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DETERMINATION OF MALATHION IN PESTICIDE FORMULATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

SUMMARY

A new, simple, fast and reliable high-performance liquid chromatography (HPLC) method for determination of an active ingredient malathion in the pesticide formulation has been developed. Successful separation and quantification of malathion were achieved using Purospher STAR RP-18e (30 x 4 mm, 3 μm) and 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 specificity, selectivity, linearity, precision, accuracy, limit of detection (LOD) and quantification (LOQ) were tested for the method validation according to the CIPAC and SANCO guidelines. The obtained values for multiple correlation coefficients (R2 > 0.99), relative standard deviation (RSD) of retention times and peak areas (RSD ≤ 0.64%), and recoveries ranged from 101.04 to 101.84 %, revealed that the developed method has an excellent linearity, precision of retention time and peak area and accuracy. The proposed method was successfully applied for determination of an active ingredient malathion in the emulsifiable concentrate (EC) "Etiol techni" for a run time of 4 min.

Keywords: HPLC method, malathion, pesticide formulation, emulsifiable concentrate.

INTRODUCTION

Malathion is a broad-spectrum, non-systemic, organophosphorus insecticide and acaricide with contact, stomach and respiratory action. Products containing malathion are used to control a wide variety of insects in agriculture, as well in mosquito control, fly eradication and for treating lice (Gervais et al. 2020, Malathion 2020, Tomlin 1997).

Malathion is one of the most widely used insecticides in our country and in many other countries around the world. It has been approved for use according to European Commission Regulation (EC) No 1107/2009 (2009) and EPA (2009), etc. As an active substance, malathion could be found in many pesticide

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formulations, such as “Etiol techni”, which is in the form of emulsifiable concentrate (EC).

New pesticide formulations are being synthetized every day, and hence the need to control their quality is growing up. It is essential to enhance the quality of pesticide formulations placed on the market, thereby reducing inefficient control of pests, crop losses and risks to human health and the environment. Consequently, the simple, fast, precise and accurate analytical methods for determination of active substances in pesticide formulations are crucial to control the quality of pesticide formulations.

There are many analytical methods for determining malathion in different matrices and chromatographic methods are the most used, such as liquid chromatography (LC) (Torosyan et al. 2018a,b, Kara and Ince 2016), high-performance liquid chromatography (HPLC) (Hadjmohammadi et al. 2013, Ramin et al. 2019), high-performance thin layer chromatography (HPTLC) (Shayeghi et al. 2007), gas chromatography (GC) (Rezaee et al. 2019, Lofty et al. 2013, Khani et al. 2011, Bezerra et al. 2010, Singh and Dogra 2009) and gas-liquid chromatography (GLC) (Tomlin, 1997). Moreover, senzors (Cao et al. 2019) and spectrophotometric methods (Venugopal et al. 2013, Venugopal et al. 2012) are also used. Hardly a few methods for determination of malathion in pesticide formulations are known, among which Fourier transform infrared spectrometry method (Khanmohammadi et al. 2007). Furthermore, CIPAC (Collaborative International Pesticides Analytical Council) has been published reference methods for the determination of an active substance malathion in different pesticide formulations using gas-liquid chromatography (GLC) (CIPAC 1983) and gas chromatography (GC) (CIPAC 2003). Additionally, in the previous work, rapid resolution liquid chromatography (RRLC) method with ultraviolet diode-array detection (UV-DAD) for the determination of malathion in pesticide formulation has been described (Velkoska-Markovska and Petanovska-Ilievska 2019).

Moreover, the new analytical methods are always welcome to control the quality of pesticide formulations. Therefore, the aim of this work was to investigate the new possibilities for developing a simple, fast, precise and accurate high-performance liquid chromatography (HPLC) method for the determination of malathion as an active substance in pesticide formulation in the form of emulsifiable concentrate (EC) using ultraviolet-diode array detection (UV-DAD).

MATERIAL AND METHODS

Reagents and Chemicals

HPLC-grade acetonitrile and water, as well as, the Pestanal analytical standard of malathion (97.2% purity) were purchased by Sigma-Aldrich (Germany). The pesticide formulation “Etiol techni” was produced by “Galenika-fitofarmacija” (Belgrade, Serbia). It was in the form of an emulsifiable concentrate (EC) and declared values for the concentration and density were 600 g/L ± 25 g/L and 1.075 g/mL, respectively.

