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Original scientific paper

DEVELOPMENT OF HIGH SPEED RRLC METHOD FOR QUANTITATIVE DETERMINATION OF SOME PESTICIDE RESIDUES IN APPLE JUICE

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Abstract
Possibilities of normal-phase (NP) and reversed-phase (RP) liquid chromatography methods for determination of methomyl, methidathion and propiconazole residues in apple juice were studied. The investigations were carried out on various analytical columns using RRLC (Rapid Resolution Liquid Chromatography) system coupled with UV-Vis diode array detector. The best conditions for separation and quantitative determination of tested pesticides were obtained using reversed-phase mode and Purospher® Star RP-18 endcapped (30 mm × 4 mm; 3 μm) column. Fast and simple method for direct determination of methidathion and propiconazole in different apple juice matrix was developed. In accordance to European Commission regulation the tested parameters for method validation (selectivity, linearity, precision, limit of detection, limit of quantification and accuracy) were satisfied.
Key words: direct determination, methidathion, propiconazole, high speed RRLC, apple juice.

Introduction

Pesticides are highly effective substances used in control of pests and vectors of human diseases. Their application in agriculture enabled increased crops yields and manufacturing of high quality products in order to satisfy the increasing food demands over the world. On the other side the use of pesticides had caused concerns about their effects on human health and the environment (Jokanović *et al*., 2012), they have been linked to a wide range of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disruption.

As food safety is among the first priorities in many countries, there is a need for determination of pesticide residues in various food commodities. Pesticides residues are usually determined by gas chromatography (GC) or liquid chromatography (LC) (Nollet *et al*., 2004; Słowik-Borowiec *et al*., 2015) coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) using a triple quadrupole (QqQ) analyzer (Rosenblum *et al*., 2001; Hernández *et al*., 2013; Martínez-Domínguez *et al*., 2015; Xiaoli *et al*., 2015; Páleníková *et al*., 2015, Takatori *et al*., 2011;). Different technique and detectors are also used with various sensitivity and repeatability as it is ultra-HPLC (UHPLC)/ESI quadrupole (Qq)-time-of-flight (TOF) MS (Wang *et al*., 2011), UHPLC-MS/MS (Moreno-González *et al*., 2015), Fourier transform infrared (FTIR), visible/near-infrared spectroscopy (Vis/NIR) (Jamshidi *et al*., 2015; Guangdong *et al*., 2015) or HPLC equipped with ultraviolet detector (UVD) (Wenbi *et al*., 2015; Tuzimski *et al*., 2016).

Because of very low contaminants quantity and complexity of sample matrix, preliminary sample preparation prior GS or LC analyses are often need. The widely used analytical methodology combining the extraction/isolation of pesticides from food matrices with extract cleanup are QuEChERS (quick, easy, cheap, effective, rugged, and safe) (Zhao *et al*., 2012; AOAC Official Method 2007.01). Supercritical fluid extraction (SFE) (Fernandes et al., 2011; Boulaid et al., 2007), matrix solid phase dispersion (MSPD) (Zhou *et al*., 2015; Liu *et al*., 2015), solid-phase microextraction (Blasco *et al*., 2008) and magnetic solid phase extraction (SPE) (del Castillo *et al*., 2012) are also used.

The most widely used pesticides over the world for apple protection are methidathion (Moura1 *et al*., 2013), methomyl and propionazole, therefore their residues are wary often present in apples or apple juice. The current status of methidathion under EU Regulation (EC) No 1107/2009 is not approved for use, against methomyl and propionazole which have opposite status.

However, studies for simultaneous determinations of methomyl, methidathion and propiconazole in apple juice using RRLC system coupled with UV-Vis diode array detector are not available. Therefore the purpose of this paper is to develop a simple and easy for use method for determination of all three pesticides in apple juices.

