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Tropane alkaloids in mint teas at the Serbian market

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Abstract

The interest in tropane alkaloids as food contaminants has been increasing. The tropane alkaloids are plant toxins that mainly occur in *Atropa, Datura* and *Hyoscyamus sp* belonging to the *Solanaceae* family. A sensitive and selective LC-MS/MS method was applied for the analysis of atropine and scopolamine in the mint tea samples from the Serbian market. Tea, which has beneficial properties thanks to the phenolic compounds, can be accidentally contaminated by many weed seeds which contain the tropane alkaloids during the harvest. Only the tropane alkaloids present in the tea bags before the tea making were analysed. Atropine and scopolamine were detected in 30% of the analysed samples in the concentration above the limit of quantification.

Key words: atropine, scopolamine, mint tea

Introduction

Some plants of the *Solanaceae* family and other related families produce tropane alkaloids (TAs) in high concentrations, particularly in the case of *Datura stramonium* and *Brugmansia arborea* seeds. TAs are a group of over 200 secondary metabolites, found in all the parts of the tropane alkaloids containing plants (Mulder et al., 2016). They have the anticholinergic effect due to being the antagonists of the acetylcholine (ACh) muscarinic receptors in mammals (EFSA, 2013).

Namely, some of the antimuscarinic effects that may occur are mydriasis and accommodation paralysis, gastric acid secretion and micturition inhibition, decrease in bronchial, salivary and sweat secretion, as well as the cardiac dysfunction (Mulder et al., 2016). However, not all the TAs have the same effects. The reason for that lies in the ability of passing the blood-brain barrier. The ones which are capable of doing that, such as scopolamine and hyoscyamine, can lead to dose-dependent psychoactive effects and hallucinations, while, on the other hand, the calystegines cannot provoke that kind of consequences due to their polarity and hydrophilicity, which make passing the barrier impossible (Kohnen-Johannsen and Kayser, 2019).

These plants can be mixed with crops used in the processed food like buckwheat, millet, or sorghum, among others. In addition to that and because these cereals are gluten free and have antioxidant properties, they are used for the cereal-based pasta with high amount of proteins, phenolic compounds, and minerals (Marín-Sáez et al., 2019).

Tea, one of the most popular drinks in the world which has many beneficial effects on the human health thanks to the phenolic compounds, can be accidentally contaminated with *Solanaceae* seeds during harvest. In fact, tea is one of the most potential matrices that can be contaminated with these type of seeds (Marín-Sáez et al., 2019).

According to the EFSA Report, drafted by Mulder et al. (2016), a total of 1709 samples of plant-derived food products, produced in Czech Republic, France, Germany, Hungary, Italy, the Netherlands, Poland, Spain, and the United Kingdom, were analysed for TAs. Out of all the samples, 121 were dry (herbal) teas.

All samples were analysed by liquid chromatography coupled with the tandem mass spectrometry (LC-MS/MS). One or more TAs were detected in 70.2% of the dry (herbal) tea. The authors emphasize that the highest TA concentration was detected in a dry herbal tea sample, with a maximum sum of atropine and scopolamine of 428.5 µg/kg.

Out of the 13 studied reports from 10 countries in the period between 1988 and 2012 at least five human intoxication cases occurred due to the contaminated or mislabelled tea (Adamse et al., 2014).

According to the RASFF (Rapid Alert System for Food and Feed), the most recent incidents dealing with TAs in tea are TAs in the organic blackberry leaves from Bulgaria (reported in 2020; atropine: 543.1 µg/kg, scopolamine: 31.4 µg/kg), as well as the TAs present in the infusion originating from the Netherlands (reported in 2021; atropine: 184 µg/kg, scopolamine: 21 µg/kg).

Taking into account the consumption of mint tea is very common in many countries, including Serbia, the aim of the study was to investigate the presence of tropane alkaloids (atropine and scopolamine) in mint tea samples by liquid chromatography coupled with the tandem mass spectrometry (LC-MS/MS) and to check the food safety of these products.

