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Original scientific paper

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF SOME PESTICIDE RESIDUES IN WATER SAMPLES[#]

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The development of a new reversed-phase high-performance liquid chromatography method (RP-HPLC) with ultraviolet-diode array detection (UV-DAD) for simultaneous determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in different water samples are described in this paper. The developed method has been validated according to European Commission guidelines for pesticide residue analytical methods, and all performance characteristics were found within acceptance criteria. The best separation and quantitative determination of the analytes were achieved using a LiChrospher 60 RP-select B (250 × 4 mm, 5 μm) analytical column, under the isocratic elution with mobile phase consisting of acetonitrile/water (60/40, *V/V*), flow rate of 1 ml/min, constant column temperature at 25 °C and UV-detection at 220 nm and 270 nm. The run time of analysis under the stipulated chromatographic conditions was about 10 min.

Key words: RP-HPLC; UV-DAD; method validation; pesticide residues; water samples

INTRODUCTION

As it is well-known, pesticides are natural or synthetic chemical compounds destined to destroy or prevent the growth of any pest (insects, weeds, diseases, fungi, etc.) that threatens the production of agricultural crops [1]. Farmers are extensively applying pesticides to increase yields while saving time and money [2]. However, only a small part of the applied pesticides reach the target plants, and the remainder remains in the air, soil and water. Excessive use of pesticides leads to pollution of water, soil and air, as well as, causes their accumulation in agricultural crops [3]. Water is the most important and crucial for life, and its pollution is a major problem nowadays. Due to the solubility of pesticides in water, they can cause serious environmental pollution (soil, water and air) and human health disorders [4]. Through primary agricultural products, they can be found in processed products for human consumption.

2,4-D and malathion are among the most used pesticides in R. Macedonia, and until several years ago, fenitrothion, parathion and atrazine were also widely used chemical plant protection products. Although atrazine, fenitrothion and parathion are forbidden for use in the EU, they are still allowed in the United States (except for parathion) and in some third countries. In addition, as a result of the unauthorized use of these pesticides, they can be found in environmental samples (water, soil, air) and food.

Herbicides from the chlorophenoxy carboxylic acids group, such as 2,4-D (Figure 1a) are characterized by relative stability and photostability in the natural waters because they are considered as persistent organic pollutants and pose a serious ecological problem [5].

Triazines, especially atrazine (Figure 1b) [6], are among the most commonly used herbicides in the world. Their use causes great concern because of their mobility and high solubility in water that allows them to pass into underground and surface waters [7]. Chemical pollution of surface waters is a threat to the aquatic environment causing negative effects such as acute and chronic toxicity to aquatic

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday

organisms, accumulation in the ecosystem, loss of biodiversity, and a threat to human health. Atrazine represents a significant risk to the aquatic environment and it is one of the 45 priority harmful substances according to Directive 2013/39/EU of the European Parliament and the Council of 2013 [8].

Organophosphate pesticides, such as malathion (Figure 1c), fenitrothion (Figure 1d) and parathion (Figure 1e) are toxic for both humans and animals, and they are also quite stable under natural environmental conditions [9]. Their improper use can cause their presence in agricultural products and the environment.

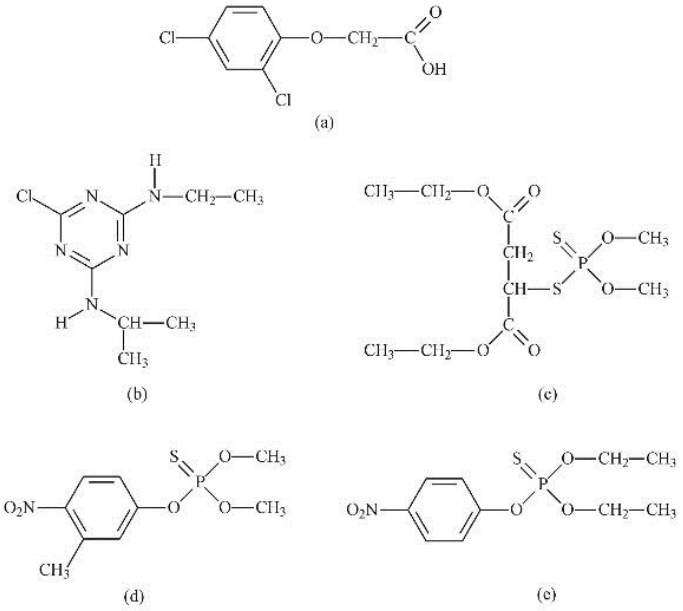

Figure 1. Structural formulas of 2,4-D (a), atrazine (b), malathion (c), fenitrothion (d) and parathion (e)

In order to protect the consumers' health from possible adverse effects, controlling the content of pesticides and their residues in environmental and food samples is necessary. In order to avoid any negative impact on human health, as well as to manage good agricultural practices, maximum residue levels of pesticides (MRLs) in food and water have been stipulated in most countries. The MRLs of pesticides in waters of class I and II, including drinking water, mineral waters and some surface waters are regulated by Directive 98/83/EC in the EU [10] and by the Water Safety Rule [11] in R. Macedonia, and they are fixed at 0.1 μg/l individually for each pesticide or 0.5 μg/l for the total quantity of all pesticides.

