

SPE-RRLC DETERMINATION OF SOME PESTICIDE RESIDUES IN APPLE JUICE

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Abstract

This paper presents the development of RRLC (Rapid Resolution Liquid Chromatography) method for simultaneous determination of pesticides methomyl, methidathion and propiconazole in different clear apple juice samples. The experiments are performed using rapid resolution liquid chromatography system coupled with UV-VIS diode array detector. The developed high speed reversed-phase (RP) liquid chromatography method is carried out on the Purospher® Star RP-18 endcapped (30 mm × 4 mm; 3 µm) column, mobile phase consists of acetonitrile and water (50/50, V/V), flow rate of 1 mL/min, column temperature at 25 °C and UV detection at 220 nm and 235 nm. Prior to RRLC analysis, the samples are cleaned up and concentrated using a solid-phase extraction (SPE). To assess the validity of the developed method, the following parameters are examined: selectivity, linearity, repeatability (precision), limit of detection, limit of quantification and accuracy.

Key words: RRLC determination, SPE, UV-DAD, pesticide residues, apple juice

Introduction

The mass production of apple fruit requires extensive use of plant protection chemicals, some of them being the pesticides methomyl, methidathion and propiconazole.

Methomyl, *S*-methyl *N*-(methylcarbamoyloxy) thioacetimidate (IUPAC) is a mixture of (*Z*)- and (*E*)-isomers, which controls a wide range of insects and spider mites which attack fruits, vines, vegetables etc. Methidathion, 3-dimethoxyphosphinothioylthiomethyl-5-methoxy-1,3,4-thiadiazol-2(3*H*)-one (IUPAC) is used to control a wide range of sucking and chewing insects and spider mites in many crops, e.g. pome fruit, stone fruit, citrus fruit, vines etc. Propiconazole, (±)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole (IUPAC) exhibits both protective and curative action towards diseases caused by fungi which attack fruits, cereals, maize, coffee and peanuts (Tomlin *et al.*, 1997).

All three substances are registered for use in the USA. Methomyl and methidathion are classified by EPA as Restricted Use Pesticides (RUP, may be used only by certified applicators), unlike propiconazole which is a General Use Pesticide (GUP), (EPA, 1998; EPA 2006a; EPA 2006b). In the European Union, only methomyl and propiconazole are

approved for use as pesticides. Both the European Union and the USA have adopted regulations concerning the MRLs (Maximum Residue Levels/Limits) for pesticides present in food. The MRLs of pesticides contained in apple are laid down by the EU (Regulation (EC) No 396/2005), and they are estimated at: 0.02 mg/kg (for methomyl), 0.03 mg/kg (for methidathion) and 0.15 mg/kg (for propiconazole).

Apple juices are one of the most used from the whole population, especially children. These juices are among the first non-dairy products that are given to infants, but also they are a favourite among older children. Therefore, the safety of these products is of particular importance. Children, especially infants fall into high risk groups in terms of the possible toxicity of pesticides.

In order to monitor food safety, it is highly necessary to develop and employ reliable methods for determination of pesticide residues.

Gas Chromatography (GC) and Liquid Chromatography (LC) are commonly used methods for the determination of pesticide residues in various matrixes (Nollet *et al.*, 2000; Stoytcheva *et al.*, 2011) using the following detectors: Flame Ionization Detector, FID (Kadifkova-Panovska *et al.*,

2000), Nitrogen Phosphorous Detector, NPD (Ay *et al.*, 2007), Mass Spectrometry, MS (He Qiang *et al.*, 2010), Tandem Mass Spectrometry, MS/MS (Guo *et al.*, 2012), Ion Trap Mass Spectrometry, ITMS (Sannino *et al.*, 1999), Fluorescent detector, FD (Sánchez-Brunete *et al.*, 2004), Electrospray Ionization Mass/Tandem Mass Spectrometry, ESI-MS (Schermerhorn *et al.*, 2005) or ESI-MS/MS (Zamora *et al.*, 2004). Also, HPLC combined with ultraviolet, UV detector (Yu *et al.*, 2011) or Diode Array Detector, DAD (Carbo *et al.*, 2008; Lagana *et al.*, 1997; Jeannot *et al.*, 2000) is used for the determination of these components.

Pretreatment of samples usually use the following procedures: liquid-liquid extraction, LLE (Jeannot *et al.*, 2000), Solid Phase Extraction, SPE (Lagana *et al.*, 1997), Solid Phase Microextraction, SPME (Hercegová *et al.*, 2011) and Matrix Solid-Phase Dispersion, MSPD (Albero *et al.*, 2003).

