DETERMINATION OF ATROPINE AND SCOPOLAMINE IN POPCORN BY THE LC-MS/MS

Gorica Vuković[1], Vojislava Bursić[2], Tijana Stojanović[2], Aleksandra Petrović[2], Bojan Konstantinović[2], Nikola Puvača[5], Dušan Marinković[2]

[1]Institute of Public Health, Bulevar despota Stefana 54a, Belgrade, Serbia
[2]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade, Serbia
[4]Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia
[5]University Business Academy in Novi Sad, Faculty of Economics and Engineering Management, Cvećarska 2, Novi Sad, Serbia
e-mail: tijana.stojanovic@polj.edu.rs

Abstract
Tropane alkaloids are secondary metabolites produced mainly by the genera of the *Solanaceae* family - *Hyoscyamus*, *Datura* and *Atropa*. For determination and quantification of the atropine and scopolamine in 12 samples of popcorn the validated rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used. Out of the studied popcorn samples 41.67% were contaminated with atropine and scopolamine, while in 58.33% of the tested samples tropane alkaloids were not detected, or the present concentrations were below the limit of detection (<1 µg/kg). Atropine was present in the range from 5.3 to 28 µg/kg, while scopolamine ranged from 2.1 to 6.3 µg/kg.

Key words: tropane alkaloids, atropine, scopolamine, LC-MS/MS, popcorn

Introduction

Animals, plants and organisms produce natural toxins which, although they are not toxic to them, may be present in food and be toxic to humans when ingested. As a possible health hazards World Health Organisation (WHO) highlighted the monitoring importance in case of relevant natural food toxins by the national authorities [1].
Those natural toxins represent the secondary metabolites which are produced, among others, by the weeds present in the fields, where they contaminate the food crops. Namely, the seeds and parts of plant organs of weeds which contain tropane alkaloids end up in the crops such as sunflower, buckwheat and maize, as well as in their products as the accidental impurities. The main weeds genera containing tropane alkaloids are the genera from the *Solanaceae* family – *Hyoscyamus*, *Datura* and *Atropa* [2].
The maximum levels of residues of the tropane alkaloids are still not determined, due to which all tropane alkaloids containing samples with concentrations above the Limit of Quantifications (LOQs) are not considered suitable for the consumption [3]. The maximum levels have not been established in case of popcorn either. That can be considered alarming since the KiESEL study ("The Children's Nutrition Survey to Record Food Consumption") done by the German Federal Institute for Risk Assessment (BfR - Bundesinstitut für Risikobewertung) determined that the single consumption intake of popcorn in children aged between 3 and 5 years can be up to 100 g [4].
The above-mentioned problems led to the liquid chromatography with tandem mass spectrometry (LC-MS/MS) determination of the atropine and scopolamine in 12 popcorn samples collected during 2020 which was done in this research.

Experimental

Chemicals and apparatus. Atropine and scopolamine reference standards were obtained from Sigma-Aldrich. The mixture working standard solution was prepared at 5 mg/L with methanol and stored in the dark at -20℃. Carbofuran D3 was used as an internal standard (IS). HPLC grade methanol and acetonitrile were obtained from J.T. Baker Chemicals. Formic acid was purchased from Fisher Scientific UK. The Agilent Bond Elut EN Buffered Extraction kit and Bond Elut QuEChERS EN Dispersive SPE kits for Fruits and Vegetables with fats and waxes were purchased from Agilent. An Agilent series 1200 HPLC system (Agilent Technologies) equipped with a G1312B binary pump, a G1367D autosampler, a G1379B degasser and G1316B column compartment temostat. The HPLC system was coupled to an Agilent triple quadrupole mass spectrometer (6410 B) coupled to an electrospray ionization source (ESI+). A Zorbax XDB C18 column (50x4.6 mm, 1.8 µm particle size) from Agilent was employed for the separation. The chromatographic determination of atropine and scopolamine was carried out employing a binary mobile phase with methanol (A) and an aqueous solution of formic acid (0.1%, v/v) (B). A gradient elution started at 90% of B and held 4 min at flow rate of 0.4 mL/min. This composition was reduced to 5% B in 10 min, and held for 5 min. The composition of the mobile phase returned to the initial conditions in 2 min and the stationary phase was equilibrated during 2 min. The total running time was 17 min. The injection volume was 5 µL and column temperature was kept at 25 ºC. The ESI source values were as follows: drying gas (nitrogen) temperature 350 °C, drying gas flow rate 10 L/min, nebulizer pressure 40 psi and capillary voltage 3500 V. The detection was performed using the multiple reactions monitoring mode (MRM). The Agilent MassHunter software (version B.06.00 Agilent Technologies, 2012) was used for the optimization and quantification.