Of particular importance is using the highly sensitive and selective analytical methods, as well as their continuous improvement for the monitoring of pesticide residues in food and water samples. The most widely used analytical techniques are gas chromatography [12] with different detectors, such as: mass spectrometry (MS) [13, 14], flame photometric detector (FPD) [15], nitrogen phosphorous detector (NPD) [16], electron capture detection (ECD) [17], and also liquid chromatography with tandem mass spectrometry (MS/MS) [18, 19] and fluorescence detector [15]. Despite that they are less sensitive, HPLC (High Performance Liquid Chromatography) methods with diode array detection (DAD) are still used [20].

Gas and liquid chromatography are very powerful techniques for analyzing pesticides in different samples, but sample preparation, such as the extraction or concentration of the analytes before their chromatographic determination, is usually required. Several extraction techniques are known that can be used to extract pesticides from different matrices, especially from water samples [21] such as liquid-liquid extraction (LLE) [22], liquid-phase microextraction (LLME) [23], solid-phase extraction (SPE) [18], solid-phase microextraction (SPME) [24], and recently used, a quick, easy, cheap, effective, rugged and safe (QuEChERS) method [25]. However, classical LLE and SPE are the most commonly used techniques for concentrating pesticides from different matrices [18]. One of the more commonly used adsorbents for solid-phase extraction of pesticides, including the investigated pesticides is C18 [26–28].

In a previous study, HPLC method was developed for the determination of 2,4-D, atrazine,

malathion, fenitrothion and parathion residues in water samples, using LiChrospher 60 RP-select B (125 × 4 mm, 5 μm) analytical column and mobile phase consisted of acetonitrile and water [26]. The purpose of this study was to investigate the other possibilities for the determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples by reversed-phase high-performance liquid chromatography (RP-HPLC) method and ultraviolet diode array detection (UV-DAD) using different analytical column and mobile phases.

EXPERIMENTAL

Reagents and Chemicals

In the development of the method, 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) were purchased by Sigma-Aldrich (Germany).

For the preparation of mobile phases, HPLC-grade acetonitrile, methanol, water, as well as buffer solutions were used. The buffer solutions were made using phosphoric acid (H_3PO_4), potassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), acetic acid (CH_3COOH) and sodium acetate (CH_3COONa) produced by Sigma Aldrich (Germany). Samples for the analysis of target pesticide residues were taken from tap water, bottled non-carbonated water, purchased from local supermarket and water from the Vardar River.

Instrumentation

The analyses were carried out using an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography (RRLC) 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. An ultrasonic bath "Elma" was used for preparing the stock solutions. The separation and determination of analytes were performed on a LiChrospher 60 RP-select B (250 × 4 mm, 5 μm, Merck) analytical column. A vacuum manifold Visiprep (Supelco) was used for the SPE and for vortexing of samples was used IKA Vortex Genius 3 (Germany).

Preparation of Standard Solutions

Stock solutions of 2,4-D, atrazine, malathion, fenitrothion and parathion were prepared by dissolving 0.0253 g, 0.0113 g, 0.0330 g, 0.0225 g and 0.0188 g of the pure analytical standards with acetonitrile in a 25 ml volumetric flask. To better dissolve the analytical standards, the prepared standard solutions were ultrasonified in an ultrasonic bath for a period of 15 minutes. According to the principles of SOP's (Standard Operating Procedure) [29], the standard solutions were stored in a refrigerator at a temperature of 4 °C. Under these conditions, the stability of the analytical standards was greater than one month. The stock solutions were used to prepare standard working solutions and standard mixtures of all examined pesticides with different pesticide concentrations (2.56 – 616.24 ng/mL for 2,4-D, 1.42 – 170.25 ng/mL for atrazine, 22.23 – 2672.5 ng/ml for malathion, 16.36 – 1967.0 ng/ml for fenitrothion and 20.90 – 2513.26 ng/ml for parathion) in 10 ml volumetric flasks by dilution with the acetonitrile/water mixture (50/50, *V/V*), as well as to enrich the water samples for method validation.

Sample preparation

The samples from the Vardar River were taken in brown glass bottles of 2.5 L, and immediately upon arrival in the laboratory, the samples were filtered through a 0.45 μm nitrocellulose membrane filter (Millipore, Ireland). Subsequently, the samples were subjected to solid-phase extraction and HPLC analysis, and each sample was injected with 5 μl.

Method Validation

Specificity, selectivity, linearity, precision, recovery and limit of quantification (LOQ) were tested for the method validation.

The calibration curves for determining the linearity of the method for determination of pesticide residues in water were obtained by threefold injection of samples of distilled water enriched with the investigated pesticides in 3 concentration levels (0.1, 0.2 and 0.5 μg/l for each pesticide analyzed) after the solid-phase extraction through Supelclean ENVI-18 columns. Each solution was injected with 5 μl.

