area of atropine and scopolamine extracted from negative control hair spiked at the final concentration of 50 pg/mg with the peak area of a methanolic standard at the same concentration. The specificity of the method was evaluated by analyzing hair from six non-drug consuming subjects (from the laboratory). The matrix effect was defined as 100 \((a-b)/a\).

Relative extraction recovery \((n = 6)\) at 50 pg/mg was 8 and 10% for atropine and scopolamine, respectively. Bias was 11 and 6% for atropine and scopolamine, respectively. Relative extraction recovery was 82 and 67% for atropine and scopolamine, respectively. As total digestion in strong base was not possible because of instability of the drugs, it was not possible to evaluate whether all drug content was extracted from the hair. The LOD was 2 pg/mg (S/N 3) with a limit of quantitation (LOQ) at 5 pg/mg (S/N 10) for both compounds. Under the chromatographic conditions used, there was no interference with the analytes by chemicals or any extractable endogenous materials present in hair. Matrix effects (ion suppression) were 27.5 and 50% for atropine and scopolamine, respectively. These are the maximum percentages of suppression of both atropine and scopolamine peaks. Because potential unexpected ion suppression can occur, caution has to be taken to interpret the generated quantitative result. However, within the same strand of hair, it is acceptable to consider that the same matrix effect will apply; therefore, putting a quantitative interpretation on different segments from the same strand of hair is possible.

Figure 1 is the chromatogram obtained after extraction of a drug-free hair spiked with both atropine and scopolamine at a final concentration of 10 pg/mg.

**Results and Discussion**

**Validation**

Linearity was observed for atropine and scopolamine concentrations ranging from 2 to 100 pg/mg with a correlation coefficient of 0.992 and 0.998, respectively. Within-batch precision \((n = 6)\) at 50 pg/mg was 8 and 10% for atropine and scopolamine, respectively. Bias was 11 and 6% for atropine and scopolamine, respectively. Relative extraction recovery was 82 and 67% for atropine and scopolamine, respectively. Total digestion in strong base was not possible because of instability of the drugs, it was not possible to evaluate whether all drug content was extracted from the hair. The LOD was 2 pg/mg (S/N 3) with a limit of quantitation (LOQ) at 5 pg/mg (S/N 10) for both compounds. Under the chromatographic conditions used, there was no interference with the analytes by chemicals or any extractable endogenous materials present in hair. Matrix effects (ion suppression) were 27.5 and 50% for atropine and scopolamine, respectively. These are the maximum percentages of suppression of both atropine and scopolamine peaks. Because potential unexpected ion suppression can occur, caution has to be taken to interpret the generated quantitative result. However, within the same strand of hair, it is acceptable to consider that the same matrix effect will apply; therefore, putting a quantitative interpretation on different segments from the same strand of hair is possible.

Figure 1 is the chromatogram obtained after extraction of a drug-free hair spiked with both atropine and scopolamine at a final concentration of 10 pg/mg.

**Case report**

A strand of hair was collected 3 weeks after the ingestion of an infusion of *Datura inoxia* flowers. The results are presented in Table III. Figure 2 is the chromatogram obtained after extraction of the 0 (root) to 3 cm segment. No atropine was detected, and the concentrations of scopolamine were 14, 48, and 43 pg/mg along the three segments that were tested. Absence of deviation in retention times versus the calibrator and simultaneous presence of the two transitions in the correct range (+20%) were the forensic criteria that were used to qualitatively determine the presence of scopolamine in the subject's hair sample. These

<table>
<thead>
<tr>
<th>Table III. Results of the Hair Segmentation</th>
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<tr>
<td>Segment</td>
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<tr>
<td>---------</td>
</tr>
<tr>
<td>0 (root) to 3 cm</td>
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<tr>
<td>3 to 6 cm</td>
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<tr>
<td>6 to 9 cm</td>
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* LOD = 2 pg/mg.
concentrations cannot be compared with previous data, as the international literature is lacking reports on this topic. The presence of scopolamine in the three consecutive segments is inconsistent with a single exposure to Datura. This is also supported by the fact that three different concentrations were measured along the hair shaft, in contrast to what is obtained because of the low concentrations to be measured.

The absence of atropine in hair is consistent with its very low dosage in the flower of Datura inoxia (0.002 µg/g versus 0.346 µg/g for scopolamine) (12).

Conclusions

The daturas are a group of plants that have an intimate association with man from time immemorial. They have been used as poisons, medicines, and ritual intoxicants. However, this old plant is more and more used for recreational experience. In case of recreational abuse, 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 use from a single exposure. Selectivity and sensitivity of MS–MS are requisite for testing alkaloids of the Datura group because of the low concentrations to be measured.

References