However, the HPLC or RRLC method for simultaneous determination of pesticides methomyl, methidathion and propiconazole residues is not found. Hence, the aim of this paper is to develop a reversed-phase RRLC method for simultaneous determination of methomyl, methidathion and propiconazole residues in apple juices using SPE and UV-DAD.

Materials and methods

Equipment and Materials

The development of the RRLC method for simultaneous determination of methomyl, methidathion and propiconazole in clear apple juice samples is performed 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. The investigations are carried out on a Purospher STAR RP-18e (30 mm x 4 mm, 3 µm, Merck) analytical column. For the better dissolving of the stock solutions an ultrasonic bath "Elma" is used. For the clean-up and concentration of apple juice samples a system for solid phase extraction is employed (Supelco, Sigma Aldrich) with Supelclean™ Envi-18 SPE columns (6 mL; 500 mg). For vortexing of samples, an IKA Vortex Genius 3 (Germany) is used.

The Pestanal grade analytical standards of methomyl, methidathion and propiconazole, K₂HPO₄ (p.a.), H₃PO₄ (85.5 %) and HPLC-grade acetonitrile are purchased by Sigma-Aldrich (Germany). Ultrapure water is produced by TKA Smart2 Pure 12 UV/UF water purification system (Germany).

The juice samples are filtered by 0.45 µm Nitrocellulose membrane filters (Millipore, Ireland), and the final extracts are filtered through 0.45 µm Iso-Disc PTFE syringe filters (Supelco), just before application.

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

Preparation of Standard Solutions

Stock solutions methomyl, methidathion and propiconazole are prepared by dissolving 0.0186 g, 0.0187 g and 0.0132 g, respectively, of the pure analytical standards in acetonitrile in 10 mL volumetric flasks. The solutions are degassed for 15 min in an ultrasonic bath and stored in a refrigerator at 4 °C. Stock solutions are used to prepare standard mixtures with different pesticide concentrations (10 – 200 mg/L for methomyl, 15 – 300 mg/L for methidathion and 75 – 1500 mg/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, 5 µL of each working solution are injected in the chromatograph three times. The obtained chromatograms are analyzed considering areas and heights of the peaks.

Solid phase extraction

For recovery experiment, 1 kg apple juice samples are spiked with concentrations corresponding to MRL: 0.02 mg/kg (for methomyl), 0.03 mg/kg (for methidathion) and 0.15 mg/kg (for propiconazole). Unspiked samples are used for blanks. After column conditioning the samples are passed through the cartridges. The retained pesticides are eluted, twice with 2 mL 100 % acetonitrile. The eluates are evaporated to dryness. The residues are redissolved with 1 mL of acetonitrile, than filtered through 0.45 µm Iso-Disc PTFE syringe filters and transferred into vials for RRLC analysis. 5 µL of each sample are injected in the chromatograph three times.

This procedure concentrated the amount of pesticides in the samples 1000 times.

Results and discussion

The UV spectra of investigated pesticides in acetonitrile/water mixture (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, RRLC analysis for simultaneous determination of these substances is performed at 235 nm (for methomyl) and 220 nm (for methidathion and propiconazole).

In preliminary experiments, different flow-rate (0.9 – 2 mL/min) and different volume ratio of acetonitrile (80 - 45 %) and water in the mobile phase on Purospher® Star RP-18 endcapped analytical column are tested. The best resolution with sharp and symmetrical peaks and satisfy purity indexes for the three analytes is achieved with the mobile phase consisted of acetonitrile/water (50/50, V/V), flow-rate of 1mL/min and column temperature of 25 °C (Figure 1).

For the method validation, specificity and selectivity, linearity, precision, accuracy, limit

of detection (LOD) and limit of quantification (LOQ) are tested.

To confirm the specificity of the developed method, UV-diode array detection is used to check the peak purity and analyte peak identity. The purity index for all analytes is greater than 999, which means that the chromatographic peak is not affected by any other compound.

The obtained values for retention times of components (t_R) under these chromatographic conditions are given in Table 1.

The linearity of the method is tested by triplicate injections (5 μ L) of five mixtures containing various concentrations of the three compounds: methomyl in the range 10 - 200 mg/L, methidathion in the range 15 - 300 mg/L and propiconazole in the range 75 -1500 mg/L. For these concentration ranges and using data for the peak areas and peak heights, calibration curves are constructed and the correlation coefficients (R^2) are calculated (Table 1). The curves followed Lambert-Beer's law and the R^2 values obtained indicated the peak area as a preferable variable for further accuracy testing.

