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HPLC METHOD FOR DETERMINATION OF SOME PESTICIDE RESIDUES IN WATER SAMPLES

Abstract

A new, accurate and suitable high-performance liquid chromatography (HPLC) method with ultraviolet-diode array detection (UV-DAD) for determination of some herbicide and organo-phosphorus insecticide residues in different water samples has been developed and validated. Separation and quantitative determination of the analytes were carried out using a Purospher STAR RP-18e (30 x 4 mm; 3 m) analytical column, under the isocratic elution with mobile phase consisted of acetonitrile/water (47/53, *V/V*), flow rate of 1 mL/min, constant column temperature at 25 °C and UV-detection at 220 nm. The obtained results showed that analysed water samples did not contain detectable residues of investigated pesticides.

Key words: *HPLC-method, pesticide residues, water samples*

INTRODUCTION

It is well known that pesticides are used to increase crop yields, but due to their widespread use, their residues are present in the environment, such as soil, water and air, and from there can easily pass into agricultural products for human consumption. Due to the adverse effects of pesticides, environmental pollution from pesticides is a cause for concern locally, regionally, nationally and globally. For these reasons, the need to develop new analytical methods for the determination of pesticide residues in the environmental samples, and especially in water, is obvious.

To control the presence of pesticides in the environment and to ensure the safety of human health, the Maximum Residue Levels (MRLs) for pesticides in food and water are legally regulated in most countries. MRLs of pesticides in I and II class of

waters, including drinking water, mineral waters and some surface waters in the EU are governed by Directive 98/83/EC (1998) and are estimated at 0.1 µg/L individually for each pesticide or the total amount of all present pesticides not to exceed 0.5 µg/L.

The most commonly used pesticides are phenoxycarboxylic acids (e.g., 2,4-D), organonitrogen, among which triazines (e.g., atrazine), and organophosphorus (e.g., malathion, fenitrothion, parathion). Owing to the fact that they are soluble in water they can lead to serious pollution to the environment (soil, water and air) and to impact on human health (Cserháti and Szőgyi, 2012).

The most widely used analytical methods for analysis of phenoxycarboxylic acids, triazines and organophosphorus residues in water samples are Gas Chromatography (GC) with Mass Spectrometry (MS) (Rocha et al., 2012), Flame Photometric Detector (FPD) (National Measurement Institute, 2013), Nitrogen Phosphorous Detector (NPD) (Food safety and standards authority of India, 2016), and Liquid Chromatography (LC) with Tandem Mass Spectrometry (MS/MS) (Donato et al., 2015) and fluorescence detector (National Measurement Institute, 2013). In spite of that they are less sensitive, HPLC (High Performance Liquid Chromatography) methods with diode array detection (DAD) are also used (Cappellini et al., 2012). In the previous papers the HPLC methods for determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples with ultraviolet diode array detection (UV-DAD) were developed and validated (Velkoska-Markovska and Petanovska-Ilievska, 2018, 2019). The previous methods were developed using analytical columns with higher length of 125 and 250 mm, and particle size of 5 µm. The aim of this paper was to develop a new HPLC method for qualitative and quantitative determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples using short, but more efficient analytical column with particle size of 3 µm. In this way, the duration of the chromatographic analysis would be reduced, and thus the consumption of organic solvents would be reduced as well.

MATERIAL AND METHODS

Reagents and Chemicals. The Pestanal analytical standards of 2,4-D (98.6 % purity), atrazine (98.8% purity), malathion (97.2 % purity), fenitrothion (95.2 % purity) and parathion (98.8 % purity), as well as, HPLC-grade acetonitrile and water were purchased by Sigma-Aldrich (Germany). Samples for the analysis of pesticide residues were taken from tap water, non-carbonated water, purchased from local supermarket and water from the Vardar River.

Equipment. The HPLC analyses were performed using an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography system equipped with: vacuum degasser (G1322A), binary pump (G1312B), autosampler (G1329B), a thermostatted column compartment (G1316A), UV-VIS diode array detector (G1316B) and ChemStation software. The experiments were carried out on Purospher STAR RP-18e (30 x 4 mm; 3 m) analytical column produced by Merck (Germany). A vacuum manifold Visiprep (Supelco, Sigma-Aldrich) was used for the solid phase exstraction (SPE).