Equipment

The HPLC analyses were accomplished on an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography (RRLC) system equipped with: vacuum degasser (G1322A), binary pump (G1312B), autosampler (G1329B), a column compartment (G1316A), UV-VIS diode array detector (G1316B) and ChemStation software. The investigations were performed on a Purospher STAR RP-18e (30 x 4 mm, 3 μm) analytical column, produced by Merck (Germany). For the better dissolving of the stock and sample solutions an ultrasonic bath "Elma" was used.

Preparation of Standard Solutions

Stock solution of malathion was prepared by dissolving 0.0330 g of the pure analytical standard with acetonitrile in a 25 mL volumetric flask. The prepared stock solution was ultrasonicated for 15 minutes in an ultrasonic bath to achieve complete dissolution of the active component. The stock solution was stored in a refrigerator at 4°C. Under these conditions the stability of the active component was greater than one month.

Stock solution was used to prepare working standard solutions with different concentrations, by diluting the appropriate volume of stock solution with a mixture of acetonitrile and water (50/50, *V/V*) in 10 mL flasks.

A series of 5 working standard solutions with a concentration of 46.75, 93.50, 187.00, 280.50 and 374.00 μg/mL were prepared in order to test the linearity of the method. 5 μL of each of these working solutions was injected in triplicate.

A series of 8 working standard solutions in a concentration range of 66.74 ng/mL - 2672.5 ng/mL were prepared to determine the limit of detection (LOD) and the limit of quantification (LOQ). Each of these working solutions was injected three times with a volume of 5 μL.

Preparation of Sample Solution

Sample solution of pesticide formulation "Etiol techni" was prepared in a 10 mL volumetric flask by dissolving the weighed amounts of 0.0333 g in mixture of acetonitrile and water (50/50, V/V). The sample solution was degassed for 15 min in an ultrasonic bath. Afterwards, 0.5 mL from sample solution was transferred to a 10 mL volumetric flask and dissolved with a mixture of equal volumes of acetonitrile and water. The sample solution was completely dissolved in the solvents used and therefore there was no need to filter. Four injections were performed with a volume of 5 μL of this solution.

The recovery of the method was determinated by dissolving 0.5 mL from sample solution in three 10 mL volumetric flasks. In each solution was added a known amount of analytical standard of malathion: 23.37, 46.75 and 93.49 μg/mL. Then the flasks were supplemented to the mark with a mixture of acetonitrile and water (50/50, V/V). Four injections were performed with 5 μL of each of these solutions.

RESULTS AND DISCUSSION

The chromatographic studies were carried out using a short analytical column of type Purospher STAR RP-18e (endcapped), based on high purity, metal-free silica gel with polymeric C18 modification and endcapping. These columns

enable high efficient separations of base, neutral, acidic or chelating compounds using simple mobile phases, producing excellent peak symmetry. Thanks to remarkable stability over a wide range of pH values from 1.5 to 10.5 these columns allow the separation of complex samples using different mobile phases under different temperature conditions (ChromBook 2011).

Malathion (Fig. 1) is a generally accepted name according to ISO (International Organization for Standardization), while according to IUPAC (International Union of Pure and Applied Chemistry) the name of this active component is diethyl (dimethoxythiophosphorylthio)succinate; S-1,2-bis(ethoxycarbonyl)ethyl O, O-dimethyl phosphorodithioate) (Tomlin 1997).