Sample collection. All analyses samples were popcorn from the local shops. The sampling was performed in accordance with 2002/63/EC for establishing MLs in the food commodities. All 12 samples were placed in polythene bags, labelled and transported to the laboratory for processing. The samples were ground into powder prior to the analysis. The blank samples were used for the preparation of fortified samples during the optimization of sample extraction procedure and method validation.

Sample preparation. A 5 g of homogenized sample was placed into a 50-mL centrifuge tube with adding of 100 µL of IS and 10 mL of water to each tube and vortexed for 1 min and equilibrated for 10 min. Next, 10 mL aliquot of acetonitrile containing acetic acid (1%, v/v) was added and shaken by vortex for 1 min. The QuEChERS EN extraction salt packet was added directly to each tube. The tubes were sealed tightly and shaken vigorously for 20 seconds by hand and 15 min/250 rpm by orbital shakers. The sample tubes were centrifuged at 4000 rpm for 5 min. The supernatants were then transferred to 15-mL QuEChERS d-SPE kits consisting of C18 and $MgSO_4$, vortexed for 5 min and centrifuged at 4000 rpm for 10 min. The obtained mixtures were transferred and dried under the nitrogen gas at 45 ºC until the volume was <0.3 mL. The residues were reconstituted in the mixture methanol/water up to 2 mL, vortexed, centrifuged at 7000 rpm and filtered prior to the LC-MS/MS analysis.

Results and discussion

The ionization was achieved using electrospray in the positive ionization mode. For each compound the detection was related to four daughter ions (atropine: m/z 290.2 to 124.2, 93.2, 77.1 and 67.1; scopolamine: m/z 304.1 to 156.1, 138.2, 103.2 and 77.1).

Validated LC-MS/MS method for the determination of atropine and scopolamine was used for the analyses of 12 popcorn samples. The validation was done according to the SANTE/1183/2017 [3]. The average recoveries of scopolamine and atropine were 87.4 and 69.2%, respectively, with the relative standard deviation (RSD) lower than 15%. The matrix–matched calibrations were with the R^2>0.99 in the calibration range from 2 to 20 µg/kg. LOQs

(limit of quantification) of atropine and scopolamine were set at 5 µg/kg. These limits were checked in terms of the recovery and repeatability.
The TIC chromatograms of popcorn samples number 7 (A) with the atropine, scopolamine and IS (carbofuran – D3) detections and blank popcorn samples (B) with only detection of IS, are shown in Figure 1.