Preparation of Stock Solutions. Stock solutions of 2,4-D, atrazine, malathion, fenitrothion and parathion were prepared by separately dissolving 0.0253, 0.0113, 0.0330, 0.0225 and 0.0188 g, respectively, of the pure analytical standards in acetonitrile in 25 mL volumetric flasks. The solutions were degassed for 15 min in an ultrasonic bath and stored in a refrigerator at 4 °C. Stock solutions were used for the fortification of water samples in order to test the method validation.

Sample preparation. The samples from the river Vardar were taken in brown glass bottles of 2.5 L. Immediately upon arrival in the laboratory, the samples were filtered through 0.45 μm nitrocellulose membrane filter.

Method Validation. Linearity, precision, recovery and limit of quantification (LOQ) were tested for the method validation. For realising that purpose, spiking samples were prepared by fortifying 1 L distilled water with three sets of concentrations (0.1, 0.2 and 0.5 μg/L for each analyte). Unspiked samples were used for blanks. After that, the samples were subjected to solid-phase extraction through the Supelclean ENVI-18 columns (0.5 g, 6 mL), before the HPLC analysis, and each sample was injected with volume of 5 μL.

RESULTS AND DISCUSSION

Chromatographic analyses were accomplished using the Purospher STAR RP-18e (endcapped) analytical column. It is suitable for efficient separation of base, neutral and chelating compounds by applying simple mobile phases, resulting in symmetrical chromatographic peaks without tails. This sorbent is made of extraordinarily pure silica gel, which surface is completely covered with C-18 radicals (ChromBook, 2011).

In order to obtain optimal conditions for separation of the analytes, a series of preliminary tests were implemented by changing the volume ratio of acetonitrile and water in the mobile phase. The best separation of the investigated pesticides with symmetrical peak shapes and satisfy purity indexes was achieved under an isocratic elution with mobile phase consisting of acetonitrile and water (47/53, *V/V*), a flow rate of 1 mL/min, a constant column temperature at 25 °C and UV detection at 220 nm. Compared to the results described in previous papers obtained using longer columns of the type LiChrospher 60 RP-select B with a length of 125 mm (Velkoska-Markovska and Petanovska-Ilievska, 2018) and 250 mm (Velkoska-Markovska and Petanovska-Ilievska, 2019), under these chromatographic conditions were obtained shorter retention times for the target pesticides, thus reducing the duration of the chromatographic analysis and the consumption of organic solvents as well.

The developed method was validated by testing specificity, selectivity, linearity, precision, accuracy, and limit of quantification (LOQ) according to the Guidance document on pesticide residue analytical methods (2010).

To confirm the specificity of the developed method, UV-diode array detection was used to check the peak purity and analyte peak identity. The purity index for all analytes was greater than 999 (the maximum value for the peak purity index (PPI) should be 1000), which means that the chromatographic peak was not affected by any other