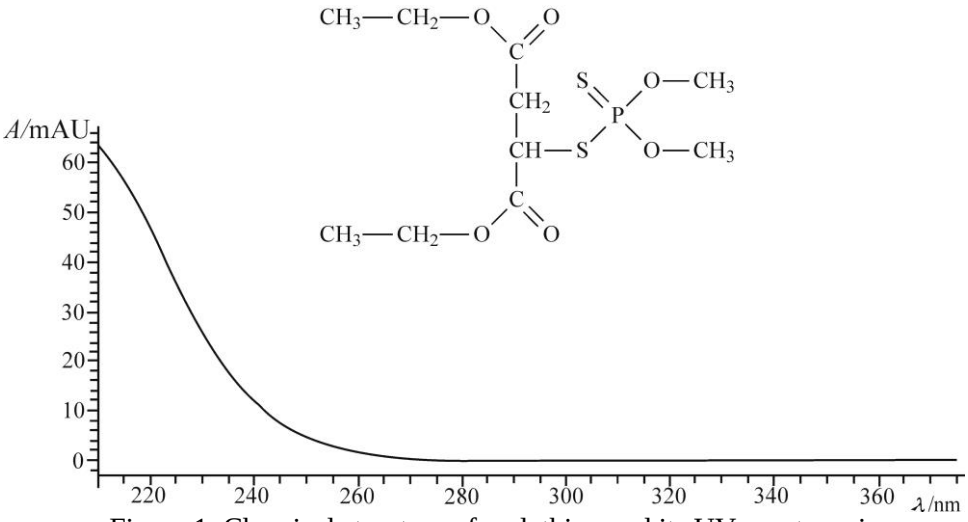

Figure 1. Chemical structure of malathion and its UV spectrum in acetonitrile/water (50/50, V/V)

On the basis of the UV spectrum of malathion recorded in acetonitrile and water solution (50/50, V/V) the wavelength was determined on which the chromatographic analyses were performed. As can be seen from the UV spectrum of malathion (Fig. 1), no maximum absorption was observed, but it was noticed that the absorption increases with decreasing wavelength. Therefore, the chromatographic analysis for the determination of malathion in the pesticide formulation was performed at 220 nm.

In order to obtain the optimum conditions for the determination of malathion in pesticide formulation, a series of preliminary tests have been performed by varying the volume ratio of acetonitrile to water in the mobile phase. In order to obtain a simple chromatographic method, isocratic elution was used. Studies have shown that the best conditions for determining malathion were obtained by using a mobile phase consisting of acetonitrile and water at a volume ratio (47/53, V/V) (Fig. 2), a flow rate of 1 mL/min, constant column temperature of 25°C and UV detection at 220 nm. At these chromatographic conditions, the dead time (t0) was

0.23 min and the malathion retention time (tR) was 2.58 min. Consequently, the calculated value for the retention factor (k') was 12.58, meaning less than 20, which is the maximum acceptable value for this parameter according to some authors (Dong 2006). At such defined chromatographic conditions of operation, a smooth base line and good peak shape of malathion were obtained. The value for malathion peak purity index was satisfactory (> 999). The time required for this analysis was approximately 4 min.

Figure 2b shows the chromatogram of the pesticide product "Etiol techni" obtained by the elaborate method. As can be seen from Figure 2b, the chromatogram of the pesticide formulation shows the presence of unknown components (X_1 and X_2) that eluted slower than malathion, with a retention time of 2.88 min and 3.20 min. Their chromatographic peaks were satisfying good separated to the baseline, and also from the malathion peak. That was confirmed by the calculated resolution values (RS = 2.56) and the separation factor (α = 1.14) of the chromatographic peak of malathion and its neighbouring peak (X1).

Specificity, selectivity, linearity, precision expressed as intra-day and inter-day repeatability of retention time and peak area, and accuracy were tested for the method validation according to CIPAC (2003) and SANCO rules (European Commission, 2019).

In addition, to confirm the specificity and selectivity of the proposed method, UV-diode array detection was used to check the peak purity and analyte peak identity. The purity index of malathion 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. Furthermore, the identification of malathion in the pesticide formulation "Etiol techni" was performed by comparing the retention time of the analyte from the standard solution and from the sample solution, and confirmed by overlaying the absorption spectra of the pure analytical standard of malathion and the absorption spectra of the malathion in the pesticide formulation sample. The match factor value obtained by overlaid spectra was 999,918, which indicates that the peak was of the same substance.

