Pesticide Residues in Maize by LC-MS/MS

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Abstract
The liquid chromatography tandem-mass spectrometry (LC–MS/MS) with ESI was applied for the detection of 60 pesticides residues in maize, extracted with QuEChERS. The average recoveries for all analites were 83.7-121.9% (RSDs 4.82-15.34%). The obtained R^2 values for all investigated pesticides were higher than 0.99. The LOQs of 0.01 mg/kg confirm that the method is appropriate for the determination of pesticide residues in all investigated vegetables according to the regulations of the Serbian and EU MRLs. One sample was with no detected pesticide residues and in three samples only one pesticide residue was detected. The multiple detections were confirmed in three analyzed samples. All the detections were below the MRLs.

Introduction
Maize (*Zea mays*) is an annual plant – a crop from grass family (*Graminae*) the cultivation of which is widespread due to the fact that it is used as food and feed. Its grains, as basic raw material, are of particular significance as they contain 70-75% of carbohydrates, 10% of proteins, about 5% of oil, 15% of mineral compounds as well as 2.5% of cellulose.
The goal of modern agricultural production is obtaining high and qualitative yields of agricultural products [1-2]. To achive these results and to obtain qualitative and quantitative justified yields it is necessary to apply plant protection products. The use of pesticides in agriculture continues to increase as much as food is needed to meet the demands of the growing population and also for the export [3]. The human exposure to low doses of pesticides through food consumption has led to chronic toxicity which can lead to birth defects, cancers, endocrine disruption or reproductive dysfunctions [4]. Crop protection problems refer to all the biotic and abiotic factors [5].
Based on the register of plant protection products sold in Serbia, 239 products have been registered for maize protection, which emphasizes the need for pesticide residues control regarding this crop [6].
That is why the aim of this study has been to determine the content of 60 pesticides in maize samples by the validated multiresidue method using liquid chromatography tandem mass spectrometry (LC–MS/MS).

Experimental
Plant material. The seven maize samples were collected directly from the farmers from Rivnica and Novi Slankamen, Vojvodina, Serbia.
The sampling was carried out in accordance with the “Regulations over the methods of food sampling and testing aimed at the determination of plant protection product residues in food” [7] which defines the sampling methods and the minimum size of laboratory samples.

Seven average samples of maize were taken at the stage of technological maturity from the farmers. The samples were put into polyethylene bags and promptly transported to the laboratory. Each sample was homogenized on the arrival in the laboratory and kept in the freezer at the temperature of -18 ºC till being analyzed [8].

Sample analyses. For LC analysis, an Agilent 1200 HPLC system with a binary pump was used. For chromatography separation, Zorbax XDB C18 analytical column of 50 × 4.6mm and 1.8 μm particle size (Agilent Technologies) was used. For the mass spectrometric analysis, an Agilent 6410B Triple-Quad LC/MS system was used. Agilent by MassHunter Workstation Software version B.04. was applied for the method acquisition and data processing.

Validation. The method was validated according to SANCO/12571/2013.

The limit of detection - LOD was determined as the lowest concentration giving a response of three times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated by MassHunter Qualitative Software.

The linearity was checked using matrix matched standards (MMS) at the concentrations of 5.0, 10.0, 25.0, 50.0 and 100.0 ng/mL.

The recovery was checked by enriching 5 g of a blank sample with the mixture of pesticide standard of 10 μg/ml in the amount of 50 and 25 μL (final mass concentration 0.10 and 0.05 mg/kg) and with the mixture of pesticide standard of 1 μg/mL in the amount of 100 μl (final mass concentration 0.01 mg/kg) with the addition of the internal standard carbofuran-D3.

Pesticide extraction. A 5 g of homogenate samples was weighed into 50 mL PP centrifuge tube. Five milliliters of water was added and mixed by vortex. For the extraction of the pesticides, 10 mL of acetonitrile was added and the tube was shaken for 30 sec. A mixture of 21 g of NaCl, 4g of $MgSO_4$, 1 g of trisodium citrate dehydrate, and 0.5 g of disodium hydrogencitrate sesquihydrate was added, and the tube was vigorously shaken for 1 min, followed by centrifugation for 5 min at 3500 rpm. An aliquot of 6 mL of the supernatant acetonitrile phase was transferred into 15 mL PP centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$, and the tube was vigorously shaken for 30 sec. One milliliter of aliquot evaporated nearly to dryness and reconstituted in 1 mL of mixture methanol/water (50:50, V/V) contained 0.1% of formic acid.