compound. Identification of the analytes was done using the values for the retention time and match factor obtained by overlaid spectra of a pure analytical standard and absorption spectra of the same analyte in the water samples.
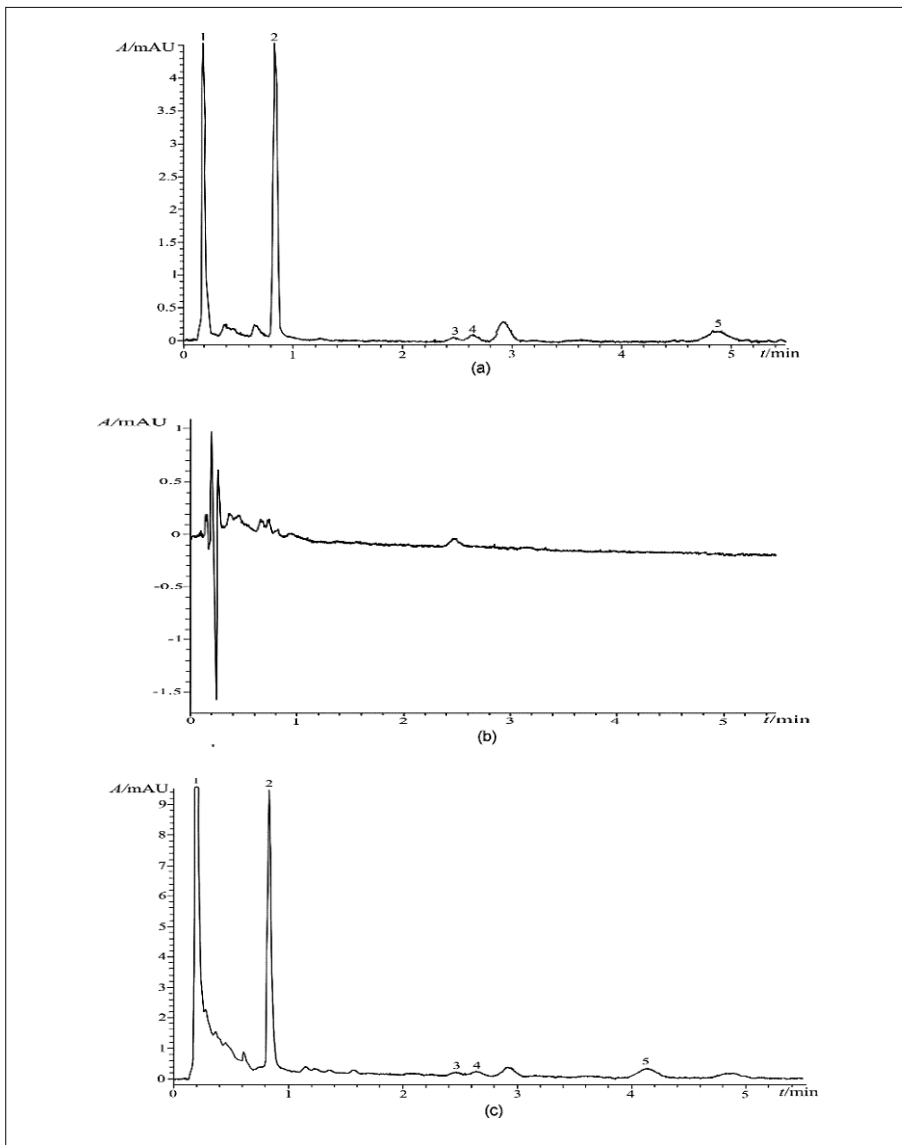
Figure 1. Chromatograms from standard mixture of 2,4-D (1), atrazine (2), malathion (3), fenitrothion (4) and parathion (5) at the concentrations which correspond to MRLs (a), blank sample (distilled water) (b) and samples of distilled water fortified at the concentration equal to MRL for each analyte (c)

Additionally, to prove selectivity of the method, in Figure 1 are presented chromatograms of standards at the concentrations which are correspond to MRLs (a), blank sample (distilled water) (b) and sample of distilled water fortified at the concentration equal to MRL for each analyte (c). The chromatographic peak that appears near the expected peak of 2,4-D (Fig. 1b) in the blank sample has a completely different spectrum and much lower intensity than the 2,4-D.

The linearity of the method was determined using calibration curves obtained by triplicate injection of samples of distilled water enriched with the examined pesticides in 3 concentrations levels (0.1, 0.2 and 0.5 µg/L for each pesticide analysed) after a solid-phase extraction through the Supelclean ENVI-18 column. Each solution was injected with 5 µL. The obtained results for multiple correlation coefficients ($R^2 \geq 0.99$) suggested that the method has a satisfactory linearity for all analytes (Table 1).

Table 1. Statistical data for linearity of the method.

Compound	Linearity range (µg/L)	Regression equation	R^2
2,4-D	0.1 – 0.5	[1] y = 52.359x + 10.744 [2] y = 26.394x + 10.555	0.9999 0.9905
atrazine	0.1 – 0.5	[1] y = 85.298x + 16.621 [2] y = 32.074x + 6.1532	1 0.9997
malathion	0.1 – 0.5	[1] y = 3.9476x + 0.8652 [2] y = 0.729x + 0.0645	0.9995 0.9983
fenitrothion	0.1 – 0.5	[1] y = 14.64x + 0.7439 [2] y = 1.8267x + 0.0972	0.9973 0.9969
parathion	0.1 – 0.5	[1] y = 19.632x – 0.032 [2] y = 1.3088x + 0.0394	0.9999 0.9999

[1] y = peak area, [2] y = peak height

The signal-to-noise ratio (S/N) at the lowest concentration level for each compound was found to be ≥ 10 for all investigated pesticides. Hence, the LOQ was estimated to be 0.1 µg/L for all examined pesticides. These obtained values for LOQs are acceptable for determining the pesticide residues in water samples according to the rules of Guidance document on pesticide residue analytical methods (2010).