A calibration curves were constructed to determine the linearity of the method, by plotting the injected amount of the standard of active ingredient as a function of the peak area and height, obtained by triplicate injection of 5 working solutions. The curves followed Beer's law in the concentration range from 46.75 μg/mL to 374.00 μg/mL (Table 1). Thereby, the maximum possible value of the multiple correlation coefficient (R^2) was obtained when the peak area was taken as the dependent variable (R^2=1), while the value of the multiple correlation coefficient when the peak height was taken as the dependent variable was significantly lower (R^2 = 0.9973). For these reasons, it was preferable that the calculations for the content of the active substance malathion in the pesticide formulation "Etiol techni" be performed according to the peak area. The results revealed excellent linearity of the proposed method.

Although, the limit of detection (LOD) and limit of quantification (LOQ) are not required according to CIPAC and SANCO guidelines, however, they were also determined.

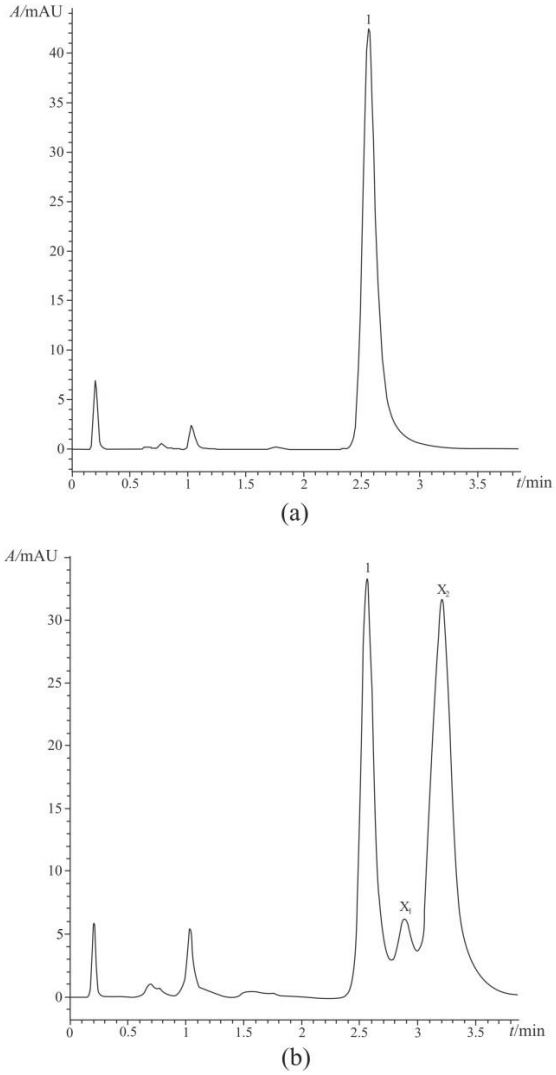

Figure 2. Chromatograms obtained from analytical standard of malathion (a) and pesticide formulation "Etiol techni" (b) on the Purospher STAR RP-18e (30 x 4 mm, 3 μm) at 220 nm using the proposed method

Although the new proposed chromatographic process was slightly longer (about 1.5 min), it could still be considered a fast and economic chromatographic method that requires insignificant higher consumption of organic solvent compared

to previously published results (Velkoska-Markovska and Petanovska-Ilievska 2020).

Figure 2b shows the chromatogram of the pesticide product "Etiol techni" obtained by the elaborate method. As can be seen from Figure 2b, the chromatogram of the pesticide formulation shows the presence of unknown components (X1 and X2) that eluted slower than malathion, with a retention time of 2.88 min and 3.20 min. Their chromatographic peaks were satisfying good separated to the baseline, and also from the malathion peak. That was confirmed by the calculated resolution values (RS = 2.56) and the separation factor ($\alpha = 1.14$) of the chromatographic peak of malathion and its neighbouring peak (X1).

Specificity, selectivity, linearity, precision expressed as intra-day and inter-day repeatability of retention time and peak area, and accuracy were tested for the method validation according to CIPAC (2003) and SANCO rules (European Commission, 2019).

In addition, to confirm the specificity and selectivity of the proposed method, UV-diode array detection was used to check the peak purity and analyte peak identity. The purity index of malathion 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. Furthermore, the identification of malathion in the pesticide formulation "Etiol techni" was performed by comparing the retention time of the analyte from the standard solution and from the sample solution, and confirmed by overlaying the absorption spectra of the pure analytical standard of malathion and the absorption spectra of the malathion in the pesticide formulation sample. The match factor value obtained by overlaid spectra was 999,918, which indicates that the peak was of the same substance.

