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Determination of malathion and its residues by normal-phase high-performance liquid chromatography method

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ABSTRACT

The quality of pesticide formulations has an impact on the crop safety, environment and human health. Therefore, the development of new analytical methods for the determination of active substances in pesticide formulations in order to control their quality, as well as, their residues in food samples in order to ensure food safety, is always welcome. A new, simple, precise and accurate normal-phase high-performance liquid chromatography (NP-HPLC) method for determination of an active ingredient malathion in the commercial emulsifiable concentrate pesticide product has been developed and validated. The analysis was carried out on a LiChrosorb CN (250 x 4 mm, 5 µm) analytical column using isocratic elution with mobile phase consisted of *n*-hexane and dichloromethane (80/20, *v/v*), flow rate of 1 mL/min, constant column temperature at 25 °C and ultraviolet diode-array detection at 220 nm. The obtained values for multiple correlation coefficients ($R^2 \geq 0.9990$), relative standard deviation of retention times, peak areas and heights (RSD ≤ 1.14%), recoveries ranged from 98.97 to 101.62%, revealed that the developed method has a satisfactory linearity, precision and accuracy. Also, the developed method was successfully applied for determination of malathion residues in apple juice samples, after preliminary sample preparation using solid-phase extraction. Specificity, selectivity, linearity, matrix effect, precision and accuracy were tested in order to validation of this method. The obtained results were in acceptable ranges and indicated that the developed method is suitable for routine determination of malathion in the pesticide formulation, as well as for determination of malathion residues in apple juice samples. The run time of HPLC analysis was about 6 min.

KEYWORDS

NP-HPLC method, malathion, residues, emulsifiable concentrate, apple juice

INTRODUCTION

Recent research has focused on the synthesis of new organophosphorus insecticides that are less toxic to humans and at the same time more effective in destroying many insects. The physico-chemical and biological properties of organophosphorus compounds allow their widespread use in agriculture to suppress various types of harmful insects that attack plants, as well as other harmful organisms that attack domestic animals and humans. Although not very toxic, their improper use can cause their presence in agricultural products and the environment. Furthermore, through primary agricultural products (e.g., fruits and vegetables) they can also be found in processed products for human consumption, such as fruit juices which are widely used, especially by children.

Among the most widely used organophosphorus insecticides in our country, as in almost all countries in the world, is malathion. Its use is approved in the USA, European Union countries and many other countries according to the EPA [1], European Commission Regulation (EC) No 1107/2009 [2] and others. Malathion is a broad-spectrum non-systemic

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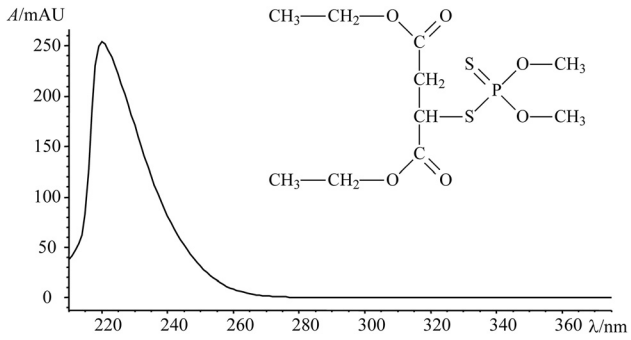

Fig. 1. Chemical structure of malathion and its UV spectrum in *n*-hexane/dichloromethane (80/20, *v/v*)

insecticide and acaricide with contact, stomach and respiratory action. Also, it is an acetylcholinesterase (AChE) inhibitor [3, 4]. The IUPAC (International Union of Pure and Applied Chemistry) name of malathion (Fig. 1) is diethyl (dimethoxythiophosphorylthio)succinate; S-1,2-bis(ethoxycarbonyl)ethyl O, O-dimethyl phosphorodithioate. It is a chiral molecule existing as two enantiomers. Malathion is moderately soluble in water and readily soluble in many organic solvents [3, 4]. It is an active substance in many plant protection products among which in the emulsifiable concentrate (EC) pesticide named "Etiol techni".

Knowledge of the potential hazards of uncontrolled use of many pesticides imposes the need of constant quality control of pesticides offered on the market. The pesticide quality is responsible not only for the crop safety, but also for the safety of the environment and human health as well. Hence, the permanent quality control of plant protection products, as well as control of pesticide residues in food, is of great importance. In order to do so, it requires simple, fast, precise and accurate analytical methods for the determination of active substances in pesticide formulations, as well as determination of pesticide residues in food samples.