Material and methods

Standard operating procedure (SOP)

Atropine and scopolamine reference standards were obtained from Sigma-Aldrich. The standard solutions of atropine and scopolamine were prepared at 1 mg/mL in methanol, each. The working standard solution mixtures were prepared at 10 µg/mL and 1 µg/mL in methanol and stored in the dark at -20℃. Carbofuran-D3, 99.54% was obtained from Dr Ehrenstorpher. Acetonitrile (MeCN) and methanol (MeOH) were purchased from J.T. Baker. Both organic solvents were Ultra Gradient HPLC grade. Formic acid was analytical grade (Fisher Scientific UK). The Hillium QuEChERS extraction pouches 550 mL (P/N QEHLL0510P) and Hillium QuEChERS dispersive kit 15 mL (P/N QDHLL15032) were used for the extraction and clean-up. HPLC Agilent 1290 Infinity II chromatograph equipped with a quaternary pump, multisampler, and thermostated column compartment was used for the analysis of atropine and scopolamine. The HPLC system was coupled to an Agilent 6495 LC/TQ triple quadrupole mass spectrometer with AJS ESI (Jet Stream Technology Ion Source). A Zorbax Eclipse Plus C18 column Rapid Resolution HD (50x2.1mm, 1.8 µm particle size) was used for the chromatographic separation. The column temperature was held at 35 ºC and the injection volume for the LC system was 2 µL. The chromatographic separation of AT and SC was carried out with the mobile phase consisting of water (A) and methanol (B), both containing formic acid (0.1%, v/v), in a gradient mode and flow rate of 0.25 mL/min. The gradient elution started at 5% of B and held for 1 min. This composition was increased to 40% B at 7 min, 90% B at 8 min and held for 2 min. The composition of the mobile phase returned to the initial conditions in 1 min and the system was equilibrated during 2 min. The total run time was 14 min. The ESI source was used with the following settings: drying gas (nitrogen) temperature 200°C, drying gas flow rate 16 L/min, nebulizer pressure 30 psi, sheath gas temperature

of 300 ºC, sheath gas flow 12 L/min, and capillary voltage 3000 V. The detection was performed using the dynamic multiple reactions monitoring mode (dMRM). The Agilent MassHunter software (version B.10.0 SR1 Agilent Technologies, 2006-2019) was used for the optimization and quantification.

Sample collection and TAs extraction

Ten mint tea samples were collected from the super markets in Novi Sad and Belgrade. The sampling was performed in accordance with the EU Directive 2002/63/EC. Prior to the extraction the samples were dry ground into powder with the particle size being less than 1 mm, after which the samples were sieved in order to obtain a homogenous sample particle size. Atropine and scopolamine were extracted from the mint tea with the QuEChERS method described below:

- 2 g sample (in tubes of 50 mL) + 10 mL water + 50 µL IS (10 µg/mL),
- 40 mL MeCN (1% HCOOH) shake 60 min on 200 rpm/min and centrifugate 5 min/7000 rpm,
- 10 mL of extract + salt ($MgSO_4$, NaCl, $C_6H_5Na_3O_7$, $C_6H_8Na_2O_8$), shake vigorously, vortex 2 min, centrifugate 10 min/7500 rpm,
- 6 mL of extract cleaned up with 150 mg $MgSO_4$ + 25 mg PSA + 25 mg C_{18},
- vortex 2 min, centrifugate 5 min/16000 rpm,
- 2 mL aliquot evaporated to dryness under a steam of nitrogen,
- reconstitution in 1 mL of mobile phase, and
- LC-MS/MS analysis.

Results and discussion

Method performance

Atropine and scopolamine were analysed using the positive electrospray ionization (ESI+) in the dynamic multiple reactions monitoring mode. The chromatographic analysis comprised the optimization of the mass spectrometer, i.e. the adjustment of the fragmentation and collision energy in order to obtain the ions with the strongest response. The fragmentation energy for all the ions was 166 V, while the collision energy is shown in Table 1. The fragmentation of the atropine and scopolamine molecules yielded 3 product ions, respectively (Tab. 1). The most intense MRM transitions for atropine m/z 290.2 > 124.2 and scopolamine 304.2 > 138.2 were monitored for the quantification, while the other transitions were used for the confirmation (Vuković et al., 2018; Vuković et al., 2020).

Tab. 1. LC-MS/MS parameters for the analysis of TAs

TA	Molecular weight (g/mol)	Precursor ion [M+H*](m/z)	Product ion (m/z)	Collision energy (V)	Rt* (min)
atropine	289.2	290.2	124.2	24	9.63
			93.2	36	
			77.1	68	
scopolamine	303.2	304.2	156	12	8.42
			138.2	24	
			103.2	44	
Carbofuran-D3	224.1	225.1	165.2	10	12.57
			123.1	22	

*Retention time

Namely, it is possible to establish LOD and LOQ in more ways than one. One of the ways for the determination is the possibility to define LOD and LOQ as the concentrations in which the signal is essentially different from the noise (Equation 1).

$$LOD = xbeg + 3\ x\ s, or\ just: LOD = 3\ x\ s$$
$$LOQ = xbeg + 10\ x\ s, or\ just: LOQ = 10\ x\ s$$

Equation 1

s – the standard deviation obtained by the analysis of the 5-10 independent blind samples or the samples with the low concentration of the analytes

xbeg – background signal or noise

The LOD values were calculated by the MassHunter Workstation Software B.04.00 Agilent Technologies 2010 and they were 0.05 µg/kg for the atropine and 0.2 µg/kg for the scopolamine.