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Although, the limit of detection (LOD) and limit of quantification (LOQ) are not required according to CIPAC and SANCO guidelines, however, they were also determined.

The limit of detection (LOD) and the limit of quantification (LOQ) for malathion was determined by construction of a calibration curves in the low concentration region at 8 concentration levels (Table 1). The limit of detection (LOD) was calculated as three times the ratio between the SD and the slope of the low concentration curve (LOD = 3·SD/slope), and the limit of quantification (LOQ) as ten times the same ratio (LOQ = 10·SD/slope) (Miller and Miller 1993). The obtained values for LOD and LOQ are listed in Table 1.

Table 1. Statistical data for linearity, LOD and LOQ

Linearity range	Regression equation	R^2	SD	LOD (mg/L)	LOQ (mg/L)
46.75 - 374.00 µg/mL	[1]y = 551.1x + 0.0975 [2]y = 52.675x + 7.2254	1 0.9973	/	/	/
66.74 - 2672.5 ng/mL	[1]y = 2.2254x – 0.2396 [2]y = 0.2401x + 0.0207	0.9991 0.9992	10.3179 1.1113	3.06	9.26
[1]Area. [2]Height.					

The precision was expressed as day-to-day (n = 3) and within-day (n = 8) repeatability of retention time and peak area of malathion. For that purpose, eight successive injections of analytical standard of malathion with concentration 187.00 µg/mL, within 3 days (Table 2) were carried out. According to CIPAC and SANCO criteria, acceptable values for RSD were based on the modified Horwitz equation and they should not exceed 1.46 %. The RSD values obtained for the retention time (RSD = 0.08 – 0.30 %) and peak area (RSD = 0.08 – 0.64 %) of malathion were within acceptable limits. The results show that the proposed method was characterized by high precision of retention time and peak area.

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 (min)	2.58 ± 0.002	0.08	2.58 ± 0.004	0.15	2.57 ± 0.008	0.30	2.58 ± 0.006	0.22
Peak area	508.56 ± 1.08	0.21	515.77 ± 0.39	0.08	514.90 ± 0.73	0.14	513.08 ± 3.31	0.64

Compared to the previously published results (Velkoska-Markovska and Petanovska-Ilievska 2020), the precision of the proposed method was better. The accuracy of the method was confirmed by standard additions (CIPAC 2003, SANCO 2019). Accuracy of the method was 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. The calculated values for the recovery were ranged from 101.04 to 101.84 % (Table 3). These values were within the acceptable values for the recovery according to the CIPAC (2003) and SANCO (2019) criteria, which should range from 98 to 102 %. Hence, it was concluded that the proposed method is accurate enough for determination of active ingredient malathion in the pesticide formulation "Etiol techni".

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

Mass of analyte (μg)	Pure analyte added (μg)	Total analyte found (μg) (±SD)	Recovery (%)	SD (%)
0.48	0.12	0.61 ± 0.003	101.54	0.51
0.48	0.23	0.73 ± 0.0007	101.84	0.09
0.48	0.47	0.96 ± 0.0009	101.04	0.09

The proposed method was applied for the quantitative determination of the active component malathion in the pesticide product "Etiol techni". The obtained mean concentration of malathion was 616.55 g/L (n = 4, RSD = 0.20 %), which corresponded to the value declared by the producer. The experimentally obtained value for the density of the pesticide product was 1.069 g/mL.

CONCLUSIONS

This paper presents the new possibility for determination of an active ingredient malathion in the pesticide formulation "Etiol techni" using high-performance liquid chromatography method and ultraviolet - diode array detection. The short analytical column of type Purospher STAR RP-18e (30 x 4 mm, 3 μm) was used for identification and quantification of malathion. An 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 was applied. The method validation was realized according to CIPAC and SANCO rules and showed that the developed method has an excellent linearity, precision of retention time and peak area, and accuracy. The obtained values for recoveries ranged from 101.04 to 101.84 %, with RSD of 0.09 – 0. 51 %, revealed that the proposed method is suitable for routine determination of malathion in the pesticide formulation in the form of emulsifiable concentrate for a run time of 4 min.

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