A lot of different analytical methods for determination of malathion in various matrices have been published. So, for example, the most commonly used are chromatographic methods, such as gas chromatography (GC) [5–8] liquid chromatography (LC) [9, 10], high-performance liquid chromatography (HPLC) with UV-detection [11, 12] and gas-liquid chromatography (GLC) [3]. Furthermore, spectrophotometric methods [13–15], colorimetric methods [16] or sensors [17, 18] are also used. Only a few publications for determination of malathion in pesticide formulations are known, among which Fourier transform infrared spectrometry method [19]. The reference methods for the determination of active substance malathion in different pesticide formulations using gas-liquid chromatography (GLC) [20] and gas chromatography (GC) [21] have been published by CIPAC (Collaborative International Pesticides Analytical Council). In the previous work, reversed-phase Rapid Resolution Liquid Chromatography (RP-RRLC) method with ultraviolet diode-array detection (UV-DAD) for the determination of malathion in pesticide formulation has been developed and validated [22].

In spite of the fact that the reversed-phase HPLC has greater application, for substances that are much more soluble in nonpolar organic solvents than in water, and for substances that are chiral isomers, the use of normal-phase chromatography has advantages. On the other hand, there is always a need for developing new analytical methods for the determination of active substances in pesticide formulations to control their quality, as well as for the determination of pesticide residues in food samples to provide the food safety and human health protection. Therefore, the aim of this paper was to develop a new, simple and suitable method for the determination of malathion in emulsifiable concentrate pesticide formulation using normal-phase high-performance liquid chromatography (NP-HPLC) method and ultraviolet diode-array detection (UV-DAD). Furthermore, because malathion is often used as an insecticide and acaricide in apple orchards, it is clear that malathion residues can be found in apple fruit, and then pass into the apple juice produced. Hence, we set another goal of the paper, to check whether the developed normal-phase liquid chromatography method for determination of malathion can be applied to the determination of malathion residues in apple juice samples. The choice of apple juice is made because it is one of the most consumed products from the smallest to the oldest population group.

EXPERIMENTAL

Reagents and Chemicals. The Pestanal analytical standard of malathion (97.2% purity) was purchased by Sigma-Aldrich (Germany). HPLC-grade *n*-hexane and dichloromethane were manufactured by Merck (Germany). The pesticide formulation "Etiol techni", which was in the form of an emulsifiable concentrate (EC) was produced by "Galenika-fitofarmacija" (Belgrade, Serbia). It was declared as containing 600 g/L ± 25 g/L of malathion and 1.075 g/mL for density. For the analysis of malathion residues in apple juice, 100% apple juice samples from three different manufacturers (A, B, C) were purchased from local supermarkets.

Equipment. The HPLC analyses were carried out 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 LiChrosorb CN (250 × 4 mm, 5 µm) analytical column, produced by Merck (Germany). For the better dissolving of the stock and sample solutions an ultrasonic bath "Elma" was used. For the solid-phase extraction (SPE) was used a vacuum manifold Visiprep (Supelco) and for vortexing of samples was used IKA Vortex Genius 3 (Germany).

Preparation of Standard Solutions. Stock solution of malathion was prepared by dissolving 0.0223 g of the pure analytical standard with *n*-hexane in a 25 mL volumetric flask. The prepared stock solution was ultrasonicated for 15 min in an ultrasonic bath to achieve complete dissolution

of the active component and stored in a refrigerator at 4 °C. Under these conditions, the stock solution was stable for more than 15 days. The stock solution was used to prepare working standard solutions with different concentrations.

A series of 7 working standard solutions with different concentration (34.60, 51.90, 86.51, 173.02, 346.03, 519.05 and 692.06 µg/mL) were prepared for the linearity estimation of the method 1. These solutions were prepared by transferring portions of a specified volume of the stock solution into 10 mL measuring flasks, supplemented with the mark with a mixture of *n*-hexane and dichloromethane (80/20, *v/v*). Each working solution was injected three times with a volume of 10 µL.

Preparation of pesticide formulation sample solution

For determination of the precision (repeatability) of the proposed method, five independently sample solutions (1) were prepared in a 10 mL volumetric flasks by dissolving the weighed amounts of 0.0034 g of pesticide formulation "Etiol techni" in mixture of *n*-hexane and dichloromethane (80/20, *v/v*). This way prepared samples solutions were degassed for 15 min in an ultrasonic bath, after which they were completely dissolved in the solvents used, so the further sample filtering was no need. Each sample solution was injected three times with volume of 10 µL.