Materials and methods

The development of the RRLC method for quantitative determination of methomyl, methidathion and propiconazole residues in apple juice samples were provided on 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. The separation of analytes were tested on different analytical columns: HS Pecosphere 3×3 Silica (3.3 cm × 0.46 cm; 3 μm) produced by Perkin-Elmer, Hypersil ODS (25 cm × 0.46 cm; 5 μm) produced by Sigma–Aldrich, and Purospher® Star RP-18 endcapped (3 cm × 4 mm; 3 μm) produced by Merck. An ultrasonic bath "Elma" was used for better dissolving of the stock solutions.

The Pestanal grade analytical standards of methomyl, methidathion and propiconazole and HPLC-grade acetonitrile were purchased by Sigma-Aldrich (Germany). Ultrapure water was produced by TKA Smart2 Pure 12 UV/UF water purification system (Germany).

Various commercial 100 % clear apple juice samples from different producers (A, B, C, D and E) were purchased in Macedonian supermarkets.

Preparation of Standard Solutions

Stock solutions of methomyl, methidathion and propiconazole were prepared by dissolving 0.0030 g, 0.0033 g and 0.0034 g, respectively, of the pure analytical standards in acetonitrile in 10 mL volumetric flasks. The solutions were degassed for 15 min in an ultrasonic bath and stored in a refrigerator at 4°C. Stock solutions were used to prepare a series of 6 working solutions with different pesticide concentrations (6 – 30 μg/L for methomyl, 9 – 45 μg/L for methidathion and 45 – 225 μg/L for propiconazole) in 10 mL volumetric flask by dilution with the mixture of acetonitrile/water (50/50, *V/V*). In order to construct the calibration plots, 20 μL of each working solution were injected in the chromatograph three times. The obtained chromatograms were analyzed considering areas and heights of the peaks.

Preparation of Sample Solutions

Before the analysis, apple juice samples were filtered through 0.45 μm Iso-Disc PTFE syringe filters (Supelco). Unspiked apple juice sample was used as blank.

The solutions for recovery experiment were prepared in three 10 mL volumetric flasks. In each volumetric flask was added a known amount of analytes which were corresponding to the MRLs, 0.03 mg/L (600 pg) of methidathion and 0.15 mg/L (3000 pg) of propiconazole) and filled to volume with apple juice. 20μL of each of these solutions was injected three times into the LC system.

Results and discussion

In order to achieve the best separation of the investigated compounds few different analytical columns for normal-phase and reverse-phase chromatography were tested.

For normal-phase chromatography the polar HS Pecosphere 3×3 Silica (3.3 cm × 0.46 cm; 3 μm) was used. The mobile phase was consisted n-hexane/dichloromethane in different volume ratio, and different flow rate. The UV spectra obtained from these chromatographic conditions showed maximum at 235 nm for methomyl, and 225 nm for methidathion and propiconazole respectively, therefore the investigations were performed at both wavelengths.

When HS Pecosphere 3×3 Silica was used with mobile phase in ratio of 40/60 *V/V* to 0/100 *V/V*, and flow-rate of 1.2 mL/min to 1.8 mL/min, at column temperature of 25 ºC, only methidathion eluted in a 6 minutes run (Fig. 1).