The recovery was determined by adding a precisely determined volume of a standard solution (at three concentration levels) from each analyzed pesticide to 1 L of distilled water, as follows: 0.1, 0.2 and 0.5 μg/l. Samples that have not been added pesticides 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 a volume of 5 μl.

Solid-phase extraction (SPE)

The solid-phase extraction was performed using columns of the type Supelclean ENVI-18 (Supelco, Sigma-Aldrich), with a volume of 6 ml and a mass of the adsorbent of 0.5 g.

The solid-phase extraction procedure consists of several steps. SPE columns conditioning was performed by passing 5 ml of acetonitrile and then 5 ml water at a flow rate of 2 ml/min. Throughout the conditioned columns, the samples (1 L water previously filtered through a nitrocellulose membrane filter with a pore size of 0.45 μm) were passed through at a flow rate of 8–10 ml/min. The retained compounds of interest and the impurities on the SPE packing were rinsed through with wash solutions (5 ml of distilled water), and then the columns were dried under vacuum for 20 min. The elution of the selected components was carried out in two portions of 2 ml of acetonitrile. The eluates were evaporated to dryness under the gentle stream of nitrogen at a temperature of 40 °C and then the dry residue was dissolved with 1 ml of acetonitrile and water mixture (50/50, *V/V*) using Vortex for 1 min. Before performing the HPLC analysis, the final extract was filtered through an Iso-Disc PTFE syringe filter (Supelco, Sigma-Aldrich) with a pore size of 0.45 μm and transferred to vials for analysis. Each sample was injected with a volume of 5 μl.

RESULTS AND DISCUSSION

The first step in the method development was the selection of the wavelength at which the chromatographic processes will be monitored.

Based on the UV spectra of the components of interest recorded in a solution of acetonitrile and water, with a volume ratio of 50/50 (Figure 2), the wavelength at which the chromatographic analysis was performed was selected. As can be seen from Figure 2a, two maxima were observed in the UV spectrum of component 2,4-D, one at about 230 nm and the other significantly less intensive at about 285 nm. In a solution of acetonitrile and water (50/50, *V/V*), atrazine exhibits maximum absorption around 220 nm (Figure 2b). In the recorded wavelength range (Figure 2c) under these conditions, maximum absorption of malathion cannot be observed, but it was noticeable that the absorption increases with decreasing wavelength. Fenitrothion shows a maximum UV absorption at about 270 nm (Figure 2d), and parathion at about 280 nm (Figure 2e). In the spectrum of the latter two compounds, absorption at a wavelength of 220 nm was observed.

For these reasons, the HPLC analysis for the simultaneous determination of 2,4-D, atrazine, malathion, fenithrothion and parathion was carried out at a wavelength of 220 nm. Additionally, the chromatographic process was followed at 270 nm, because at this wavelength fenithrothion and parathion exhibit maximum absorption, which means that the intensity of their chromatographic peaks at this wavelength was higher.

In order to develop a simple HPLC method for separation and determination of the investigated components in water samples, the chromatographic process was conducted using isocratic elution, *i.e*, the use of a constant composition of the mobile phase.

Chromatographic analysis was performed using the LiChrospher 60 RP-select B (250 × 4 mm; 5 μm) analytical column, which is characterized by a higher number of theoretical plates (55 000 plates/m) [30], and hence with higher efficiency compared to the shorter column with the same C-8 stationary phase, LiChrospher 60 RP-select B (125 × 4 mm; 5 μm), in which the number of theoretical plates is 44 000 plates/m, used in a previous study [26].

To obtain optimal conditions for separating analytes with satisfactory purity index values, a series of preliminary experiments were accomplished by changing the composition of the mobile phase. Namely, acetonitrile, methanol and water, as well as, 0.1 % acetic acid, phosphate buffer and ammonium acetate buffer were used for the preparation of mobile phases. The following mobile phases were used: acetonitrile/water (45–80 % acetonitrile), methanol/water (60–80 % methanol), acetonitrile/0.1 % acetic acid, methanol/0.1 % acetic acid, as well as methanol/phosphate buffer (pH = 2.5, 3.5 and 4.5) and methanol/ammonium acetate buffer (pH = 4.5, 5.5 and 6.0) (Figure 3). The performed investigations showed that when using methanol as a constituent of the mobile phase, longer retention times for analytes and a noisy baseline were obtained.

