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QUANTITATIVE DETERMINATION OF 2,4-D IN PESTICIDES MONOSAN HERBI AND DMA-6

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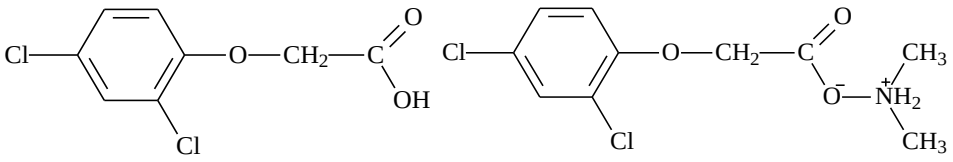
Abstract
A rapid and reliable method for determination of active ingredient 2,4-D ((2,4-dichlorophenoxy)acetic acid) in the pesticide formulations Monosan herbi and DMA-6 is presented. The procedure utilizes high-performance liquid chromatography (HPLC) followed by UV diode array detection and two analytical columns with different stationary phases and dimensions. The better results for identification and quantitation of the active ingredient in two pesticides are achieved using LiChrospher 60 RP-select B (250 x 4 mm, 5 µm) column, UV detection at 220 nm, temperature at 25 ^{0}C, mobile phase consisted of acetonitrile and water (60/40; *V/V*) and flow rate of 1 mL/min. The method is validated by testing linearity, precision, recovery, LOD and LOQ. The values for multiple correlation coefficient ($R^2 > 0.999$), relative standard deviation (RSD) of retention time and peak area (RSD ≤ 1.18 %), recoveries ranged from 98.16 % - 101.38 %, with RSD of 0.10 % - 1.96 %, revealed that the developed method has a good linearity, precision and accuracy. The proposed method is applicable for fast and accurate determination of active ingredient 2,4-D in the pesticides Monosan herbi and DMA-6.
Key words: HPLC-method, UV-detection, 2,4-D, Monosan herbi, DMA-6.

Introduction

2,4-D, (2,4-dichlorophenoxy)acetic acid (IUPAC) belongs to aryloxyalkanoic acid (phenoxy carboxylic) acid group of herbicides (Figure 1a) that is used post-emergence for control of annual and perennial broad-leaved weeds in cereals, maize, sorghum, grassland, established turf, grass seed crops, orchards (pome fruit and stone fruit), cranberries, asparagus, sugar cane, rice, forestry, and non-crop land (Tomlin, 1997).



(a) (b)

Figure 1. Chemical structure of 2,4-D (a) and 2,4-D-dimethyl ammonium salt (b)

2,4-D is an ingredient in approximately 660 agricultural and home use products, as a sole active ingredient and in conjunction with other active ingredients. 2,4-D is formulated primarily as an amine salt in an aqueous solution or as an ester in an emulsifiable concentrate (EC), but, also exists in the form of granular, soluble concentrate/solid, water dispersible granules, and wettable powder (EPA, 2005).

Several products containing 2,4-D as an active substance, including Monosan herbi and DMA-6, which are in the form of a liquid solution concentrate (SL) are registered in R. Macedonia.

The actual CIPAC (Collaborative International Pesticides Analytical Council) handbook (1985) referee method for determination of 2,4-D is by reversed-phase HPLC, using 4-bromophenol as an internal standard and UV detection at 280 nm.

A chromatography is a widely used analytical method for the determination of 2,4-D and its residues in different matrices. For example, for determination of 13 phenoxy acid herbicide residues in soybean is used a gas chromatography with an electron capture detector (Hua*et al*., 2006). Ion chromatography is employed for analysis of some pesticides, including 2,4-D in agrochemicals (Gangal*et al*., 2000). The determination of chlorophenoxy herbicides (2,4-D and related compounds) in biological specimens is performed by HPLC and UV detection at 240 nm (Flanagan and Ruprah, 1989). For determination of 2,4-D in environmental water samples are used liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Lagana*et al*., 2002; Raina*et al*., 2010), HPLC and UV detection (Jafari and Marofi, 2005) or HPLC-UV DAD (Nestorovska-Krsteska *et al*., 2008). Velkoska-Markovska and Petanovska-Ilievska (2013) have been developed RP-HPLC method for quantitative determination of 2,4-D in pesticide formulations by UV-DA detection.