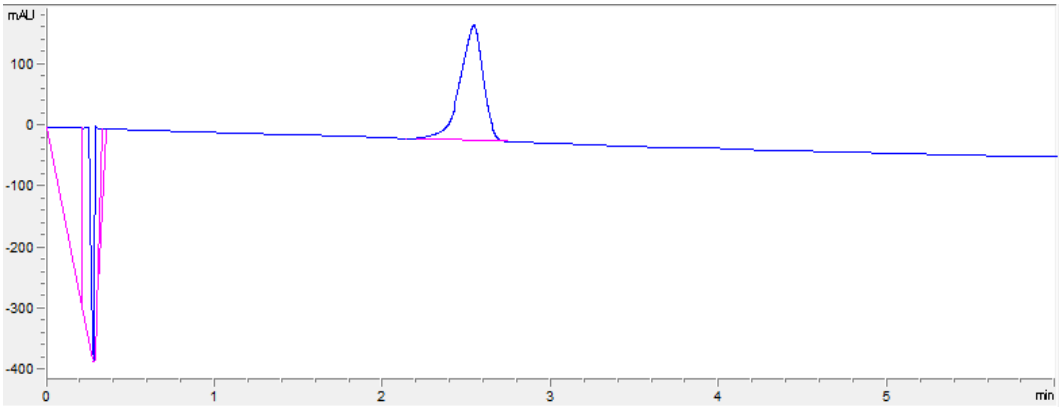

Figure 1. Chromatogram obtained on HS Pecosphere 3×3 Silica (3.3 cm × 0.46 cm; 3 µm) column of a standard mixture containing methidathion (I) methomyl (II), and propiconazole (III); mobile phase n-hexane/dichloromethane 30/70 (*V/V*), flow rate 1.6 mL/min, temperature 25 ºC and UV detection at 230 nm

Because of methomyl and propiconazole precipitation on the HS Pecosphere 3×3 Silica column, the possibility for reverse-phase chromatography was tested on Hypersil ODS and Purospher® Star RP-18 endcapped columns. The UV spectra of methomyl, methidathion and propiconazole in acetonitrile/water (50/50 *V/V*) show that methomyl has an absorption maximum at 235 nm, while methidathion and propiconazole have their absorption maxima at 220 nm. Therefore the further investigations were providing on both wavelengths.

The best resolution of analytes using Hypersil ODS column was achieved with a mobile phase acetonitrile/water 80/20 (*V/V*), flow-rate of 1 mL/min and column temperature of 25 ºC. Under using chromatography conditions the tested column shoved unsatisfied peak symmetry, and peak splitting for methidathion (Figure 3).

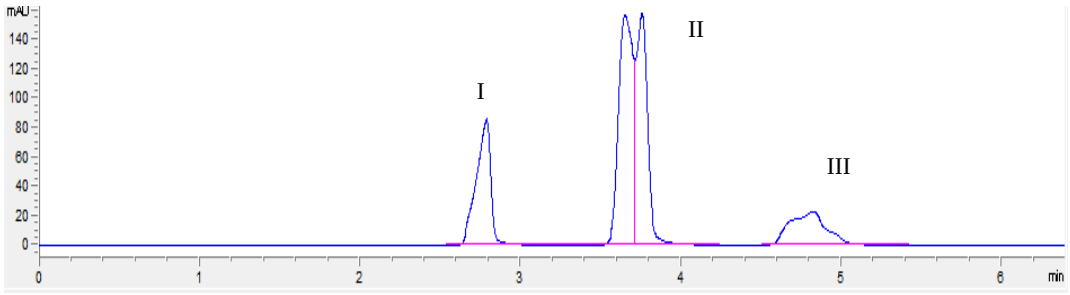

Figure 2. Chromatogram obtained on Hypersil ODS (25 cm × 0.46 cm; 5 µm) column from a standard mixture containing methomyl (I), methidathion (II) and propiconazole (III); mobile phase acetonitrile/water 80/20 (*V/V*), flow1 mL/min, temperature 25 ºC and UV detection at 220 nm

The best resolution with sharp and symmetrical peaks for all three pesticides was achieved on Purospher® Star RP-18 endcapped (30 mm × 4 mm; 3 µm) column, mobile phase consisting acetonitrile/water 50/50 (*V/V*) flow-rate of 1mL/min and column temperature of 25 ºC (Figure 3).

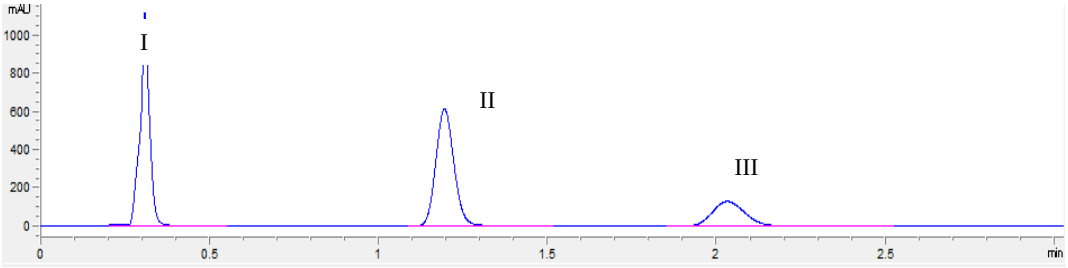

Figure 3. Chromatogram obtained on Purospher® Star RP-18 endcapped (3 cm × 4 mm; 3 µm) column from a standard mixture containing methomyl (I), methidathion (II) and propiconazole (III); mobile phase acetonitrile/water 50/50 (*V/V*), flow1 mL/min, temperature 25 ºC and UV detection at 220 nm