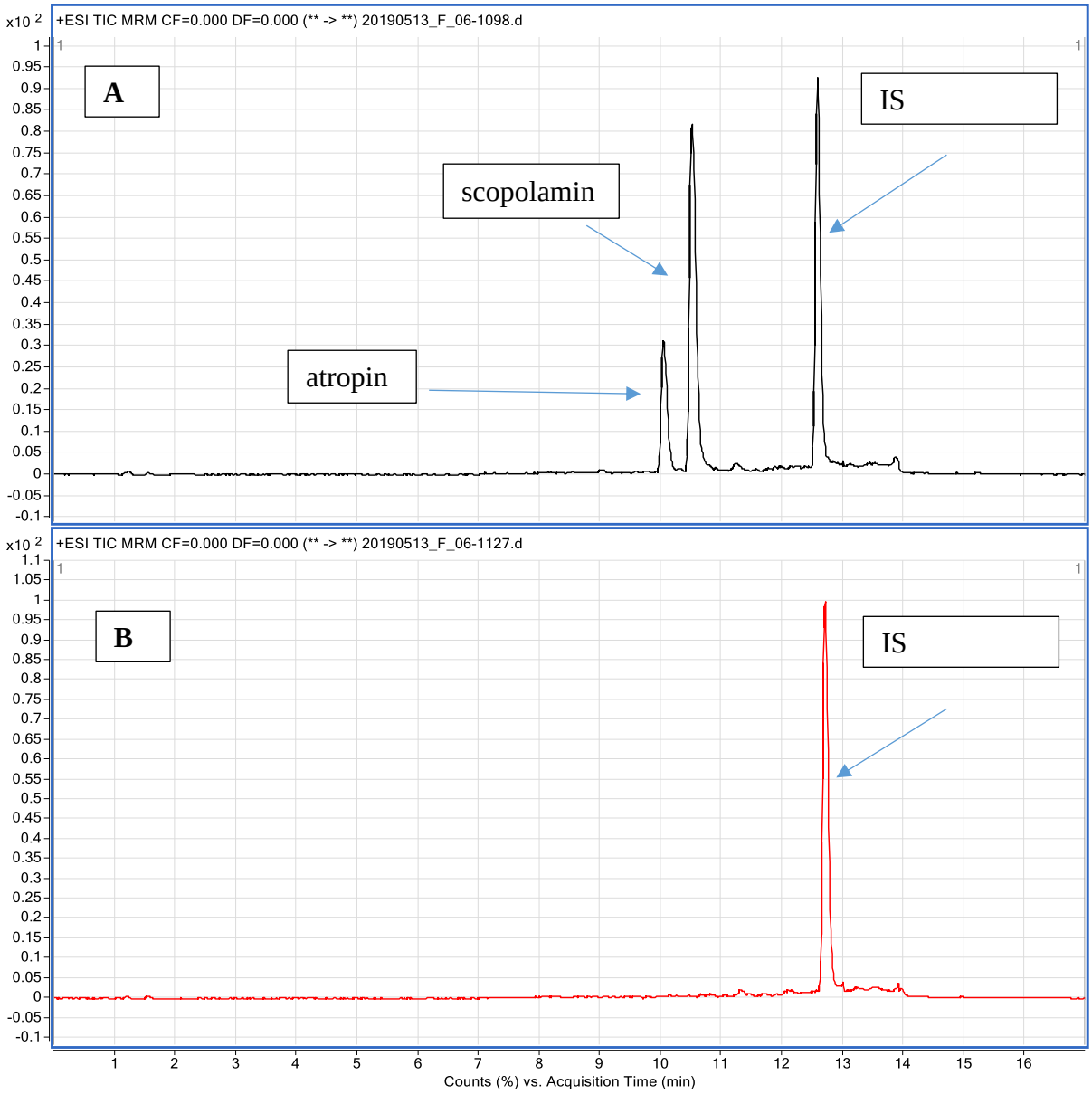

Figure 1. TIC chromatograms of samples

Table 2. Atropine and scopolamine residues in popcorn samples (µg/kg)

Sample	**1**	**2**	**3**	**4**	**5**	**6**
Atropine	< 1	5.4	5.3	< 1	< 1	< 1
Scopolamine	< 1	2.8	2.1	< 1	< 1	< 1
Sample	**7**	**8**	**9**	**10**	**11**	**12**
Atropine	16.3	28	< 1	18.8	< 1	< 1
Scopolamine	3.3	6	< 1	6.3	< 1	< 1

In almost half of the tested samples (5 out of 12 in total) atropine and scopolamine were detected in concentrations above the LOD (1 μg/kg). Atropine was present in the range from 5.3 to 28 μg/kg, while scopolamine ranged from 2.1 to 6.3 μg/kg. The obtained results may be considered alarming since the RASFF (Rapid Alert System for Food and Feed) reported three cases of atropine and/or scopolamine in popcorn in similar concentrations to those detected in our research, which ended with the withdrawal from the market in every of the mentioned cases. Namely, in 2015 in microwave popcorn from Spain atropine was detected in the concentration of 29 μg/kg, while scopolamine was present with 6 μg/kg. In 2018 two incidents were noted. In the first 6.6 μg/kg of atropine and 1.8 μg/kg of scopolamine were detected in popcorn from France, while in the second incident 10.3 μg/kg of scopolamine was detected in popcorn from Argentina [5]. Since Perharič et al. [6] reported loss of atropine (37%) and scopolamine (58%) during cooking, it is unknown how much of these substances actually remains in the popcorns.

The European Food Safety Authority (EFSA) determined 0.016 μg/kg body weight as the acute reference dose for the sum of atropine and scopolamine [7]. As the maximum concentration for atropine and scopolamine in the infants and young children food 1 μg/kg has been established by the EU Regulation 2016/239, while the preferred LOQ for the same tropane alkaloids in agricultural commodities is below 5 μg/kg and not above 10 μg/kg and below 2 μg/kg for the finished foods (e.g. breakfast cereals), as stated by the EU commission recommendation 2015/976 [8].

Conclusion

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Acknowledgements

The authors acknowledge the financial support of the Ministry of Education and Science, Republic of Serbia.

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