To determine the precision of the developed method, five consecutive injections (5 µL) of a distilled water sample fortified with the investigated pesticides at the MRL level (0.1 µg/L) were done. The precision of the method was expressed as the repeatability of the results obtained for the retention time and the peak area for each analyte. The computed values of RSD (Table 2) for retention time (0.06 – 0.27 %) and peak area (0.39 – 4.51 %) indicated an excellent precision of the proposed method.

The recovery of the developed method was determined by adding 0.1, 0.2 and 0.5 µg/L of each analyte in 1 L distilled water. Samples of distilled water in which pesticides have not been added were used as blank samples. For each concentration level,

4 samples were prepared ($n = 4$). Subsequently, the samples were subjected to solid-phase extraction and HPLC analysis, and each sample was injected with 5 μL.

Table 2. Statistical data for Intra-day precision
of retention time and peak area (n = 5)

Compound	t_R (min) ± SD	RSD (%)	peak area ± SD	RSD (%)
2,4-D	0.21 ± 0.004	0.22	16.09 ± 0.35	2.18
atrazine	0.83 ± 0.005	0.06	25.08 ± 0.10	0.39
malathion	2.62 ± 0.006	0.24	1.26 ± 0.04	3.14
fenitrothion	2.92 ± 0.004	0.13	2.38 ± 0.09	3.97
parathion	4.88 ± 0.01	0.27	1.90 ± 0.08	4.51

Table 3. Results from recovery experiments (n = 4)

Compound	Fortification level (μg/L)	Total analyte found (μg/L ± SD)	Recovery (%)	RSD (%)
2,4-D	0.1	0.102 ± 0.007	102.15	6.56
	0.2	0.200 ± 0.012	100.22	6.02
	0.5	0.495 ± 0.008	99.04	1.55
atrazine	0.1	0.096 ± 0.002	95.51	1.75
	0.2	0.202 ± 0.007	101.16	3.72
	0.5	0.498 ± 0.001	99.61	0.22
malathion	0.1	0.099 ± 0.010	99.30	10.06
	0.2	0.198 ± 0.019	99.14	9.59
	0.5	0.499 ± 0.041	99.75	8.31
fenitrothion	0.1	0.111 ± 0.006	111.47	5.78
	0.2	0.186 ± 0.006	92.87	3.03
	0.5	0.505 ± 0.003	98.55	4.43
parathion	0.1	0.098 ± 0.004	107.15	8.16
	0.2	0.207 ± 0.014	103.66	6.74
	0.5	0.496 ± 0.006	99.27	1.31

The obtained values for recovery and for relative standard deviation (Table 3) were within the following ranges 92.87 – 111.47 % and 0.22 – 10.06 %, respectively. The mean recovery at each fortification level in the range of 60 – 120 % and relative standard deviation (RSD) ≤ 30 % per level are acceptable according to the Guidance document on pesticide residue analytical methods (2010). Consequently, it can be concluded that the proposed method was accurate and suitable for the determination of the target pesticide residues in water samples.

The developed high-performance liquid chromatography method was applied for the determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in different water samples (tap water (a), non-carbonated water (b) and water from Vardar River (c)). Before HPLC analysis, samples were concentrated and clean-up using SPE. Each analysis was repeated five times, and each sample was injected with 5 µL. Typical chromatograms of the tested water samples are presented in Figure 2.