Although, there are analytical methods for determination of 2,4-D in pesticide formulations and other matrices, constantly thinking about their improvement, or to create new analytical methods. For these reasons, the purpose of this paper is to investigate new opportunities for developing a suitable, simple and fast HPLC-method for determination of a content of active ingredient 2,4-D in pesticide formulations Monosan herbi and DMA-6 using reverse-phase liquid chromatography (RP-HPLC) and UV diode array detector (UV-DAD).

Material and methods

Reagents and Chemicals

The Pestanal analytical standard of 2,4-D (98.6 % purity) and HPLC-grade acetonitrile and methanol are purchased by Sigma-Aldrich (Germany). Ultrapure water is produced by TKA Smart2 Pure 12 UV/UF water purification system (Germany).

The pesticide formulation Monosan herbi in form of a soluble concentrate (SL) is procured free of charge from Galenika-fitofarmacija (Serbia). It is declared as containing 464 ± 23.2 g/L of 2,4-D (corresponding to the concentration of 2,4-D-dimethyl ammonium salt of 588 ± 23.2 g/L). The declared value for the density of the pesticide formulation Monosan herbi is 1.15 g/mL which is very close to the experimentally determined value of 1.16 g/mL.

The pesticide formulation DMA-6 (in the form of a soluble concentrate (SL)) contains 67 % of 2,4-D as an active ingredient, in the form of dimethyl ammonium salt, manufactured by "Dow AgroSciences", France.

Equipment

The chromatographic analysis are performed on 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. For better dissolving of the stock solutions an ultrasonic bath "Elma" is used. The investigations are carried out on a Purospher STAR RP-18e (30 mm x 4 mm, 3 μm, Merck) and LiChrospher 60 RP-select B (250 mm x 4 mm, 5 μm, Merck) analytical columns.

Preparation of Standard Solutions

Stock solution of 2,4-D is prepared by dissolving 0.0253 g of the pure analytical standard with acetonitrile in a 25 mL volumetric flask. The prepared solution is ultrasonicated for 15 min, and stored in a refrigerator at 4 ^{o}C. Stock solution is used to prepare a series of 5 working solutions with different analyte concentrations (1.82 μg/mL – 14.59 μg/mL) in 10 mL volumetric flask by dilution with the mixture of acetonitrile/water (50/50, *V/V*).

Preparation of Sample Solutions

Sample solutions of pesticide formulations Monosan herbi and DMA-6 are prepared in 10 mL volumetric flasks by dissolving the weighed amounts of 0.0096 g and 0.0072 g, respectively, in the mixture of equal volumes of acetonitrile and water. The samples are degassed for 15 min in an ultrasonic bath. From each sample solution 0.1 mL is transferred in a 10 mL volumetric flask and dissolved with the mixture of acetonitrile/water (50/50, *V/V*), and four injections are performed with 5 μL each. The sample solutions are clear, therefore filtering is not necessary.

The solutions for recovery experiment are prepared by dissolving 0.1 mL from each sample solution in a 10 mL volumetric flask. In each solution is added a known amount of

analyte (0.91 mg/mL, 1.82 mg/mL and 3.65 mg/mL) and diluted to volume with the same solvent mixture. 5 μL of each of these solutions is injected four times.

Results and discussion

Two analytical columns with different stationary phases and dimensions, such as Purospher STAR RP-18e (30 x 4 mm, 3 μm) and LiChrospher 60 RP-select B (250 x 4 mm, 5 μm), different mixtures of methanol/water (10 – 90 % methanol) and acetonitrile/water (10 – 90 % acetonitrile) as mobile phases, at different column temperature (20 – 30 ^{0}C) are used for identification and quantitation of the active ingredient 2,4-D in two pesticides Monosan herbi and DMA-6.

Under the conditions tested on the Purospher STAR RP-18e column the obtained chromatographic peak of 2,4-D is asymmetric, i.e. with tailing (Figure 2). There are many reasons for tailing phenomenon, such as unsuitable choice of mobile or stationary phases which can be remedied by change the mobile and/or stationary phases (Meyer, 1994). Therefore, the further investigations are performed on LiChrospher 60 RP-select B (250 x 4 mm, 5 μm). LiChrospher 60 RP-select B is a versatile reversed-phase sorbent based on spherical silica particles with excellent properties for the determination of basic, neutral and acidic substances (Chrombook, 2011).