Results and discussion

The LC-MS/MS was used for the simultaneous residue determionation of 60 pesticides in the maize samples. The validated method which uses the LC-MS/MS provides a very high sensitivity, good reproducibility, appropriate linearity and can be applied with the high reliability to the analysis of investigated pesticide residues in maize samples.

The LOQs was 10 mg/kg with the average recoveries for all analites from 83.7 to 121.9% (RSDs 4.82-15.34%). The obtained coefficients of correlations (R^2) values for all investigated pesticides were higher than 0.99.

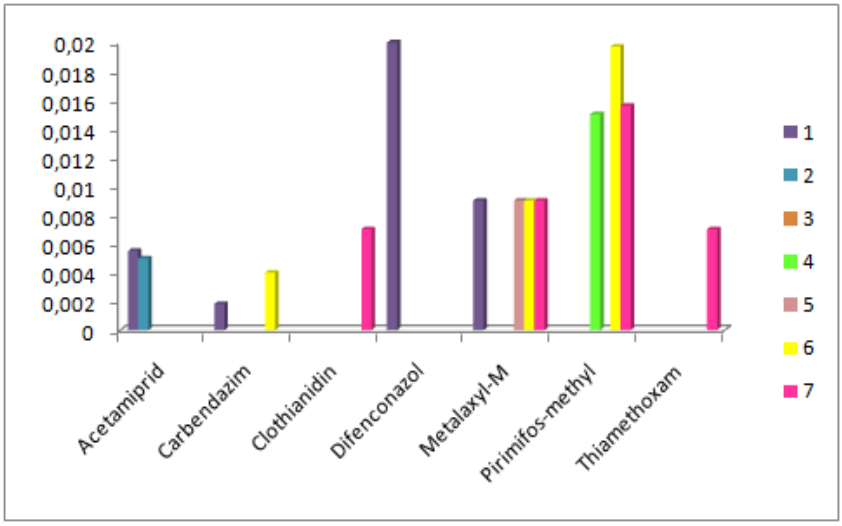
The analyses of the obtained data from the LC-MS/MS analyses show that the most frequently detected pesticides were pirimifos-methyl, metalaxyl-M and acetamiprid.

One maize sample was with no pesticide residues detection, or detections were below the LOQ. In three samples one pesticide was detected: acetamiprid, pirimifos-methyl and metalaxyl-M, respectively. Three samples were with multiple detections, two with four and one with three detected pesticides (sample number 1. acetamiprid, difenoconazol and metalaxyl-M; sample number 6. carbendazim, metalaxyl-M and pirimifos-methyl and sample number 7. clothianidin, metalaxyl-M, pirimifos-methyl and thiametoxam).

Figure 1. LC-MS/MS chromatogram of a maize sample

Off. Gazzete RS No 29/2014 regulates the maximum limits the amount of pesticide residues in agricultural products whereby the Regulations are in compliance with the MRLs as regulated in the European Union, i.e. by Regulation 396/2005.
When comparing the detected values of pesticides in the analyzed maize samples with the MRL values all the detections were found to be in accordance with their limits.

Figure 2. Detected pesticides in maize samples (mg/kg)



Conclusion

The high percentage of samples (85.71%) positive to the pesticide residues which are below the MRL values, emphasizes the need for the continuous food safety control in the production, in order to successfully prevent harmful effects of pesticides on the healts of humans and animals, as well as to secure the obligatory adherence to the rules of GAP-a (Good Agricultural Practice).
However, the alarming fact is that farmers, in their production, tend to use the compounds, i.e. active substances which have not been registered and are not allowed for use in maize protection. Among the detected insecticides there are acetamiprid, thiamethoxam, clothiandin and pirimifos-methyl which are not registered for the application on maize as well as the

detected fungicides carbendazim and difenoconazol. The only detected pesticide permitted to be used on this crop is the fungicide metalaxyl-M.
It is interesting to note that, although a large number of herbicides are used for maize protection from weeds, none were registered in the investigated samples.

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