For determination of the content of an active substance malathion in the pesticide formulation, a sample solution (2) of pesticide formulation "Etiol techni" was prepared in a 10 mL volumetric flask by dissolving the weighed amounts of 0.0682 g in mixture of *n*-hexane and dichloromethane (80/20, *v/v*). The sample solution was degassed for 15 min in an ultrasonic bath. Subsequently, 0.5 mL from sample solution was transferred to a 10 mL volumetric flask and dissolved with a mixture of *n*-hexane and dichloromethane (80/20, *v/v*). Four injections ($n = 4$) were performed with a volume of 10 µL of this solution.

The recovery of the method was determinated by dissolving 0.5 mL from sample solution (2) in three 10 mL volumetric flasks. In each solution was added a known amount of analytical standard of malathion: 47.90, 95.80 and 191.60 µg/mL. Then the flasks were supplemented to the mark with a mixture of *n*-hexane and dichloromethane (80/20, *v/v*). Four injections were performed with 10 µL of each of these solutions.

Preparation of apple juice samples for malathion residues analysis

For validation of the method for determination of malathion residues in 100% apple juice samples, specificity, selectivity, linearity, precision expressed as repeatability of the retention time, peak area and peak height, and accuracy were tested. For this purpose, 1 kg of 100% apple juice samples were prepared, by fortified with 0.014, 0.020 and 0.024 mg/kg of malathion. Unspiked samples were used for blanks. 5 samples ($n = 5$) were prepared for each concentration level. Samples were then subjected to solid-phase extraction using Supelclean ENVI-18 columns (6 mL, 0.5 g, Supelco). The solid-phase extraction procedure consists of the following steps: conditioning the columns, sample passing, column washing, column drying, and elution of malathion residues. Conditioning of the SPE columns was performed by 5 mL of acetonitrile, followed by 5 mL of water at a flow rate of 2 mL/min. Before the SPE, the prepared apple juice samples were filtered through a 0.45 µm Nitrocellulose membrane filters. After that, 1 kg of filtered apple juice samples were passed through the cartridges at a flow rate of 8–10 mL/min. Then, the columns were rinsed of any residual amount of the sample, as well as of the interfering substances present in the sample, with 5 mL of water. The columns were then vacuum dried for 20 min. Subsequently, elution of the malathion residues was performed with two portions of 2 mL of acetonitrile each. The eluates were evaporated to dryness under the gentle stream of nitrogen at 40 °C. Then, the dry residue was dissolved in 1 mL of mixture of *n*-hexane and dichloromethane (80/20, *v/v*) by vortexing for 1 min. Prior to HPLC analysis, the final extracts were filtered through a 0.45 µm Iso-Disc PTFE syringe filters and transferred into vials for HPLC analysis. Each sample was injected three times with volume of 20 µL.

Matrix effect evaluation. The matrix effect estimation was performed by comparing the peak areas from standard solutions ($n = 5$) of the malathion in solvent (*n*-hexane/dichloromethane, (80/20, *v/v*)) with the peak areas obtained from standard solutions of the malathion prepared in blank apple juice extract, at concentration of 0.02 mg/kg for malathion. For calculation of the matrix effect (ME) the following equation [23] was used:

$$ME(\%) = (X_2 - X_1)/X_1 * 100 \quad (1)$$

where X_1 = average area of the malathion standard in solvent (*n*-hexane/dichloromethane, (80/20, *v/v*)), at 0.02 mg/kg malathion; X_2 = average area of the malathion standard in blank apple juice extract, at the same concentration. Utilizing this formula, it was possible to estimate the positive or negative matrix effect, which is an increase or decrease of the detector response.

RESULTS AND DISCUSSION

Chromatography study

Typical stationary phases in normal-phase chromatography include bare silica as well as cyano, diol, and amino bonded phases, and the most used constituent of mobile phase is the non-polar organic solvent, such as hexane [24].

The chromatographic studies were carried out using a LiChrosorb CN (250 x 4 mm, 5 µm) analytical column, made of totally porous, irregular silica particles with a cyano derivative. It is a highly reliable and versatile sorbent that offers both polar and hydrophobic properties, making it suitable for polar or weakly hydrophobic interactions in normal-phase or reversed-phase HPLC. Also, this sorbent is suitable for selective charged interactions [25].