It is found that the optimum separation and symmetrical peak shape of the investigated pesticide is achieved with mobile phase consisted of acetonitrile/water (60/40, *V*/*V*) in isocratic elution with flow rate of 1.0 mL/min and column temperature at 25 ^{0}C (Figure 3a). UV detection is performed at 220 nm. Under these chromatographic conditions, the obtained values for column dead time is 1.09 min and the retention time of 2,4-D is 1.31 min, so the calculated values for the retention factor (*k*') is 0.20.

Specificity, selectivity, linearity, precision expressed as repeatability of retention time and peak area, limit of detection (LOD), limit of quantification (LOQ) and accuracy are tested for the method validation.

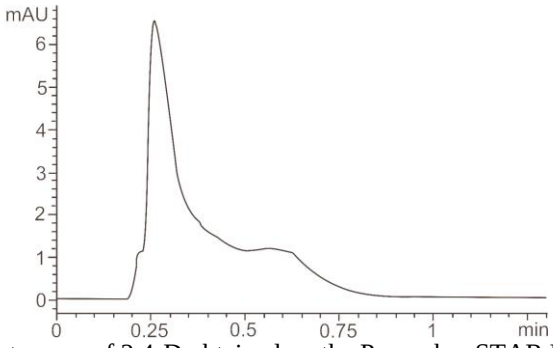
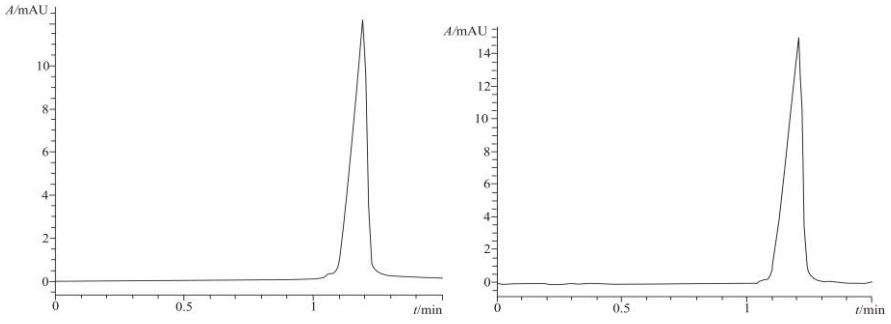
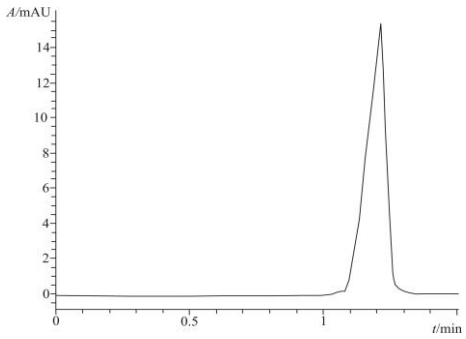


Figure 2. Chromatogram of 2,4-D obtained on the Purospher STAR RP-18e column



(a) (b)



(c)

Figure 3. Chromatograms of 2,4-D obtained from standard solution (a), pesticide formulation Monosan herbi (b) and pesticide formulation DMA-6 (c)

In addition, to confirm the specificity and selectivity of the developed method, the UV diode array detection is used to check the peak purity and analyte peak identity (Jenkie, 1996). The specificity and selectivity of the developed method are estimated by identifying the peak of interest and value for the index of peak purity.

The identification of the analyte is performed by comparing its retention time in the standard solution and the sample and confirmed by overlaid spectra of pure analytical standard of the active substance and the absorption spectra of the same substance in pesticide formulations (Jenkie, 1996).

As can be seen from the chromatograms of the pesticides (Fig. 3b and c) besides the chromatographic peak of the active ingredient there are no other coeluted peaks that interfere on its determination. Moreover, the value of the match factor obtained by overlaid spectra is 999.008 (for Monosan herbi) and 999.293 (for DMA-6), indicating that the peak is of the same substance.

The calibration curve of 2,4-D is obtained with triplicate injections (5 μL each) of working solutions. The area and height of chromatographic peak and the corresponding amount of 2,4-D are used to construct the standard curve using the least-squares method. The curve followed Beer's law in the mentioned range. The obtained results for multiple correlation coefficients ($R^2 \geq 0.9995$) indicated that the method has an excellent linearity. The results are given in Table 1.