As can be seen from Figure 2, a chromatographic peak (X) was noticed at the beginning of all three chromatograms, with a retention time of 0.2 min (similar to 2.4-D), but with overlapping of its UV spectrum and that of the standard it was proved that this peak was not originate from the 2,4-D. Chromatographic peak (X_1) with a retention time of 0.79 min (similar to atrazine, $t_R = 0.83$ min) was present in the samples of drinking water (Figure 2b) and that of the Vardar River (Figure 2c), but the presence of this component has not been proven, because their spectra were quite different. The analysis performed by comparison of retention times and UV spectra shows that in the analysed samples no residues of the examined pesticides were found in a concentration corresponding to MRL or higher.
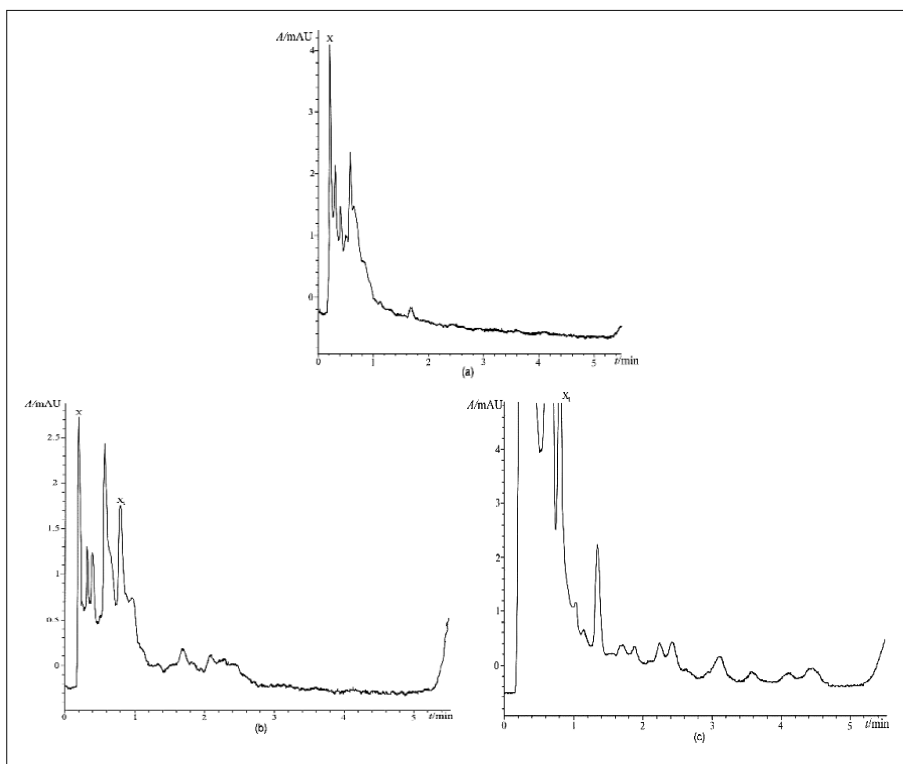

Figure 2. Typical chromatograms of water samples obtained from tap water (a), non-carbonated water purchased at the local market (b) and water from the Vardar River (c) at 220 nm

CONCLUSIONS

This paper describes a new possibility of successful determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples using high-performance liquid chromatography (HPLC) method and ultraviolet – diode array detection (UV-DAD). Specificity, selectivity, linearity, precision, recovery and limit of quantification (LOQ) were examined to assess the validity of the developed method. The developed method was successfully applied for the determination of target pesticide residues in tap water, non-carbonated water and water from Vardar River. The obtained results showed that analysed water samples did not contain detectable residues of investigated pesticides.

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ХПЛЦ (HPLC) МЕТОДА ЗА ОДРЕЂИВАЊЕ НЕКИХ ОСТАТАКА ПЕСТИЦИДА У УЗОРЦИМА ВОДЕ

Извод

Развијена је и потврђена нова, тачна и погодна течна хроматографија високих перформанси (ХПЛЦ) са детекцијом ултраљубичасто-диодне матрице (УВ-ДАД) за одређивање остатака органофосфорних инсектицида и хербицида у различитим узорцима воде. Одвајање и квантитативно одређивање аналита извршено је употребом Пуроспхер СТАР РП-18е (30 x 4 mm; 3 m) аналитичке колоне, под изократском елуцијом са мобилном фазом која се састојала од ацетонитрила / воде (47/53, *V/V*), брзина протока од 1 мЛ / мин, константна температура колоне на 25 °C и УВ детекција на 220 nm. Добивени резултати су показали да анализирани узорци воде не садрже остатке испитиваних пестицида.

Кључне речи: *ХПЛЦ метода, остаци пестицида, узорци воде*