On the basis of the UV spectrum of malathion recorded in mixture of *n*-hexane and dichloromethane (80/20, *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), maximum absorption was observed at 220 nm. Therefore, the chromatographic analyses for the determination of malathion were 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 *n*-hexane and dichloromethane in the mobile phase. 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 *n*-hexane and dichloromethane at a volume ratio (80/20, *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 (t_0) was 2.68 min and the malathion retention time (t_R) was 5.49 min. Dead time is the time required to elute a substance that is not retained in the column. This is usually determined by the first peak that appears in the chromatogram. In this study, it was the time of the negative peak which was about 2.68 min. Consequently, the calculated value for the retention factor (k') was 1.05, a value that belongs to the range of optimal values for this parameter [26]. At such defined chromatographic conditions of operation, a smooth baseline and good peak shape of malathion were obtained. The time required for this analysis was approximately 6 min.

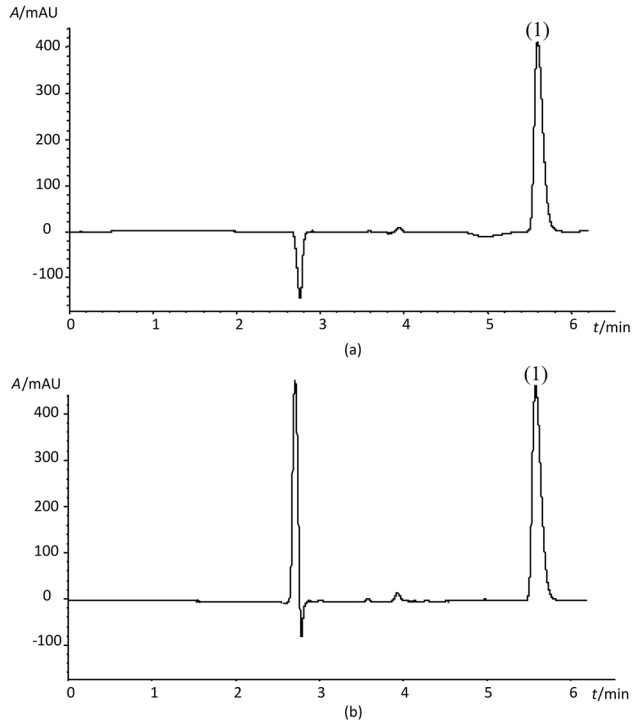

Fig. 2. Chromatograms obtained from analytical standard of malathion (1), (a) and pesticide formulation "Etiol techni" (b) on the LiChrosorb CN (250 x 4 mm, 5 µm) column with mobile phase consisted of *n*-hexane and dichloromethane (80/20, *v/v*), flow rate of 1 mL/min, constant column temperature at 25 °C and UV detection at 220 nm

Figure 2b shows the chromatogram of the pesticide product "Etiol techni" obtained by the elaborate method. As can be seen from Fig. 2b, the chromatogram of the pesticide formulation shows no presence of other components except the peak of the active substance malathion (1).

Method 1 validation for determination of malathion in pesticide formulation

Specificity, selectivity, linearity, precision expressed as intra-day repeatability of retention time, peak area and peak height and accuracy were tested for the method 1 validation in accordance with the CIPAC and SANCO rules [27, 28].

Specificity and Selectivity. In addition, to confirm the specificity and selectivity of the proposed method 1, UV-diode-array detection was used to check the peak purity and analyte peak identity. The purity index of malathion was greater than 998 (the maximum value for the peak purity index (PPI) should be 1,000), 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 (Fig. 3). The match factor value obtained by overlaid spectra was 998.110, which indicates that the peak was of the same substance.

Linearity. Calibration curves were constructed to determine the linearity of the method 1, 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 7 working solutions. The curves followed Beer's law in the concentration range from 34.60 to 692.06 µg/mL (Table 1). The values obtained for the multiple correlation coefficients (R^2) were 0.9999 when the peak area was taken as dependent variable, and 0.9990 when the peak height was taken as dependent variable. For these reasons, it was preferable that the calculations for the content of the active substance

