

## A novel method for the detection of pyrrolizidine alkaloids in bottled tea and tea leaves by LC-MS/MS

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### Abstract

Pyrrolizidine alkaloids are products of secondary metabolism in plants. They have been detected in honey, as well as in herbal and green tea. However, these molecules are toxic and carcinogenic, and hence, their presence in commercial products should be regulated. In this study, a quantitative analytical method for the detection of 19 pyrrolizidine alkaloid compounds in bottled tea and tea leaves was developed. The samples were prepared via the QuEChERS method and the analysis was performed with a tandem of high performance liquid chromatography and mass spectrometry. Monitoring tests were conducted on commercially available bottled tea and tea leaves. The results showed that in Japanese commercial bottled tea, all pyrrolizidine alkaloids were below the detection limit. Tea leaves experiments, in contrast, revealed that some types of tea (rooibos and chamomile) contain pyrrolizidine alkaloids.

Keywords : LC-MS/MS, simultaneous analysis, QuEChERS method, pyrrolizidine alkaloid, tea

### I Introduction

Pyrrolizidine alkaloids (PAs) including their N-oxide (NO), a group of pyrrolizidine derivatives, are natural toxins produced by plants<sup>1)</sup>. Over 600 PAs compounds have been reported to date<sup>2)</sup>, in more than 6,000 plant species such as Asteraceae, Leguminosae, and Murasakiaceae<sup>3)</sup>. The distribution of PAs in plants greatly varies depending on the climatic conditions, sampling season, and geography<sup>4)</sup>. These compounds are reportedly more abundant in new shoots than in ripe leaves, and in roots and rhizomes than in leaves<sup>5, 6)</sup>.

PAs are hepatotoxic, causing a venous occlusive disease (VOD) of the liver<sup>7)</sup>, which progresses to cirrhosis and liver failure<sup>8)</sup>. When ingested, PAs are absorbed through the intestinal wall and subsequently metabolized in the liver by enzyme CYP3A4<sup>9)</sup>. The resulting metabolite acts as an alkylating agent and crosslinks to proteins and DNA to induce cell death. The specific toxicity pathway of PAs is currently under investigation<sup>10)</sup>.

Exposure to PAs through ingestion can occur in four different ways:

- (1) ingesting cereals, contaminated with PAs-containing plants<sup>11)</sup>;
- (2) consuming honey, containing nectar from PAs-containing plants<sup>12)</sup>;
- (3) eating meat from livestock fed with PAs-containing food<sup>12)</sup> and
- (4) intake of products made from PAs-containing plants<sup>12)</sup>.

One of the fatal cases of PAs toxicity was reported in Mexico, where an infant ingested a herbal liquid extract and as a result developed Reye's syndrome, which subsequently led to its death<sup>13)</sup>. In addition to the high toxic effects that PAs have on infants, symptoms such as VOD have been observed in newborns of women who took herbal tea during their pregnancy<sup>14)</sup>. Another case of injury was reported in the United States, where people who ingested comfrey as the main dietary supplement for a long time were diagnosed with VOI<sup>15)</sup>. Even though there have been many other cases of toxicity caused by the ingestion of PAs from herbal tea, honey, and comfrey, there are currently no regulations on the presence of these chemicals in human food or animal feed. However, research institutions and governments have issued PA-toxicity