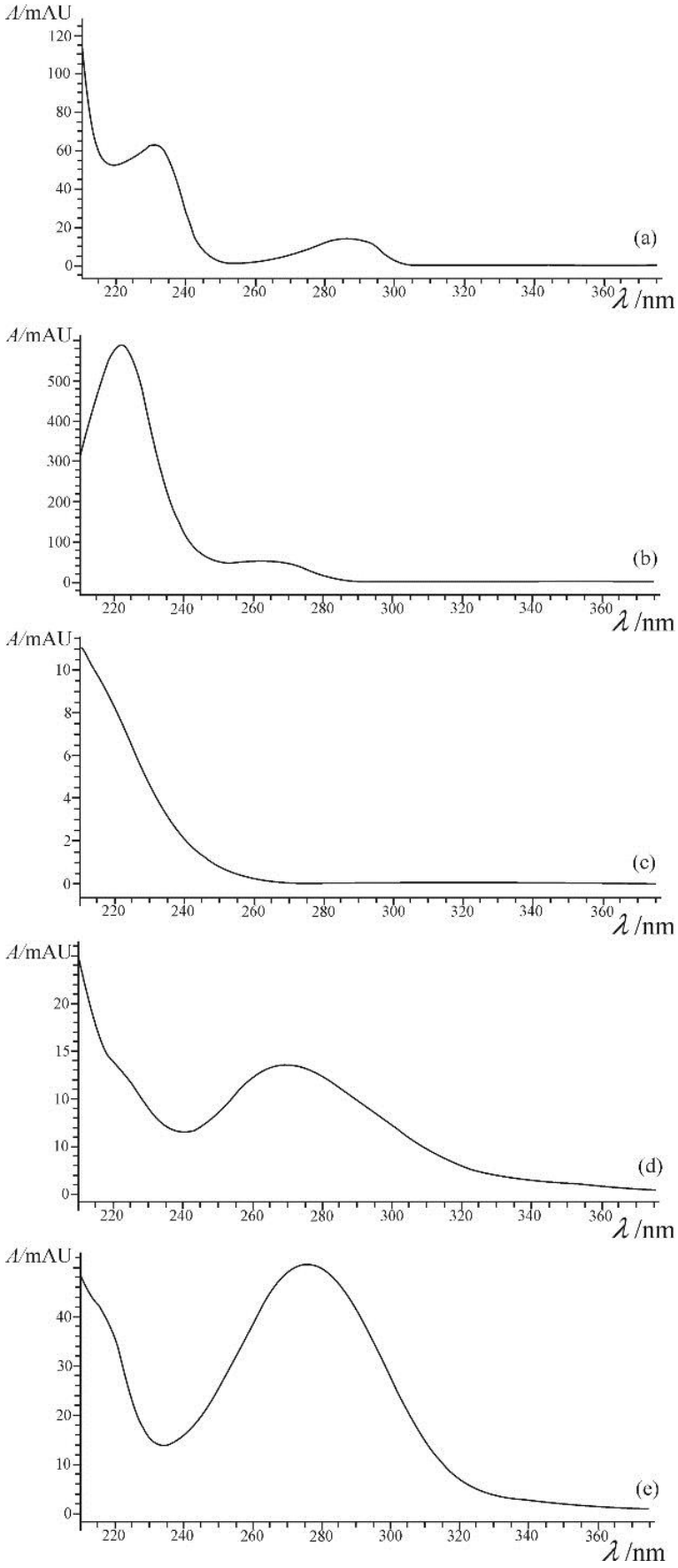

Figure 2. The UV spectra of pure analytical standards of 2,4-D (a), atrazine (b), malathion (c), fenitrothion (d) and parathion (e) in acetonitrile/water (50/50, *V/V*)