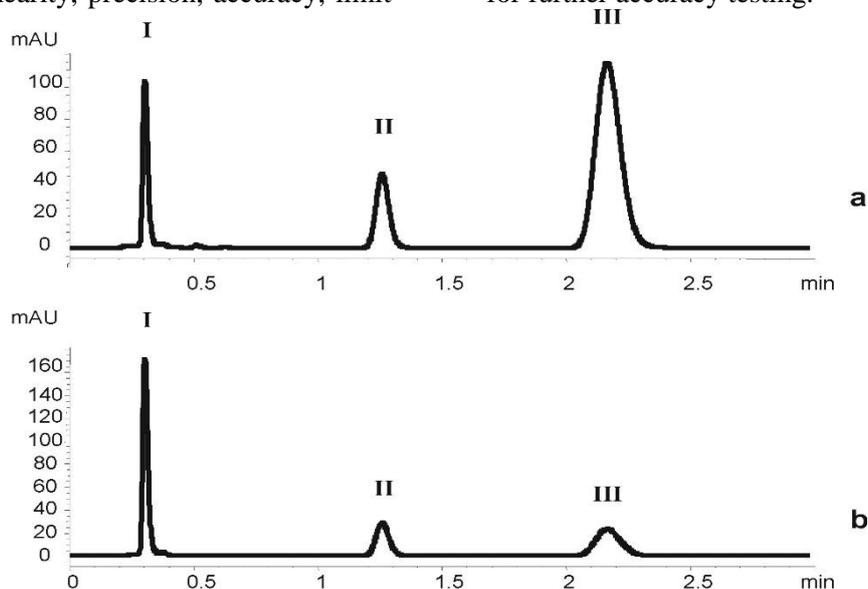


Figure 1. Chromatogram obtained from a standard mixture of methomyl (I), methidathion (II) and propiconazole (III) at 220 nm (a) and 235 nm (b) on Purospher® Star RP-18 endcapped column with acetonitrile/water (50/50, V/V) as a mobile phase, flow 1 mL/min and temperature 25 °C

Table 1. Data from experiments of investigated pesticides

Compound	t_R /min	Regression equation	R^2	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)
Methomyl (235 nm)	0.30	$^1y = 4473,8x + 24,217$ $^2y = 1933,3x + 134,67$	0,9993 0,9878	0.12	0.4
Methidathion (220 nm)	1.25	$^1y = 2175,8x + 22,005$ $^2y = 417,4x + 38,832$	0,9999 0,9868	4.5	15
Propiconazole (220 nm)	2.13	$^1y = 2006,8x + 152,53$ $^2y = 248,27x + 53,249$	0,9999 0,9980	6	20

1y = peak area; 2y = peak height

The precision is expressed as repeatability of obtained results which is evaluated for peak areas peak, heights and retention times of the analytes from eight successive injections (5 μL) of the mixture containing 20 mg/L methomyl, 30 mg/L methidathion and 150 mg/L propiconazole within 3 days. The percent of relative standard deviation (RSD) for intra-day repeatability is less than 0.65 %, while RSD values for inter-day repeatability is less than 2.55 % for each component. The

obtained results (Table 2) indicated a very good precision of peak area, height and retention time under the conditions used in the tested method.

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 for each compound are listed in Table 1.

Table 2. Precision data for investigated pesticides

		Intra-day repeatability (n = 8)		Inter-day repeatability (n = 3)	
		$\bar{x} \pm \text{SD}$	RSD (%)	$\bar{x} \pm \text{SD}$	RSD (%)
Retention time	Methomyl	0.30 \pm 0.005	0.15	0.30 \pm 0.003	0.44
	Methidathion	1.24 \pm 0.003	0.22	1.23 \pm 0.02	1.93
	Propiconazole	2.13 \pm 0.005	0.25	2.10 \pm 0.05	2.18
Peak area	Methomyl	526.17 \pm 0.58	0.11	536.10 \pm 7.22	1.35
	Methidathion	339.32 \pm 1.46	0.43	334.82 \pm 7.90	2.36
	Propiconazole	1671.71 \pm 1.80	0.11	1694.11 \pm 33.54	1.98
Peak height	Methomyl	320.67 \pm 1.04	0.32	316.65 \pm 6.05	1.91
	Methidathion	94.03 \pm 0.31	0.33	92.36 \pm 2.33	2.53
	Propiconazole	244.45 \pm 1.55	0.64	248.67 \pm 6.21	2.50

