# Concentrations of tetrodotoxin and aconitine using a column-switching LC-ESI-MS method in mouse serum

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Since toxicity of aconitine is high and at times it has been used for homicide in Japan. In 1986, there was a murder in which both aconitine and tetrodotoxin (TTX) were used as the poison. Sensitive analyses are required for determination of aconitine and TTX since serum concentration at the time of intoxication is low. The authors have already reported on the liquid chromatography-mass spectrometry-electrospray ionization (LC-ESI-MS) method coupled with a column switching technique for the determination of TTX in mouse serum. In this study, a mixture of aconitine and TTX was administered to the mouse and using this method of analysis, serum concentrations of both aconitine and TTX were determined.

An on-column column switching technique was employed to analyze TTX and aconitine without pretreatment of the serum. Combination of a multimode column with reversed phases and cation exchange were used for TTX determination, and the use of a multimode column with reversed phases and a hydrophobic polymer column for determination of aconitine and benzoylaconine provided successful separation and MS determination in the ESI positive mode. A 100-µ l serum sample was directly injected into a the precolumn. For TTX monitored at m/z 320.1 in the SIM mode, the calibration curve was linear within the range of 0.1-100 ng/ml and the limit of detection was 0.1 ng/ml. For aconitine monitored at m/z 646.3 and for benzoylaconine at m/z 604.3 in the SIM mode, linear calibration curves were obtained up to 500ng/ml and the limit of detection ranged of 0.2-1 ng/ml. Positive detection from serum samples were within the range 78-119% for all the compounds studied.

Male mice of the ICR strain (age, 5 weeks; weight, approximately 30 g,) were used for the animal experiments, which were conducted according to the guidelines of the Ethical Committee on Animal Experimentation of the Nippon Medical School (Tokyo, Japan). Mice were divided into three groups: 1) In the TTX group, TTX was injected into the mice intraperitoneally at a dose of 15 µg/kg. 2) In the aconitine group, aconitine was injected at a dose of 0.4 mg/kg. 3) In the mixed toxicity group, TTX and aconitine were injected at a dose of 15µg/kg for TTX and 0.4 mg/kg for aconitine. Then the blood samples were collected from the mice hearts, and centrifuged for 10 min at 16,000 rpm. After filtration through a 0.45-µm membrane filter, a-100-µL aliquot was injected automatically into the instrument used. For aconitine analysis a-50-µL aliquot was injected.

In the aconitine group, aconitine demonstrated maximum drug concentration in 15 minutes after administration and benzoylaconine demonstrated maximum drug concentration in 30 minutes after administration. Comparison of serum concentration between the aconitine group and the mixed toxicity group revealed a persistent low value of aconitine for the mixed toxicity group. As for benzoylaconine concentration, there were no significant differences noted between the aconitine and mixed toxicity groups. The TTX group demonstrated maximum drug concentration in 30 minutes after administration. When the serum concentration of TTX group and a mixed toxicity group was measured, slightly higher values were demonstrated by the mixed administration group but was not of significance and the same serum concentration curve was demonstrated. It is speculated that in the mixed toxicity group, TTX had affected or influenced the metabolism of aconitine in the body.

**KEY WORDS:** *Tetrodotoxin, Aconitine, Mixed toxicity, Serum, Mouse, Column-switching, LC/MS/ESI*

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