The mean value of retention times obtained under describes chromatography conditions were: 0.302 min, 1.248 min and 2.130 min for methomyl, methidathion and propiconazole, respectively.

Using the chromatography condition set on Purospher® Star RP-18 column (Fig. 7), the estimated values for limit of detection (LOD) (detrmined as signal to noise ratio 3:1) was 0.6 pg (0.12 µg/L) for methomyl, 22.5 pg (4.5 µg/L) for methidathion and 30 pg (6 µg/L) for propiconazole. The obtained values for the limit of quantification (LOQ) (detrmined as signal to noise ratio 10:1) were: 2 pg (0.4 µg/L) for methomyl, 75 pg (15 µg/L) for methidathion and 100 pg (20 µg/L) for propiconazole. Because of high sensitivity of develop method in relation to investigated analytes, the tested apples juice were used without concentration and cleanup procedures.

The chromatogram obtained from apple juice spiked with 0.02 mg/L (400 pg) methomyl, 0.03 mg/L (600 pg) methidathion and 0.15 mg/L (3000 pg) propiconazole (concentrations corresponding to their EU MRLs)[8] showed that methomyl peak overlap with the coeluting peaks from apple juice matrix. (Figure 5.). This confirmed that under the given chromatographic conditions, methomyl can not be determined in this matrix.

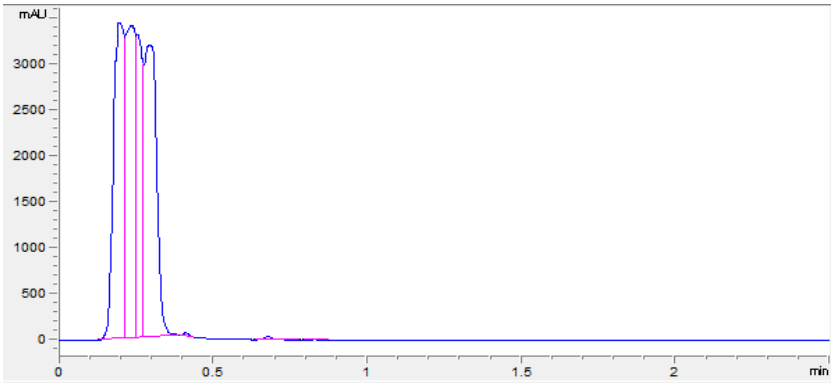

Figure 4. Chromatogram obtained on Purospher® Star RP-18 endcapped column from apple juice; mobile phase acetonitrile/water 50/50 (*V/V*), flow1 mL/min, temperature 25 ºC and UV detection at 220 nm

The linearity of the method was tested in different concentrations level up and below to analytes MRL's (metholmyl in the range 6-30 µg/L, methidathion in the range 9-45 µg/L and propiconazole in the range 45-225 µg/L) revealed lower values of the correlation coefficients (R^2). The calculated R^2 values were: 0.8180 for methomyl, 0.9528 for methidathion and 0.9898 for propiconazole for the peak areas and 0.9556 for methomyl, 0.9640 for methidathion and 0.9892 for propiconazole for the peak heights. These R^2 values indicated the peak area as preferable variable for methidathion and the peak height as preferable for propiconazole during linearity testing.