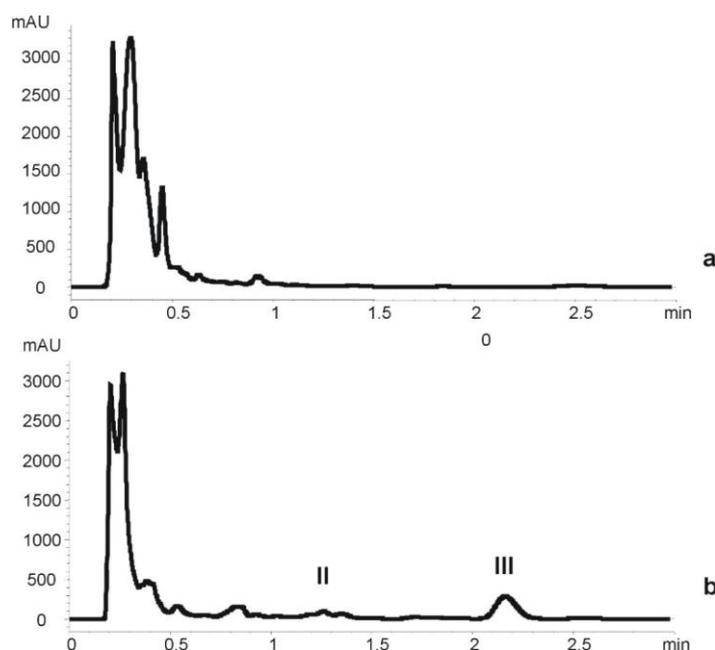


Figure 2. Chromatogram obtained from unspiked apple juice (a) and sample of apple juice fortified at the concentration equal to MRL for methomyl (I), methidathion (II) and propiconazole (III) (b) on Purospher® Star RP-18 endcapped column with acetonitrile/water (50/50, V/V) as mobile phase, flow 1 mL/min, temperature 25 °C and UV detection 220 nm

The developed method based on solid-phase extraction is applied for the determination of investigated pesticide residues from apple juice samples. The chromatograms obtained under the same conditions from the cleaned-up and concentrated apple juice, after SPE, show a number of tall peaks (originating from the apple juice matrix) in the area around 0.3 minutes, where the methomyl peak appears (Figure 2a). So we continued investigations aimed at determining only methidathion and propiconazole residues.

The values for the retention time and match factor obtained by overlaid UV spectra of a pure analytical standard and absorption spectra of the same analyte in the spiked apple juice samples are used for the identity of the analytes. The estimated values for the match factor are 994.092 for methidathion and 999.899 for propiconazole. Being high above 990, these values confirm the identity of methidathion and propiconazole. On Figure 2 are presented chromatograms of matrix blank (unspiked apple juice that is apple juice free of investigated pesticides) (a) and sample of apple juice fortified at the concentration equal to MRL for each analyte (b). The efficiency of the extraction method using ENVI-18 SPE cartridges expressed as recovery. The obtained values for recovery are 114.46 % for

methidathion and 121.48 % for propiconazole. In cases where pesticide residues in food are analyzed recoveries in the range 70 - 120 % and even outside this range are acceptable in cases of multiresidue methods (European Commission, 2010; SANCO, 2011). The methidathion and propiconazole residues in concentrations which correspond to MRLs or higher are not detected in none of the five samples of apple juice.

Conclusion

The developed RRLC method with UV-DAD is selective for two of the three pesticides examined, them being methidathion and propiconazole. The matrix contains interfering compounds that disable the determination of methomyl. The proposed method is simple, linear, precise and sufficiently accurate for qualitative and quantitative determination of methidathion and propiconazole residues in apple juices after the SPE procedure.

This method can be used for determination pesticide residues in laboratories for control of chemical hazards in food which will contribute to food safety.

References

Albero, B., Sánchez-Brunete, C., Tadeo, J. L. (2003). Determination of organophosphorus