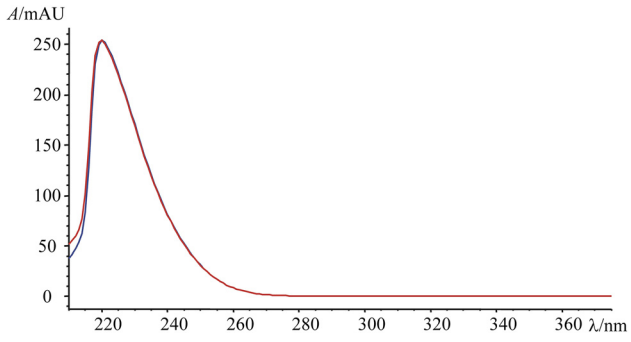

Fig. 3. The overlaid UV spectra obtained by comparing the absorption spectra of a pure analytical standard of malathion and absorption spectra of the same analyte in the pesticide product

Table 1. Statistical data for linearity of the method 1 and method 2

	Linearity range	Regression equation	R^2
Method 1	34.60–692.06 (μg/mL)	*y = 506.87x + 4.4722	0.9999
		**y = 68.359x + 8.1282	0.9990
Method 2	0.014–0.024 (mg/kg)	*y = 18,401x - 174.57	0.9986
		**y = 5,545.8x - 77.62	0.9927

*Area.
**Height.

malathion in the pesticide formulation "Etiol techni" be performed according to the peak area. The results revealed excellent linearity of the proposed method.

Precision. The precision was expressed as intra-day (n = 5) repeatability of retention time, peak area and peak height of malathion (Table 2). The results were obtained by triplicate injections from each of the five prepared sample solutions (n = 5) with volume of 10 μL. In accordance with the CIPAC and SANCO criteria [27, 28], 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 = 0.21%), peak area (RSD = 0.49%) and peak height (RSD = 1.14%) of malathion were within acceptable limits. These results indicate satisfactory precision of the method 1.

Accuracy. The accuracy of the method 1 was confirmed by standard additions [27, 28]. 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 98.97 to 101.62% (Table 3). These values were within the acceptable values for the recovery according to the CIPAC and SANCO criteria [27, 28], which should range from 98 to 102%. Hence, it was concluded that the proposed method 1 was accurate enough for determination of active ingredient malathion in the pesticide formulation "Etiol techni".

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

		$\bar{x}$	SD	RSD (%)
Method 1	retention time (min)	5.49	0.01	0.21
	peak area	1861.22	12.28	0.66
	peak height	259.12	2.96	1.14
Method 2	retention time (min)	5.59	0.026	0.46
	peak area	76.38	11.82	15.48
	peak height	1.03	0.17	16.83

Table 3. Results from recovery experiments (n = 4) for the method 1

Mass of analyte (μg)	Pure analyte added (μg)	Total analyte found ±SD (μg)	Recovery (%)	RSD (%)
3.69	0.96	4.72 ± 0.016	101.62	0.35
3.69	1.91	5.62 ± 0.046	100.22	0.83
3.69	3.83	7.45 ± 0.004	98.97	0.06

The proposed method 1 was applied for the quantitative determination of the active component malathion in the pesticide product "Etiol techni". The obtained mean concentration of malathion was 578.33 g/L (n = 4, RSD = 0.88%), 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.

Also, the developed NP-HPLC method has been tested for the determination of malathion residues in apple juice samples (method 2), after preliminary sample preparation. For this purpose, a solid-phase extraction (SPE) was carried out using Supelclean ENVI-18 columns, as described in the experimental section. SPE is one of the most commonly used sample preparation procedure for concentrating analytes of interest in the sample, as well as removing interfering components from a sample.

Method 2 validation for determination of malathion residues in apple juice samples

Method 2 validation was performed according to EU regulations and EU documents [29, 30]. For that purpose, specificity, selectivity, linearity, matrix effect, precision expressed as repeatability of retention time, peak area and peak height, and accuracy were evaluated.

Specificity and selectivity. As in the method 1 validation for determination of malathion in the pesticide formulation, to confirm the specificity of the developed method for determination of malathion residues in apple juice samples, UV-diode array detection was used to check the peak purity and analyte peak identity. The purity index for malathion was greater than 999, which means that the chromatographic peak was not affected by any other compound. As before, malathion identification was performed by comparing the retention time of the malathion analytical standard with that of the apple juice sample and by monitoring the match factor values obtained by overlaid the UV spectra of the pure analytical standard of malathion and the absorption spectrum of malathion present in apple juice samples. The obtained values for match factors (>998) confirmed the identity of the analyte. Additionally, on the recommendation of EU [30], to prove selectivity of the method, in Fig. 4 are presented chromatograms of analytical standard at the concentration which is correspond to MRL (a), matrix blank (unspiked apple juice sample) (b) and sample of apple juice fortified with analyte at the concentration equal to MRL (c). The MRL of pesticides contained in apple (and apple juice) was set up by the Regulation (EU) 2015/399 [31] and it was estimated at 0.02 mg/kg for malathion.