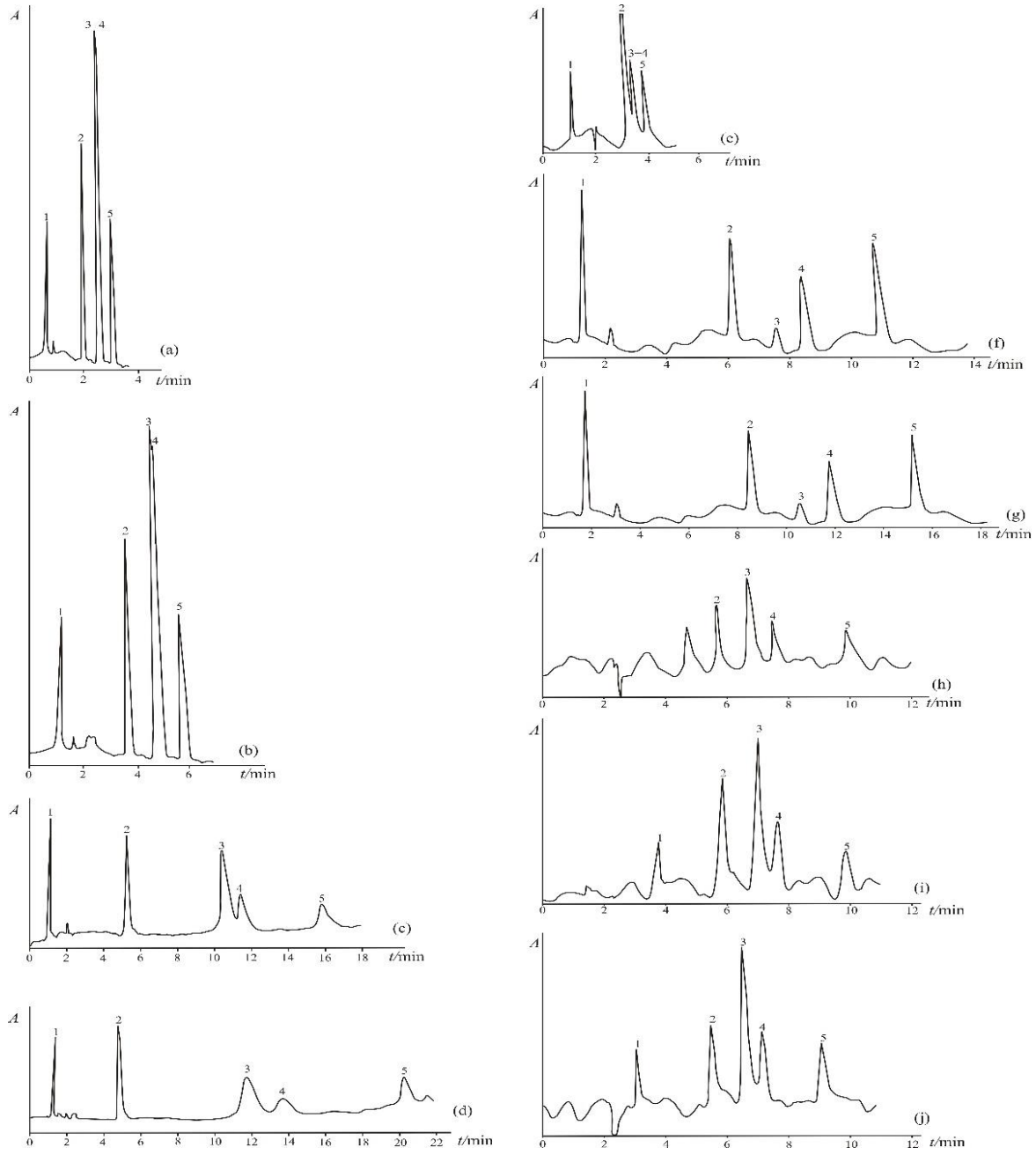

Figure 3. Chromatograms obtained from standard mixture of 2,4-D (1), atrazine (2), malathion (3), fenitrothion (4) and parathion (5) at 220 nm on LiChrospher 60 RP-select B (250 x 4 mm; 5 μm) column with mobile phase consisted of acetonitrile/water (80/20, *V/V* (a), 70/30, *V/V* (b), 50/50, *V/V* (c), 45/55, *V/V* (d)), methanol/water (80/20, *V/V* (e), 70/30, *V/V* (f), 60/40, *V/V* (g)), methanol/0.1 % acetic acid (70/30, *V/V* (h)), methanol/phosphate buffer (70/30, *V/V*, pH = 3.5 (i)) and methanol/ammonium acetate buffer (70/30, *V/V*, pH = 6.0 (j))

Using a mobile phase consisting of acetonitrile and water with a volume ratio (60/40, *V/V*), a flow rate of 1 ml/min, a constant column temperature at 25 °C and UV detection at 220 nm and 270 nm were shown to be the optimum separation conditions of the tested components with symmetrical peak shapes and satisfactory resolution purity index (Figure 4). Table 1 shows the obtained values for the column dead time (t_0), the retention times (t_R) of the analytes, their retention factors (k'), the separation factors (α) and the resolution (R_S) of the adjacent peaks. According to these data, the calculated values for the retention factors (k') were less than 10, the separation factor (α) of two adjacent chromatographic peaks was greater than 1, and the resolution (R_S) at the adjacent peaks was higher than 1.5. Consequently, it can be concluded that the proposed method allows optimal conditions for separation of analytes [31] for a total run time of 10 min.

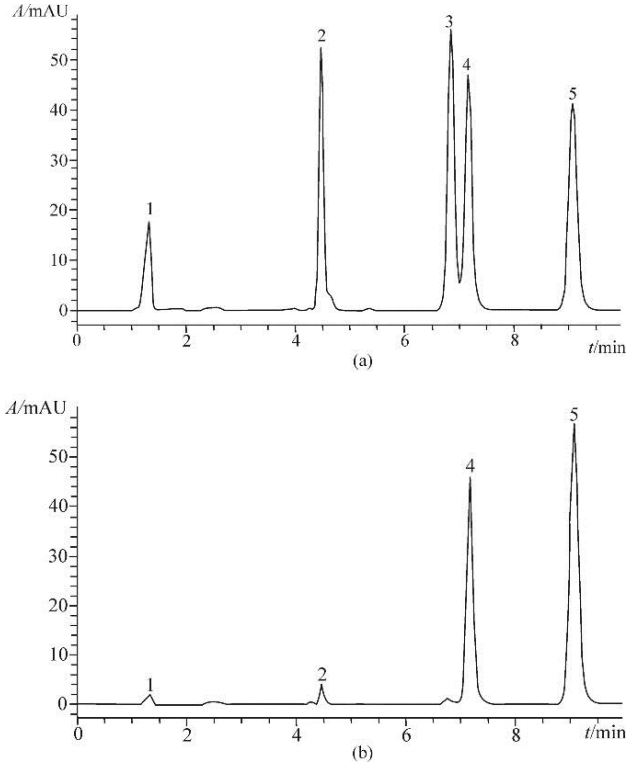

Figure 4. Chromatograms obtained from standard mixtures of 36.48 ng 2,4-D (1), 12.48 ng atrazine (2), 935.00 ng malathion (3), 223.68 ng fenitrothion (4) and 257.02 ng parathion (5) at 220 nm (a) and 270 nm (b) with developed method

