INTRODUCTION

Plants of the genus Aconitum L (family of Ranunculaceae) are known to be among the most toxic plants of the Northern Hemisphere and are widespread across Europe, Northern Asia and North America. Two plants from this genus are of particular importance: the blue-blooded Aconitum napellus L. (monkshood) which is cultivated as an ornamental plant in Europe and the yellow-blooded Aconitum vulparia Reich. (wolfsbane) which is commonly used in Asian herbal medicine (Figure 1).

Many of the traditional Asian medicine preparations utilise both the aconite tubers and their processed products for their pharmaceutical properties, which include anti-inflammatory, analgesic and cardiotoxic effects. These effects can be attributed to the presence of the alkaloids; the principal alkaloids are aconitine, mesaconitine, hypaconitine and jesaconitine.

The use of the alkaloids as a homicidal agent has been known for more than 2000 years. Although intoxications by aconitine are rare in the Western Hemisphere, in traditional Chinese medicine, the use of aconite-based preparations is common and poisoning has been frequently reported. Poisoning has occurred both during clinical use and also as consequence of accidental ingestion e.g. by eating plant material or Aconitum preparations. The use of aconite tubers for suicide and homicide purposes has also been reported.

The first symptoms of aconitine poisoning appear ~20 min to 2 hours after oral uptake and include paraesthesia, sweating and nausea. This leads to severe vomiting, colicky diarrhea, intense pain and then paralysis of the skeletal muscles. Following the onset of life-threatening arrhythmia, including ventricular tachycardia and ventricular fibrillation, death finally occurs as a result of respiratory paralysis or cardiac arrest.

Clearly in the case of suspected aconitine intoxication there is a need for rapid analytical techniques to enable prompt diagnosis and treatment. To this end we have developed a simple LC/MS/MS method for the determination of aconitine in various body fluids.

The method was fully validated for the determination of aconitine from whole blood samples and applied in two cases of fatal poisoning.

METHODS AND INSTRUMENTATION

Sample preparation

Biological samples were prepared for LC/MS/MS by means of a solid-phase extraction (SPE) procedure. Blood and tissue samples (0.5 g each) were mixed with 3 mL of 0.15 M phosphate buffer pH 6.0, homogenised and centrifuged at 5000 g for 10 min. The supernatants were decanted and loaded on a prepared SPE cartridge. Cartridges were pre-conditioned with 3 mL methanol, 3 mL water and 1 mL of 0.15 M phosphate buffer pH 6.0. Samples were allowed to pass through the cartridge under gravity, before an initial wash step (3 mL water followed by 1 mL 0.01 M HCl) was performed.

Two further washing steps i.e. 2 mL dichloromethane, followed by 2 mL methanol, were performed before elution of the aconitine. Cartridges were dried under vacuum between each of the 3 wash steps. Aconitine was eluted (2 x 1.5 mL) with a mixture of dichloromethane:2-propanol:25% aqueous ammonia (80:20:2). Eluents were pooled and evaporated to dryness under a stream of nitrogen at 40 °C before reconstitution with 100 μL LC mobile phase.
LC/MS/MS

A Quattro micro™ tandem mass spectrometer fitted with ZSpray™ ion interface was used for all analyses. Ionisation was achieved using electrospray in the positive ionisation mode (ES+). Detection of aconitine was performed using multiple reaction monitoring (MRM). The transition MRM transition m/z 646.4 > m/z 586.5 was used for quantification purposes and a further two transitions i.e. m/z 646.4 > m/z 526.4 and m/z 646.4 > m/z 368.4 were monitored for confirmatory purposes.

LC analyses were performed using an Alliance® 2695 separations module (Waters). Chromatography was achieved using a XTerra® RP8 pre-column (2.1 x 10 mm, 3.5 μm) and a XTerra® RP8 analytical column (2.1 x 150 mm, 3.5 μm). The column was maintained at 40 °C and eluted isocratically with 0.1 % ammonium acetate (adjusted to pH 6.0 with 1 M acetic acid) and methanol (50:50) at 200 μL/min. The injection volume was 10 μL and a total run time of 10 min was used. All aspects of system operation and data acquisition were controlled using MassLynx™ NT 4.0 software with automated data processing using the QuanLynx™ program (Waters).

RESULTS

A series of calibrators (0.1 – 25 ng/g) were prepared in duplicate by adding aconitine standards to control blood. Samples were then extracted, using the SPE method described above, prior to LC/MS/MS analysis.

Following analysis, the areas under the specific MRM chromatograms were integrated. The response was linear (r² = 0.999) over the range investigated. The limit of detection (LOD) of the assay was estimated at 0.1 ng/g blood. Figure 2 shows the responses for the quantifier and qualifier ions of aconitine obtained with a calibrator spiked at the LOD.

In two forensic cases of suspected aconitine intoxication, aconitine was detected in the blood samples and also in the stomach content and urine of the deceased (Table 1). Figure 3 shows the chromatogram of the blood sample of aconite victim no 2. At the time of autopsy the body was already in an advanced state of putrefaction. Despite these difficult circumstances, the chromatogram shows a strong signal for aconitine.

SUMMARY

We have developed a rapid and sensitive method for the quantification of aconitine in biological specimens. The method involves a simple SPE purification prior to analysis using LC/MRM.

The utility of the method was demonstrated by its application to authentic samples in 2 fatal cases of suspected aconitine poisoning. Blood, urine and stomach contents were collected during autopsy and analysed using the developed LC/MS/MS method. Aconitine could be detected in the blood of both victims, in the stomach content of one individual and in the urine of the other.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Blood (ng/g)</th>
<th>Stomach content (ng/g)</th>
<th>Urine (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>10.0</td>
<td>3.0</td>
<td>Not available</td>
</tr>
<tr>
<td>2</td>
<td>12.1</td>
<td>Not available</td>
<td>180.0</td>
</tr>
</tbody>
</table>

Table 1: Concentrations of aconitine in autopsy samples from two cases of fatal aconite intoxication.

Figure 2: MRM chromatograms for a blood calibrator spiked at 0.1 ng aconitine/g blood. Peak intensity is given in the top right-hand corner of the trace.
REFERENCES


Figure 3: MRM chromatograms of the blood sample from the victim in case 2, with 12.1 ng aconitine/g. The chromatograms show no interferences although the body was in an advanced state of putrefaction at the time of the autopsy.