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 acetonitrille and water (50/50, V/V), flow rate of 1 mL/min, column temperature at 25 ºC 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-dimethoxyphosphinolthioylthiomethyl-5-methoxy-1,3,4-thiadiazol-2(3H)-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]-1H-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 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 (Schermterhorn 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, KH2PO4 (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 ºC (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 µL) 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.

![Chromatogram](image)

*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 ºC*
Table 1. Data from experiments of investigated pesticides

<table>
<thead>
<tr>
<th>Compound</th>
<th>$t_0$/min</th>
<th>Regression equation</th>
<th>$R^2$</th>
<th>LOD (μg/L)</th>
<th>LOQ (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl (235 nm)</td>
<td>0.30</td>
<td>$y = 4473.8x + 24.217$ $y = 1933.3x + 134.67$</td>
<td>0.9993</td>
<td>0.12</td>
<td>0.4</td>
</tr>
<tr>
<td>Methidathion (220 nm)</td>
<td>1.25</td>
<td>$y = 2175.8x + 22.005$ $y = 417.4x + 38.832$</td>
<td>0.9999</td>
<td>4.5</td>
<td>15</td>
</tr>
<tr>
<td>Propiconazole (220 nm)</td>
<td>2.13</td>
<td>$y = 2006.8x + 152.53$ $y = 248.27x + 53.249$</td>
<td>0.9999</td>
<td>6</td>
<td>20</td>
</tr>
</tbody>
</table>

$y$ = peak area; $y$ = 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

<table>
<thead>
<tr>
<th></th>
<th>Intra-day repeatability $(n = 8)$</th>
<th>Inter-day repeatability $(n = 3)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ ± SD</td>
<td>RSD (%)</td>
</tr>
<tr>
<td><strong>Retention time</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methomyl</td>
<td>0.30 ± 0.005</td>
<td>0.15</td>
</tr>
<tr>
<td>Methidathion</td>
<td>1.24 ± 0.003</td>
<td>0.22</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>2.13 ± 0.005</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Peak area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methomyl</td>
<td>526.17 ± 0.58</td>
<td>0.11</td>
</tr>
<tr>
<td>Methidathion</td>
<td>339.32 ± 1.46</td>
<td>0.43</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>1671.71 ± 1.80</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Peak height</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methomyl</td>
<td>320.67 ± 1.04</td>
<td>0.32</td>
</tr>
<tr>
<td>Methidathion</td>
<td>94.03 ± 0.31</td>
<td>0.33</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>244.45 ± 1.55</td>
<td>0.64</td>
</tr>
</tbody>
</table>
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 are 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
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