Table 1. Data for retention times (t_R), retention factors (k'), separation factors (α) and resolution (Rs) for the investigated pesticides

Compound	t_R (min)	k'	α	Rs
dead time	1.09	–	–	–
2,4-D	1.39	0.27	12.92	29.68
atrazine	4.49	3.49	1.54	19.02
malathion	6.89	5.32	1.05	2.18
fenitrothion	7.20	5.60	1.32	11.54
parathion	9.17	7.41	–	–

In order to be able to perform qualitative and quantitative analysis of the investigated pesticide residues in water samples, their prior concentration was necessary. This occurs as a result of the fact that the calculated values for LOD and LOQ, from the analysis carried out with the standard mixture of the tested pesticides in the lowest concentration area, without concentrating the analytes were greater than 0.1 μg/L, which is equal to MRLs of pesticide residues in water, prescribed by the law in the Republic of Macedonia [11] and with the European Regulation [10].

Before the HPLC analysis, the concentration of the analytes and sample clean-up were carried out by solid-phase extraction using Supelclean ENVI-18 columns.

The development and validation of an analytical method for simultaneous determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples were performed according to the Guidance document on pesticide residue analytical methods [32]. Consequently, specificity, selectivity, linearity, precision expressed as repeatability of retention time and peak area, recovery and limit of quantification (LOQ) for all analytes were tested.

Specificity and selectivity. UV-diode array detection was applied to check the peak purity and analyte peak identity, in order to prove the specificity of the developed method. The purity indexes for all analytes were not less than 999 (the maximum value for the peak purity index (PPI) should be 1000), meaning that no other component influenced the chromatographic peaks of the analytes. Furthermore, the identification of the components of interest was accomplished by comparing the retention times of the analytical standards with those of the same components in the water samples. Additionally, the values of the match factors obtained by overlapping the UV spectra of the pure analytical standard and the absorption spectrum of the same analyte present in water samples were used. Moreover, in accordance with the EU criteria [32], to demonstrate the selectivity of the method, chromatograms of a standard mixture of investigated pesticides with a concentration corresponding to MRL (a), a matrix blank (distilled water) (b) and a sample of distilled water spiked with pesticides with a concentration equal to the MRL for each analyte (c) are presented in Figure 5. It can be seen that by applying the proposed method, the examined components can be determined in water samples after solid-phase extraction.

Linearity. The linearity of the method was determined by the construction of calibration curves which represented the dependence of the concentration of analytes and the obtained response as peak area or peak height.

As can be seen from Table 2, the proposed method was linear for all components of interest (R^2 > 0.99) using the peak areas and peak heights. The calculation of the results was done using the peak areas for each analyte.

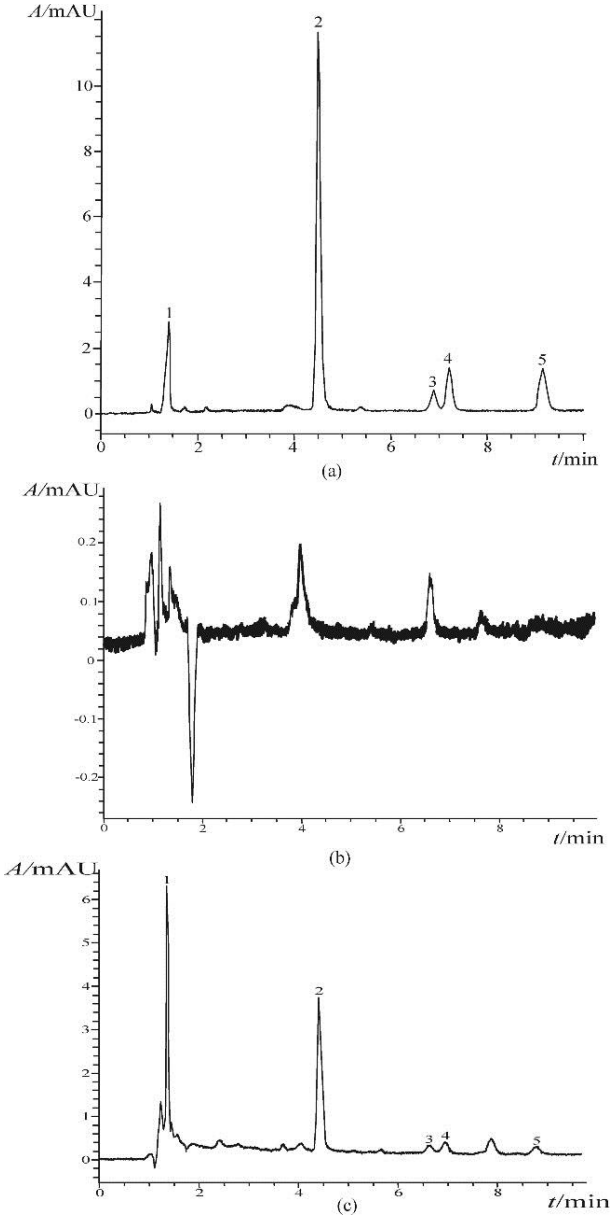

Figure 5. Chromatograms from standard mixture of 2,4-D (1), atrazine (2), malathion (3), fenitrothion (4) and parathion (5) at the concentrations which correspond to MRLs (a), matrix blank (b) and samples of distilled water fortified at the concentration equal to MRL for each analyte (c).