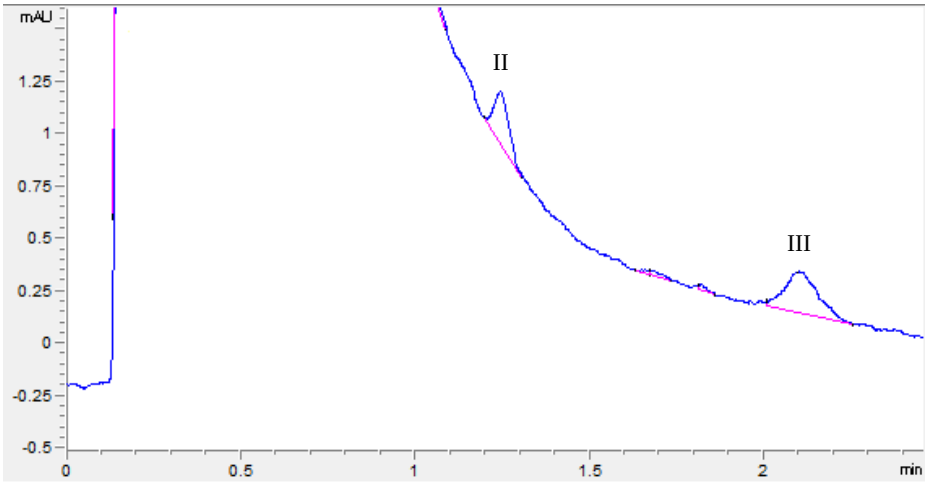

Figure 5. Chromatogram obtained on Purospher® Star RP-18 endcapped column from spiked apple juice; methidathion (II) and propiconazole (III); mobile phase acetonitrile/water 50/50 (*V/V*), flow 1 mL/min, temperature 25 ºC and UV detection at 220 nm

Table 1. Results for linearity of the method

Compound	Regression equation	
Methomyl (235 nm)	^{1}y = 0.0017x + 0.5331 ^{2}y = 0.0007x + 0.1193	0.8180 0.9556
Methidathion (220 nm)	^{1}y = 0.0015x + 0.0612 ^{2}y = 0.0004x + 0.0113	0.9528 0.9640
Propiconazole (220 nm)	^{1}y = 0.0007x - 0.2216 ^{2}y = 9E-05x - 0.0143	0.9898 0.9892

^{1}y = peak area; ^{2}y = peak height

The precision was expressed as repeatability of obtained results which was evaluated for peak areas, peak heights and retention times of the analytes from eight successive injections (20 µL) of the mixture containing 400 pg methomyl, 600 pg methidathion and 3000 pg propiconazole.

Table 2. Statistical data for precision of the method

	Intra-day repeatability (n = 8)								
	Retention time			Peak height			Peak area		
Compound	$\bar{x}$	SD	RSD (%)	$\bar{x}$	SD	RSD (%)	$\bar{x}$	SD	RSD (%)
Methomyl	0.9595	0.0190	1.98	0.3426	0.0057	1.65	0.3049	0.0004	0.12
Methidathion	1.1644	0.0473	4.06	0.2912	0.0062	2.13	1.22	0.0019	0.15
Propiconazole	1.7255	0.0873	5.06	0.2244	0.0052	2.31	2.0815	0.0061	0.29

The accuracy was tested by the method of standard additions. Three apple juice samples were spiked with 600 pg methidathion and 3000 pg for propiconazole. The calculated values for analyte recovery ware 98.82 % with 2.51 % of RSD and 76.03 with 6.36 % of RSD. The obtained values for recovery are in accordance with method performance acceptability criteria (70-120 %) given by SANCO Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.

Using developed method no residues of methidathion and propiconazole were found in all tested clear apple juice samples (A, B, C, D and E) taken from Macedonian markets.

Conclusions

The develop method described in this paper is suitable for direct qualitative and quantitative determination of methidathion and propiconazole in 100 % clear apples juices. Due to strong matrix effect under developed chromatography condition there was no possibility for determination of methomyl in apples sample. The proposed method is fast, simple, linear, precise and sufficiently accurate for quantitative and qualitative determination of methidathion and propiconazole in apple juice. The obtained values for all tested parameters are in accordance with SANCO Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.

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