Linearity. The linearity of the developed method 2 was determined by construction of calibration curve with triplicate injections (20 μL) of the spiked malathion standard in the apple juice samples in the range from 30% less than MRL to 20% above (Table 1), i.e. 0.014–0.024 mg/kg. For these concentration range and using the data for the peak areas

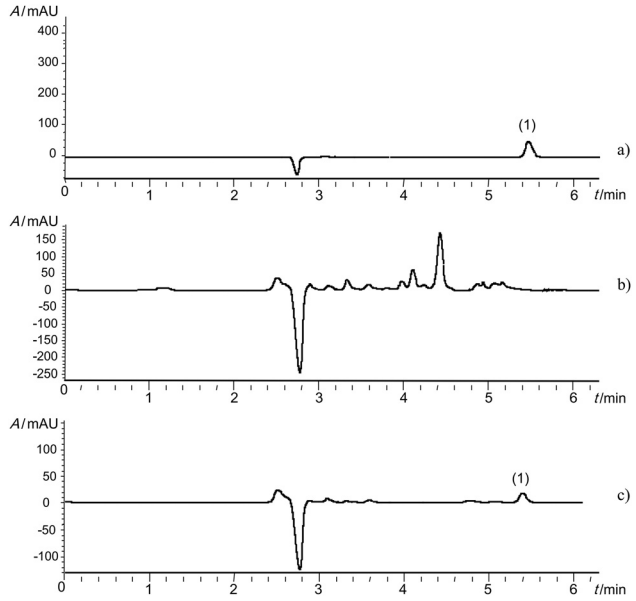

Fig. 4. Chromatograms obtained from the analytical standard of malathion (1) at a concentration equal to MRL (a), a blank sample (b) and an apple juice sample fortified with malathion at a concentration equal to MRL (c)

and peak heights the curves were constructed and the multiple correlation coefficients (R^2) were calculated (Table 1). The curves followed Lambert-Beer's law and the calculated results for multiple correlation coefficients ($R^2 \geq 0.99$) suggested that the method 2 has a satisfactory linearity (Table 1). However, the values obtained for the multiple correlation coefficients (R^2) when the peak area was taken as dependent variable were greater than that obtained when the peak height was taken as dependent variable. Hence, the further calculations were performed according to the peak area.

Matrix effect. Matrix effect represents the noticed effect of an increase (enhancement) or decrease in detector response (a positive or negative matrix effect, respectively) of a pesticide present in a matrix extract compared with the same pesticide present in just solvent [23]. The quantitative determination of matrix effect was carried out using equation (1). The calculated matrix effect for malathion was -70.85% (Table 4) and indicated a significant negative matrix effect. Whenever matrix effects are significant (i.e. > 20%), calibration should be made using standards prepared in blank matrix extracts (matrix matched standards) [29, 30]. Therefore, the calibration was carried out this way.

Limit of quantification. The limit of quantification (LOQ) for malathion was determined by spiking an apple juice sample with 0.014 mg/kg of malathion, which concentration correspond to 30% less of MRL for malathion.

The signal-to-noise ratio (S/N) at this concentration level was found to be >10 and therefore, the LOQ was estimated to be 0.014 mg/kg for malathion in apple juice sample. This result is acceptable for determining the pesticide residues, according to the EU rules [30].

Precision. The precision was expressed as repeatability of obtained results from five successive injections (20 µL) of the spiked apple juice samples at MRL of malathion (Table 2). The computed values of RSD for retention time, peak area and peak height indicated a good precision of the proposed method 2.

Accuracy. The accuracy of the method 2 was determined by recovery studies in apple juice samples (malathion free) fortified with the malathion at three concentration levels (Table 5). The obtained values for recovery and for relative standard deviation were within the following ranges 95.05–97.41% and 4.71–7.59%, respectively. The mean recovery at each fortification level in the range of 70%–120% and relative standard deviation (RSD) ≤ 20% per level are acceptable according to EU criteria [30]. Consequently, it can be concluded that the proposed method 2 is convenient to determination of the malathion residues in apple juice samples.