The LOQ was studied at the level of 1 µg/kg, which is in accordance with the European Recommendation 20015/976/EC, for the atropine and scopolamine.

R^2 for both TAs was >0.99 for the calibration in the range from 1 to 20 µg/kg. The recoveries for atropine and scopolamine were 83.6 and 86.4, respectively, with RSD less than 17%.

The linearity of the atropine and scopolamine in the mint tea was studied by five calibrations levels ranging from 1 to 20 µg/kg. All the determinations were done in two replicates. In order to determine the influence of the matrix, the calibration was performed in the mobile phase and in the matrix of the sample (a method of the standard addition to the sample). The calibration of the standard addition was performed by adding the defined mixture of the standards into the sample before the extraction, after which the samples were prepared in the same way as the studied samples. It is necessary to emphasize that 100 µL of the

internal standard was added to each sample before the analysis. The extraction yield was examined by enriching the samples which did not contain the TAs. The samples were enriched at three concentration levels (2, 5, and 100 μg/kg) in six replicates, by adding the working solution of the TAs to the blank sample. After being spiked the samples were vortexed for 60 seconds and left to set for 5 minutes before the extraction.

The occurrence of tropane alkaloids in mint teas

A total of 10 mint tea samples (tea bags) were analysed for the presence of atropine and scopolamine. As shown in Table 2, both atropine and scopolamine were detected above the LOQ in three samples. All the other detections were below the LOQ.

The maximum level was established only in cereal-based foods for the infants and young children, containing millet, sorghum, buckwheat, or their derived products (Regulation 2016/239), limiting the presence of atropine and scopolamine at 1 μg/kg for each (EC, 2016). Discussions are continuing to define the maximum levels of TAs of different origins. The herbal infusions are also under consideration.

Tab. 2. TAs detections in mint tea samples (μg/kg)

Tea sample	1	2	3	4	5
Atropine	<1.0	5.41	<1.0	7.23	<1.0
Scopolamine	<1.0	1.92	<1.0	2.66	<1.0
Tea sample	6	7	8	9	10
Atropine	<1.0	<1.0	<1.0	<1.0	4.27
Scopolamine	<1.0	<1.0	<1.0	<1.0	1.08

Since the detections in the analysed samples are not so high, the results do not indicate a serious health concern related to the exposure to tropane alkaloids through the consumption of mint tea. But, Goncalves et al. (2020) indicated that the intoxications by the TAs have been reported several times during the past decades, which were the result of the consumption of contaminated herbal teas. The main ingestion route was through contaminated or mislabelled tea. Drinking the marshmallow root tea (*Althaea officinalis*) contaminated with the deadly nightshade (*Atropa belladonna*) led to the hospitalization of four persons in the Netherlands in 2013. The Israeli market survey in 2015 showed that 80% of the peppermint samples were contaminated with atropine and scopolamine with the mean values being 171 μg/kg (range: 20–208 μg/kg) and 81 μg/kg (range: 14–171 μg/kg), respectively.

Conclusion

Out of the 10 tested mint tea samples three of them contained atropine and scopolamine in concentrations above the LOQ, which leads to conclusion that the serious health concern is not indicated in this case. However, this research was conducted on a relatively small number of mint tea samples and the obtained results are undoubtedly accentuating the need for monitoring the TAs presence in herbal teas.

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Присуство тропанских алкалоида у чајевима од нане на тржишту Србије

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Сажетак

Све је веће интересовање за тропанске алкалоиде као загађиваче хране. Тропански алкалоиди су биљни токсини који се углавном јављају у *Atropa*, *Datura* и *Hiosciamus* sp. које припадају породици *Solanaceae*. За анализу атропина и скополамина у узорцима чаја од нане са тржишта Србије примијењена је осјетљива и селективна LC-MS/MS метода. Чај, који има благотворно дејство захваљујући фенолним једињењима, може током бербе да буде случајно контаминиран многим сјеменима корова која садрже тропанске алкалоиде. Анализирани су тропански алкалоиди присутни у кесицама чаја прије прављења чаја. Атропин и скополамин су детектовани у 30% анализираних узорака у концентрацији изнад границе квантификације.

Кључне ријечи: атропин, скополамин, чај од нане

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