Table 2. Statistical data for linearity of the method

Compound	Linearity range (μg/L)	Regression equation	R^2
2,4-D	0.1 – 0.5	$^1y = 59.1x + 9.542$ $^2y = 8.3008x + 6.0438$	0.9975 0.9919
atrazine	0.1 – 0.5	$^1y = 83.824x + 14.765$ $^2y = 12.901x + 2.2897$	0.9992 0.9998
malathion	0.1 – 0.5	$^1y = 2.4476x + 1.1549$ $^2y = 0.4031x + 0.1056$	0.9990 0.9978
fenitrothion	0.1 – 0.5	$^1y = 14.877x + 0.614$ $^2y = 1.6061x + 0.0819$	0.9974 0.9981
parathion	0.1 – 0.5	$^1y = 17.767x + 0.7515$ $^2y = 1.5217x + 0.0836$	0.9992 0.9992

^1y = peak area, ^2y = peak height

Limit of quantification (LOQ). 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 the European Commission Guidance document on pesticide residue analytical methods [32].

Precision. 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 made. Table 3 shows the precision of the method expressed as the repeatability of the results obtained for the retention time and the peak area for each analyte. From the calculated values for RSD of retention times (0.12–0.25 %) and the peak areas of the analytes (0.61–5.85 %), it is evident that the method was characterized by a satisfactory precision for quantitative determination of the analyzed pesticide residues in water.

Recovery. The obtained results of the recovery of the developed method, as well as the relative standard deviation (RSD) of the recovery for each concentration level, are shown in Table 4. The calculation of the recovery results was done using the peak areas for each of the components.

Table 3. 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	1.34 ± 0.002	0.12	15.95 ± 0.40	2.53
atrazine	4.40 ± 0.007	0.17	22.77 ± 0.14	0.61
malathion	6.61 ± 0.011	0.17	1.40 ± 0.02	1.83
fenitrothion	6.92 ± 0.017	0.25	2.27 ± 0.08	3.43
parathion	8.75 ± 0.019	0.22	2.65 ± 0.15	5.85

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

Compound	Fortification level (μg/L)	Total analyte found (μg/L ± SD)	Recovery (%)	RSD (%)
2,4-D	0.1	0.108 ± 0.007	108.51	6.29
	0.2	0.186 ± 0.013	92.83	7.29
	0.5	0.464 ± 0.065	92.81	13.94
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	0.100 ± 0.010	100.41	10.44
	0.2	0.201 ± 0.013	100.77	6.32
	0.5	0.505 ± 0.014	101.08	2.79
fenitrothion	0.1	0.112 ± 0.005	111.67	4.71
	0.2	0.185 ± 0.009	92.53	4.76
	0.5	0.505 ± 0.003	100.92	0.63
parathion	0.1	0.107 ± 0.009	107.15	8.16
	0.2	0.190 ± 0.010	94.99	5.47
	0.5	0.506 ± 0.010	101.15	2.04

The recovery values for each concentration level (92.53–111.67 %) and the relative standard deviation (RSD ≤ 13.94 %) were within the acceptable values for these parameters according to the EU criteria [32]. They confirm that the method was precise and accurate enough for determining analyzed pesticide residues in water samples.

The developed reversed-phase high-performance liquid chromatography method based on solid-phase extraction was applied for the determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in different water samples (tap water, non-carbonated water and water from Vardar River). Typical chromatograms of the tested water samples are presented in Figure 6. As can be seen from Figure 6, the analyzed samples did not found residues of the investigated pesticides at a concentration corresponding to the MRL or higher.