The developed method 2 was applied for the determination of the malathion residues in apple juice samples under the stipulated experimental conditions. The typical chromatograms of apple juice samples from three different producers marked as: A, B and C are presented in Fig. 5. The samples were concentrated and the clean-up using SPE prior to NP-HPLC analysis. Each analysis was repeated five times.

Table 4. Average matrix effect (%) for malathion (n = 5)

Compound	Concentration (mg/kg)	$X_1 \pm$ SD	$X_2 \pm$ SD	Matrix effect (%)
malathion	0.02	621.93 ± 4.13	181.21 ± 25.10	−70.85

X_1 = average peak area of the malathion standard solution in solvent (*n*-hexane/dichloromethane, (80/20, *v/v*)) at concentration of 0.02 mg/kg.
X_2 = average peak area of the malathion standard solution in blank apple juice extract at concentration of 0.02 mg/kg.

Table 5. Results from recovery experiments (n = 5) of the method 2

Compound	Fortification level (mg/kg)	Total analyte found (mg/kg ± SD)	Recovery (%)	RSD (%)
malathion	0.014	0.0136 ± 0.00064	97.41	4.71
	0.02	0.0193 ± 0.00136	96.67	7.05
	0.024	0.0228 ± 0.00173	95.05	7.59

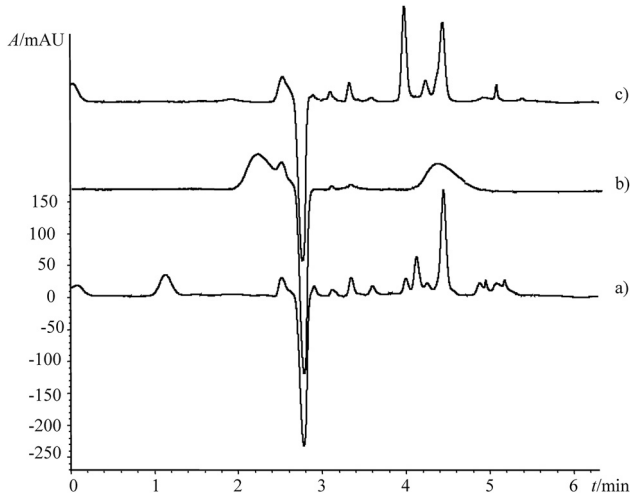

Fig. 5. Typical chromatograms of apple juice samples A (a), B (b) and C (c) obtained by the developed method at 220 nm

The investigations show that malathion residues in concentrations which correspond to MRL or higher were not detected in the analysed apple juice samples.

CONCLUSIONS

This study presents a new, simple and reliable normal-phase high-performance liquid chromatography (NP-HPLC) method with ultraviolet diode-array detection (UV-DAD) for determination of an active ingredient malathion in the commercial pesticide formulation "Etiol techni" (method 1). Successful separation and quantification was achieved using the LiChrosorb CN (250 × 4 mm, 5 µm) analytical column and isocratic elution with mobile phase consisted of *n*-hexane and dichloromethane (80/20, *v/v*), flow rate of 1 mL/min, constant column temperature at 25 °C and UV detection at 220 nm. Specificity, selectivity, linearity, precision and accuracy were tested for the method 1 validation in accordance with the CIPAC and SANCO rules. The elaborated method was successfully applied for determination of an active ingredient malathion in pesticide formulation "Etiol techni" within 6 min chromatographic run. The obtained mean concentration of malathion was 578.33 g/L, which corresponded to the value declared by the manufacturer. Also, the developed method has been successfully applied for the determination of malathion residues in apple juice samples, after performed preliminary sample preparation using solid-phase extraction (method 2). Method 2 validation was performed according to EU regulations and EU documents, and for that purpose, specificity, selectivity, linearity, matrix effect, precision expressed as repeatability of retention time, peak area and peak height, and accuracy were evaluated. The obtained results from validation of the both, method 1 and method 2, indicated that all tested parameters were found within acceptance criteria.

This paper represents contribution in the field of new analytical methods for determination of active ingredients in pesticide formulation and thus to implement more efficient quality control of plant protection products. The application of the normal-phase high-performance liquid chromatography (NP-HPLC) method with ultraviolet diode-array detection (UV-DAD) for determination of malathion residues in apple juice samples was also presented. The control of pesticide residues in food is also of outstanding significance to ensure the food safety and human health protection.

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