- pesticides in fruit juices by matrix solid-phase dispersion and gas chromatography. *Journal of agricultural and food chemistry*. No. 51(24), pp. 6915-6921.
- Ay, R., Yasar, B., Demirözer, O., Aslan, B., Yorulmaz, S., Kaya, M., Karaca, I. (2007). The determination of the residue levels of some commonly used pesticides in Isparta apple orchards. *Turkish Journal of Entomology*. No. 31(4), pp. 297-306.
- Carbo, L., Souza, V., Dores, E. F. G. C., Ribeiro, M. L. (2008). Determination of pesticide multiresidues in shallow groundwater in a cotton-growing region of Mato Grosso, Brazil. *Journal of the Brazilian Chemical Society*, Vol.19. No. 6, pp.1111-1117.
- European Commission, Directorate General Health and Consumer Protection. (2010). Guidance document on pesticide residue analytical methods. pp. 9-11.
- EPA. Registration Eligibility Decision (RED) for Methomyl. (1998). pp.14.
- EPA. Registration Eligibility Decision (RED) for Methidathion. (2006a). pp.5.
- EPA. Registration Eligibility Decision (RED) for Propiconazole. (2006b). pp. 5.
- Guo, M., Tan, Z., Wu, H., Li, J., Wang, Y., Shi, J., Li, J., Jiang, L., Yao, X., Fang, L. (2012). Determination of 11 triazole fungicides in fruits using solid phase extraction and gas chromatography-tandem mass spectrometry. *Chinese Journal of Chromatography*. Vol. 30. No. 3, pp. 262-266.
- He, Q., Li, J., Kong, X., Yue, A., Dong, H., Zhao, J. (2010). GC-MS Determination of Residual Amount of Propiconazole in Foodstuffs with Separation by Solid Phase Extraction. *Physical Testing and Chemical Analysis*. No. 2, pp. 178-183.
- Hercegova, A., Möder M. (2011). Determination of some selected pesticide residues in apple juice by solid-phase microextraction coupled to gas chromatography – mass spectrometry. *Acta universitatis agriculturae et silviculturae mendelianae brunensis*. Vol. LIX. No. 1, pp. 121-127.
- http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=activesubstance.selection
- Jeannot, R., Sabik, H., Sauvard, E., Genin, E. (2000). Application of liquid chromatography with mass spectrometry combined with photodiode array detection and tandem mass spectrometry for monitoring pesticides in surface waters. *Journal of chromatography A*. No. 879, pp. 51-71.
- Kadifkova-Panovska, T., Kavrakovski, Z., Bauer, S. (2000). Determination of propiconazole residues in tomatoes by gas chromatography. *Bulletin of the Chemists and Technologists of Macedonia*. Vol. 19. No.1, pp. 27-23.
- Lagana, A., D'Ascenzo, G., Fago, G., Marino, A. (1997). Determination of organophosphorus pesticides and metabolites in crops by solid-phase extraction followed by liquid chromatography/diode array detection. *Chromatographia*. No. 46(5/6), pp. 256–264.
- Nollet, L. (2000). *Food Analysis by HPLC*, Marcel Dekker Inc., New York, Basel, pp. 693-715.
- Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Text with EEA relevance.
- Sánchez-Brunete, C., Albero, B., Tadeo, J. L., (2004). HPLC multiresidue method for the determination of N-methyl carbamates in fruit and vegetable juices. *Journal of food protection* No. 67 (11), pp. 2565-9.
- SANCO. (2011). Method validation and quality control procedures for pesticide residues analysis in food and feed, Document N° SANCO/12495/2011 (implemented by 01/01/2012). pp. 13.
- Sannino, A., Bandini, M., Bolzoni, L. (1999). Multiresidue Determination of 19 Fungicides in Processed Fruits and Vegetables by Capillary Gas Chromatography after Gel Permeation Chromatography. *Journal of AOAC International*. Vol. 82. No. 5, pp. 1229-1238.
- Schermerhorn, P., Golden, P. E., Krynskiy, A. J., Leimkuehler, W. M. (2005). Determination of 22 triazole compounds including parent fungicides and metabolites in apples, peaches, flour and water by Liquid Chromatography/Tandem Mass Spectrometry. *Journal of AOAC International*. Vol. 88. No. 5, pp. 1491-1502.
- Stoytcheva, M., Zlatev, R. (2011). Pesticides in the Modern World - Trends in Pesticides Analysis. In *Tech*. pp. 1-526
- Tomlin, C. (1997). *The Pesticide Manual Incorporating the Agrochemicals Handbook*. 11th Edition. Crop Protection Publications. pp. 675-676, 679-680, 855-857.

Yu, X., Wang, B. (2011). Rapid HPLC Analysis Method for Prochloraz and Propiconazole Content in Bactericidal Suspension Emulsion. *Agrochemicals*. No. 10, pp. 734-736.

Zamora, T., Pozo, O. J., López, F. J., Hernández, F. (2004). Determination of tridemorph and other fungicide residues in fruit samples by liquid chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography A*. No. 1045(1-2), pp. 137-143.