Table 1. Results for linearity and sensitivity of the method

	Linearity range (μg/mL)	Regression equation	R^2	LOD (ng/mL)	LOQ (ng/mL)
Area	1,82 - 14,59	y = 3.983x +1.1818	0.9999	2.56	7.68
Height		y = 0.3143x + 5.4309	0.9995		

The limits of detection (LOD) is defined as the amount of analyte for which the signal-to-noise ratio (*S/N*) is 3 whereas the limits of quantification (LOQ*)* is defined as the amount of analyte for which *S/N* =10. The LOD and LOQ are listed in Table 1.

The precision is expressed as repeatability of obtained results (Meyer, 1994; Lough and Wainer, 1996) which is evaluated for retention times and peak areas of the analyte from eight successive injections with concentration 7.30 μg/mL within 3 days (Table 2). The results are tested according to the criteria laid down in CIPAC Document 3807 (2011). The obtained values of RSD for retention times ranged from 0.11 to 0.59 % and from 0.43 to 1.18 % for peak areas indicated a very good precision of the tested method.

Table 2.Statistical data for repeatability

	Intra-day repeatability (n = 8)						Inter-day repeatability (n = 3)	
	I day		II day		III day			
	$\bar{x}$ ± SD	RSD (%)	$\bar{x}$ ± SD	RSD (%)	$\bar{x}$ ± SD	RSD (%)	$\bar{x}$ ± SD	RSD (%)
Retention time	1.31 ± 0.005	0.41	1.30 ± 0.003	0.23	1.31 ± 0.001	0.11	1.31 ± 0.008	0.59
Peak area	146.81±1.74	0.18	146.28 ± 1.12	0.76	146.36 ± 0.62	0.43	146.48 ± 1.27	0.86

The accuracy of the method is confirmed by standard additions (CIPAC, 2011; Snyder *et al.*, 1997). Accuracy of the method is expressed as the deviation between the calculated mean value obtained by examination and the true value of the spiked amounts of the analyte into a sample matrix that already contains some quantity of the analyte (Table 3). As it is shown in Table 3 the obtained values for recovery are within the following ranges (98.16 – 99.68 % for the pesticide Monosan herbi, and 100.58 – 101.38 % for the DMA-6) which are according to CIPAC criteria (CIPAC, 2011). Consequently, it is concluded that the proposed method is accurate enough for determination of active ingredient in the pesticide formulations Monosan herbi and DMA-6.

Table 3. Results from recovery (n = 4)

	m (analyte) before addition (µg)	m (analyte) added (µg)	m (analyte) after addition (µg) (±SD)	Recovery (%)	RSD (%)
Monosan herbi	17.46	4.56	21.70 ± 0.27	98.54	1.25
	17.46	9.12	26.50 ± 0.52	99.68	1.96
	17.46	18.24	35.05 ± 0.35	98.16	0.99
DMA-6	18.70	4.56	23.39 ± 0.28	100.58	1.18
	18.70	9.12	28.20 ± 0.28	101.38	0.98
	18.70	18.24	37.16 ± 0.04	100.61	0.10

The obtained mean concentrations of 2,4-D in the pesticide formulation Monosan herbi are 448.92 g/L (n = 4, RSD = 0.76 %), which is corresponding to the concentration of 2,4-D-dimethyl ammonium salt of 568.89 g/L) and 54.10 % (n = 4, RSD = 0.63 %), which is corresponding to the concentration of 2,4-D-dimethyl ammonium salt of 68.56 %. These values corresponded to the values declared by the manufacturer.

Conclusion

This study shows the new possibility for identification and quantitation of the active ingredient 2,4-D in the pesticides Monosan herbi and DMA-6 by the reversed-phase HPLC-DAD method using LiChrospher 60 RP-select B column (250 x 4 mm, 5 µm). The proposed method showed high value of multiple correlation coefficient for calibration equation and repeatability of retention time and peak area. The developed method is simple, fast, precise and accurate for a routine analysis of active ingredient 2,4-D in the pesticide formulations Monosan herbi and DMA-6 according to CIPAC rules.

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