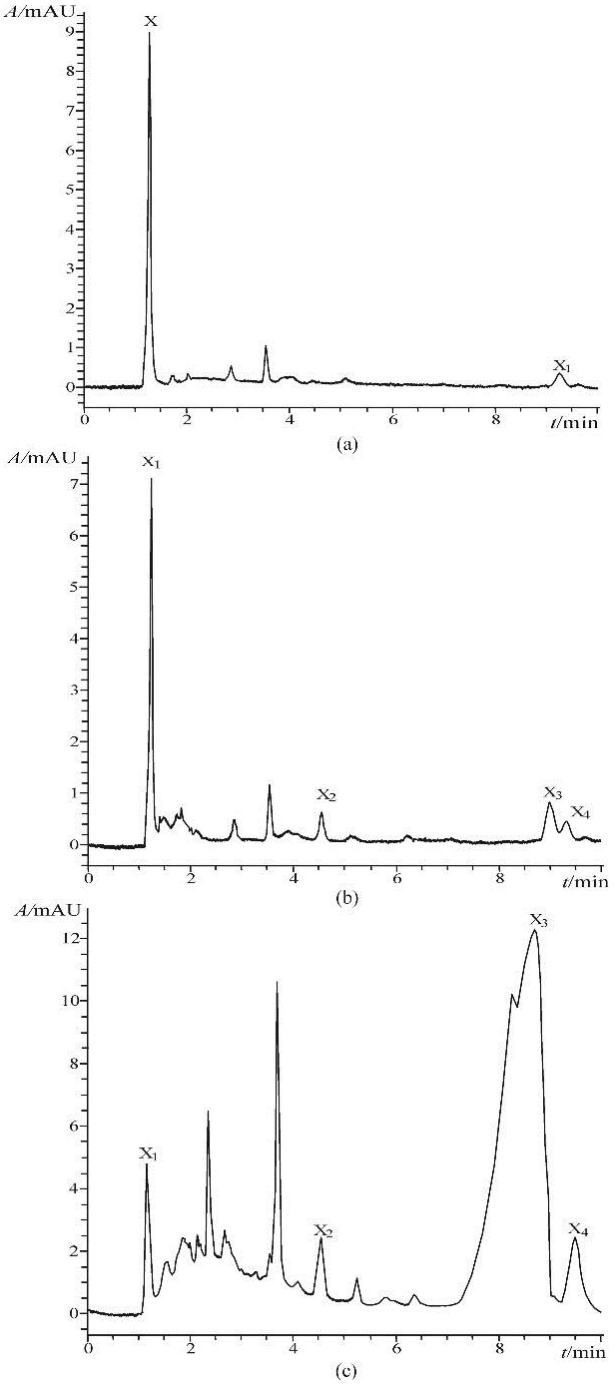

Figure 6. Typical chromatograms of water samples obtained from tap water (a), non-carbonated water purchased at the local market (b) and water from the Vardar River (c) at 220 nm.

On the chromatogram of tap water (Figure 6a) chromatographic peaks with a similar retention time of 2.4-D (1.25 min (X_1)) and parathion (9.27 min (X_2)) can be seen. Chromatographic peaks with retention time similar to the peaks of 2,4-D, atrazine and parathion (1.23 min (X_1), 4.55 min (X_2), 9.01 min (X_3), and 9.33 min (X_4)) also occur in the sample of purchased non-carbonated water (Fig. 6b). In the sample of the Vardar River (Fig. 6c) there are peaks at 1.16 min (X_1), 4.56 min (X_2) and 9.5 min (X_4). Also, on this chromatogram, a broad irregularly formed a chromatographic peak with two peaks (X_3), which starts at about 7.5 min and ends at 9 min, can be observed. This high-intensity peak did not overlap the peaks of malathion, fenitrothion, and parathion. Comparing the UV-spectra of the unknown substances to those of the analytical standards confirms that no residues of pesticides of interest were found in the analyzed water samples.

This paper describes a new possibility for successful determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples using reversed-phase high-performance liquid chromatography (RP-HPLC) method and ultraviolet - diode array detection (UV-DAD). Prior to HPLC analysis, a solid-phase extraction (SPE) was used for analytes concentration and sample clean-up. Specificity, selectivity, linearity, precision, recovery and limit of quantification (LOQ) were examined to assess the validity of the developed method. The method had satisfactory values for all correlation coefficients for calibration curves ($R^2 > 0.99$) and excellent precision for the retention times and peak areas for all examined pesticides. Under the established condition, the recovery of analytes was 92.53–111.67 %, with relative standard deviations below 13.94 %.

The developed method was successfully applied for the determination of selected pesticide residues in tap water, non-carbonated water and water from Vardar River. The obtained results showed that analyzed water samples did not contain detectable residues of investigated pesticides above 0.1 μg/L.

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РАЗРАБОТКА И ВАЛИДАЦИЈА НА RP-HPLC МЕТОД ЗА ОПРЕДЕЛУВАЊЕ НА НЕКОИ ОСТАТОЦИ ОД ПЕСТИЦИДИ ВО ВОДНИ ПРИМЕРОЦИ

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Во овој труд е опишана разработката на нов метод со реверзно-фазна високоефикасна течна хроматографија (RP-HPLC) и ултравиолетов детектор со низа од диоди (UV-DAD) за истовремено определување на остатоци од 2,4-Д, атразин, малатион, фенитротион и паратион во различни водни примероци. Разработениот метод е валидиран во согласност со насоките на Европската комисија за аналитички методи за остатоци од пестициди и добиените резултати за сите тестирани параметри се во границите на прифатливи вредности. Најдобро раздвојување и квантитативно определување на аналитите се постигнати со помош на аналитичката колона LiChrospher 60 RP-select B (250 × 4 mm, 5 μm), при изократско елуирање со мобилна фаза составена од ацетонитрил/вода (60/40, *V/V*), проток од 1 ml/min, константна температура на колоната од 25 °C и UV-детекција на 220 nm и 270 nm. Времето на спроведување на анализата под пропишаните хроматографски услови е околу 10 min.

Клучни зборови: RP-HPLC; UV-DAD; валидација на методот; остатоци од пестициди; водни примероци