## Occurrence of tropane alkaloids in teas. Effect of tea making on atropine and scopolamine

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Tropane alkaloids (TAs), particularly atropine and scopolamine, are hazardous secondary metabolites found in toxic plants such as those from Brassicaceae and Solanaceae families. Concerns arise from reported cases of food poisoning due to these toxins, which often contaminate crops in sustainable farming with limited herbicide use. TAs, when consumed through teas and herbal teas, exhibit anticholinergic effects. Despite high contamination, data on TAs in these beverages are scarce. Thus, this study aims to evaluate the presence of atropine and scopolamine in 33 teas and herbal teas purchased in Portugal and Spain.

Infusions (black, green, white-tea, and mixed herbal tea) were prepared, cooled, and purified with a method previously validated and developed by our group in similar samples [1]. This methodology involved a microextraction technique ( $\mu$ -SPEed<sup>®</sup>) with a PS/DVB cartridge, where 5×500 µL of sample was loaded and eluted with methanol (2×100 µL) before analysis by HPLC-MS/MS.

The analytical performance showed recoveries (at 2.5 ng/mL) of 89-109% for atropine and 73-114% for scopolamine, and good linearity (0.1-25 ng/mL, R<sup>2</sup> 0.995-0.998). The method assessed contamination levels in infusions, revealing that 63.6% of samples contained one or both analytes, with 45.5% exceeding the 0.2 ng/g limit for liquid herbal infusions.

In addition, the impact of heating conditions in standard solutions (0.2 ng/mL and 4 ng/mL) and naturally contaminated green, white and black teas were evaluated by brewing with different boiling times (5 and 10 min) at 97°C. The infusion conditions described in the international standard ISO 3103 were also compared with the contaminated samples. In the experiment with standard solutions, these remained stable under boiling. In the other experiment, the results showed a higher extraction of the analytes. Overall, this study provides crucial insights into the contamination levels of TAs and demonstrates that there is no degradation of TAs by tea brewing.

[1] González-Gómez, L.; Pereira, J.A.M.; Morante-Zarcero, S.; Câmara, J.S.; Sierra, I. Green extraction approach based on  $\mu$ SPEed® followed by HPLC-MS/MS for the determination of atropine and scopolamine in tea and herbal tea infusions. *Food Chem.* **2022**, 394, 133512.

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