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**SCREENING OF NEONICOTINOIDS IN SURFACE WATER SAMPLES BY LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY**

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**Abstract**

The EU Decision 2015/495 concerning the watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC, among others, includes neonicotinoid insecticides, as well. The LC-QToF-MS technique was used for screening analysis of the surface water samples from Serbia. The residues of thiamethoxam, acetamiprid and imidacloprid were detected.

**Introduction**

Water is one of the essential resources for life and its multiple uses are indispensable for a series of activities, such as agriculture, generation of energy, public and industrial supply, among others. However, the technological development of mankind has not taken into account the risks to the environment. The pollution of surface and groundwater worldwide has become a major topic of discussion among scientists and experts in the field of environmental protection. Unfortunately, this problem has been observed relatively late (late eighties of the previous century), after the hazardous substances caused many damages to the aquatic ecosystems. Pesticides deserve special attention because they can migrate to the surface and ground water, after their application to the plants or the soil [1].

Very often farmers after the pesticides application throw empty packaging into the local channels or leave them next to the fields, which is another source of pollution [2, 3].

In the European Union, Directive 2000/60/EC sets out the basis for joint action in the field of water policy (Water Framework Directive - WFD). WFD constitutes the legal framework for the protection and improvement of the quality of all water resources such as rivers, lakes, groundwater, water and other coastal waters in the European Union. The main goal is to achieve "good chemical and ecological status in all waters" by 2015. The presence of priority and priority hazardous substances in water is regulated under Directive 2008/105/EC and Directive 2013/39/EU amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.

Directive 2013/39/EC [4] brings us environmental quality standards (EQS) for 45 hazardous substances in surface water, including 21 pesticides (atrazine, simazine, terbutryn, isoproturon, diuron, etc.). The presence in the environment of the pesticides that are not encompassed in this Directive is regulated by maximum allowable concentration of 0.1 µg/L (sum 0.5 µg/L).

The latest Commission implementing Decision (EU) 2015/495 [5] from 20 March 2015, established a watch list of substances for union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC. This Decision establishes additional substances and their maximum acceptable method detection limits like oxadiazon (8.8 ng/L), methiocarb (10 ng/L) and neonicotinoids (9-500 ng/L). Neonicotinoids are nowadays the most widely used insecticides in the world and include imidacloprid, thiamethoxam, acetamiprid, thiacloprid and clothianidin, as well as a metabolite 6-chloro nicotinic acid.

The multi-residue methods, lately, have been using LC/MS-MS which gives high sensitivity and selectivity operating in selective reaction monitoring mode. Triple quadrupole is the primary choice for pesticides because of the selectivity and sensitivity, as well as the wide dynamic range. Mass detectors with high mass resolution and high mass accuracy coupled with liquid chromatography allows us to get valuable information for substance identification. Time of flight (TOF) detectors are used for the identification of transformation products. Their intrinsic characteristic of highly accurate mass measurement and sensitivity in full-scan acquisition mode allows the reliable identification of a large number of degradation products in a single chromatographic run. The hybrid systems are also used for the identification of non-target and transformation products. In our work, we have investigated the surface water samples for the presence of neonicotinoids, by liquid chromatography with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) [6].

## **Experimental**

### **Samples and chromatographic conditions**

One hundred surface water samples were taken from rivers in Serbia according to ISO 5667-6 during autumn 2016. Samples are taken in 1L amber glass bottles, transported to the laboratory and kept in the refrigerator on 4 °C until the analysis. Before analysis samples are filtered through 1µm glass fiber filters and off-line pre-concentrated on Oasis HLB cartridges. The pesticides were eluted from cartridges with 5 ml of dichlormethane and 5 ml methanol. Extracts were evaporated to dryness at 40 °C on TechneDry-block under stream of nitrogen and reconstituted in 0.5 ml of initial mobile phase. Then, they were filtered through the 0.22 µm PTFE filters into the auto-sampler vials for LC-QTOF-MS. The LC-QTOF-MS conditions are given in Table 1.

Table 1. The LC-QToF-MS conditions

<b>LC1290Agilent Tehn.</b>	
Column	Eclipse Plus C18 150x2,1mm, 1,8µm
Flow	0.400 ml/min
Mobile phase composition	A:95% Acetonitrile + 0,1% Formic acid
	B: Water +0.1% Formic acid
<b>LC6550 QTOF Agilent Tehn.</b>	
<b>LC6550 QTOF Agilent Tehn.</b>	
Auto MS/MS settings	
<ul style="list-style-type: none"> <li>• Full Scan, Mass range 10-450 m/z, Absolute threshold 500 Acquisition rate 3 spectra/sec</li> </ul>	
MS/MS	
<ul style="list-style-type: none"> <li>• Inclusion list 125 pesticides, <math>\Delta</math> m/z 20 ppm, <math>\Delta</math> Rt 0.35 min</li> <li>• Isolation width 1.3 m/z, Mas range 35-450 m/z, Acquisition rate 2 spectra/sec, Absolute threshold 50, Max 5 precursor per cycle</li> </ul>	

The validation was done in accordance with SANTE/11945/2015 [7].

Limit of detection (LOD) and Limit of Quantification (LOQ) are defined by the regulation and experimentally confirmed through the spiking of real water sample with pesticide mix solution. The final concentration was from 4 to 25ng/L for different pesticide. S/N ratio was calculated with software in obtained chromatograms for LOQ. LOD values are obtained through statistical calculation.

Tabela 2: Accurate masses for  $[M + H]^+$  ions, LOD and LOQ for neonicotinoid pesticides

<b>Compound</b>	<b>Precursor ion</b>	<b>LOD (ng/L)</b>	<b>LOQ (ng/L)</b>
Thiamethoxam	292.0270	10	25
Clothianidin	250.0164	10	25
Imidacloprid	256.0597	2	5
Acetamiprid	223.0748	10	25
Thiacloprid	253.032	10	25

## Results and discussion

Data obtained from this investigation are given in Table 3. The most frequent found pesticide was imidacloprid in 39% of all analyzed samples. Eight water samples have got values which were above PNEC value. Other four pesticides are detected in amount less than 10% of all analyzed samples, and their concentrations are under the PNEC value for each of them.

Table 3. Pesticide residue detected in analyzed samples of surface water samples (ng/L)

Compound	PNEC value*	Max found concentration	Number of Postive results
Thiamethoxam	140	15	4
Clothianidin	130	12	5
Imidacloprid	9	16	39 (8 over limit)
Acetamiprid	500	19	9
Thiacloprid	50	47	6

\*The Predicted no effect concentration (PNEC) is the concentration of a chemical which marks the limit at which below no adverse effects of exposure in an ecosystem are measured

## Conclusion

For the extraction of pesticides and other contaminants from surface water samples, solid phase extraction techniques have been employed, which is a well-known technique used for the extraction of numerous compound classes in water, with high accuracy and precision. The polymeric Oasis HLB sorbent are the most used for extraction of pesticides. The use of LC-QToF-MS technique for qualitative and quantitative analyses of aqueous samples is growing. The rapid investigation in the field of LC-MS have transformed this technique into a key technique for the analysis of pesticides in environmental matrices.

EU Decision 495/2015 extends the standards pertaining to environmental quality in water protection of surface water, including neonicotinoid group of insecticides. LC-QToF-MS analysis of surface water samples is very helpful to detect low concentrations of neonicotinoids inspite of their very low PNEC values (for imidacloprid 9 ng/L). The results of the study suggest the need for constant monitoring of surface waters, especially if the water will be used for human consumption or recreation – bathing.

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