

OPTIMIZATION OF HPLC CONDITIONS FOR SIMULTANEOUS DETERMINATION OF CAPTAN, TERBUMETON AND DELTAMETHRIN

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A high performance liquid chromatographic method for the simultaneous determination of the pesticides captan, terbumeton and deltamethrin was developed. The best separation was achieved with analytical column Lichrosorb RP 18, 200 × 4.6 mm, 5 μm, by isocratic elution with mobile phase acetonitrile/water (80/20, V/V), flow rate 1.0 ml/min, UV diode array detection at 210 nm. The calibration curves, constructed in the range of 20 – 400 ng mass of pesticide injected in the column, followed linear dependence in the whole range and the values of the coefficient of correlation were higher than 0.9971. The sensitivity of the method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ) for each pesticide. Intra day precision of retention time and the peak area of each pesticide was also determined.

Key words: high performance liquid chromatography; pesticides; captan; terbumeton; deltamethrin

INTRODUCTION

Several hundred pesticides of different chemical nature are used for agricultural and non-agricultural purposes throughout the world today. Because of their widespread use, multiresidue analytical methods are needed for analyzing environmental and food samples which may contain residues of pesticides, pesticide transformation products and other environmental toxicants. The high performance liquid chromatography (HPLC) and gas chromatography (GC) have found increasing uses in pesticide residue analysis today [1–5].

Deltamethrin is a synthetic pyrethroid used to control insect pests in crops. Pyrethroid insecticides are generally very toxic. To study the environmental fate of these pesticides, a selective and very sensitive analytical method is essential. Mao et. al. [6] described an analytical method for the simultaneous determination of the synthetic pyrethroids tralomethrin and deltamethrin in water, sediment and fish tissue by normal phase HPLC (using a hexane/dioxane gradient solvent system on a silica column) with radiometric detection. In

addition, deltamethrin was determined in wine by using solid phase extraction (SPE) and gas chromatography with electron capture (ECD) and nitrogen-phosphorus (NPD) detectors [7], in wool after pour-on or plunge dipping application by the reverse phase (RP) HPLC [8]. The pesticide formulations producer ROUSSEL UCLAF for determination of deltamethrin in the pesticide formulation K-OBIOL PRIMATEX recommend HPLC method on Lichrospher Si 60 column by isooctane/dioxane as mobile phase and UV detection.

Analytical determinations of volatile fungicides such as captan are generally carried out by GC-ECD [9] and GC-MS (mass spectrometry) [10]. However, the liquid chromatography (LC) permits the direct analysis of some fungicides without the need of derivatization. LC methods combined with the liquid-liquid extraction (LLE) [11] and SPE [12] have been reported. Captan was determined in environmental waters by using HPLC-DAD (diode array detector), GC-ECD and GC-MS [13], in air and water by normal phase

(NP) HPLC and capillary GC [14], in various commodities (apples, tomatoes, potatoes) by GC-CIMS (chemical ionization mass spectrometry) [15], and in wine by GC [7] and by reversed phase (RP) HPLC [16]. The pesticide formulations producer CIBA-GEIGY for determination of captan in the pesticide formulation TOPAS C 50 WP recommend the LC chromatographic method on Lichrosorb RP 18 column by methanol/water as mobile phase and UV detection.

Triazines, such as terbutometon, are still widely used as herbicides worldwide. Analytical methods for determination of terbutometon in ground and sur-

face waters by LC-MS [17] and RP/HPLC-UV [18] have been reported.

In this paper, we introduce optimization of chromatographic conditions for the quantitative and simultaneous determination of captan, terbutometon and deltamethrin. The method consists of reversed-phase HPLC with diode array detection.

These pesticides are some of the most widely used in viticulture in Macedonia. So, we wanted to optimize the chromatographic method, which can be used, in future, for determination of the residues of these pesticides in wine, grape and other matrices.

EXPERIMENTAL

Standards and reagents

Acetonitrile (HPLC-grade) was from Sigma-Aldrich (Germany). Water was deionized and after that distilled from a glass apparatus. All solvents and solutions for the HPLC analysis were vacuum degassed by sonication before use. Pesticide standards were from BASF (Germany).

Stock solutions (1.0 mg/ml) were prepared by dissolving the appropriate amount of the standard pesticide in methanol and stored frozen. These methanolic solutions were stable during a long time. Working solutions (5 µg/ml) were prepared daily by diluting aliquots of the stock solutions with a mixture of acetonitrile / water (volume ratio 50 : 50) and stored at 4°C. The working solution was directly introduced in the HPLC system during the measuring.

HPLC analysis

Chromatography was performed on a HPLC system Varian equipped with a ternary gradient pump Model 9012, a 20 µl sample loop (Rheodyne) and a UV-Diode Array detector Model 9065. The analytical column was Lichrosorb RP 18, 200 × 4.6 mm, 5 µm (Hewlett-Packard). The composition of the mobile phase was acetonitrile / water,

volume ratio 80/20. The flow rate of the eluent was 1.0 ml/min., and the column temperature was ambiental. The diode array detector was set to monitor the signals of the analyte at a wavelength of 210 nm, where the compounds of interest have an absorption maximum.

Calibration curves

A set of working standard solution was prepared by diluting aliquots of the stock solutions, with a mixture of acetonitrile/water (volume ratio 50:50) to give concentrations ranging from 1.0 to 20.0 µg/ml for each compound studied. The calibration graphs were constructed by plotting the peak areas versus the amounts (ng) injected in the column. The curves followed the Beer's law in the investigated range of 20 – 400 ng mass of pesticide injected in the column.

Repeatability

The within day repeatability (intra day precision) of the retention time and the peak area was performed by 7 successive injections of the analytical standard of each pesticide respectively. The obtained results were statistically treated.

RESULTS AND DISCUSSION

The investigated pesticides were captan [*N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide], terbutometon [*N*2-*tert*-butyl-*N*4-ethyl-6-methoxy-1,3,5-triazine-2,4-diamine] and deltamethrin [(*S*)- α -cyano-3-phenoxybenzyl(1*R*,3*R*)-3-(2,2-di-

bromovinyl)-2,2-dimethylcyclopropanecarboxylate], which are fungicide, herbicide and insecticide, respectively. Their chemical structures are given on Fig. 1.

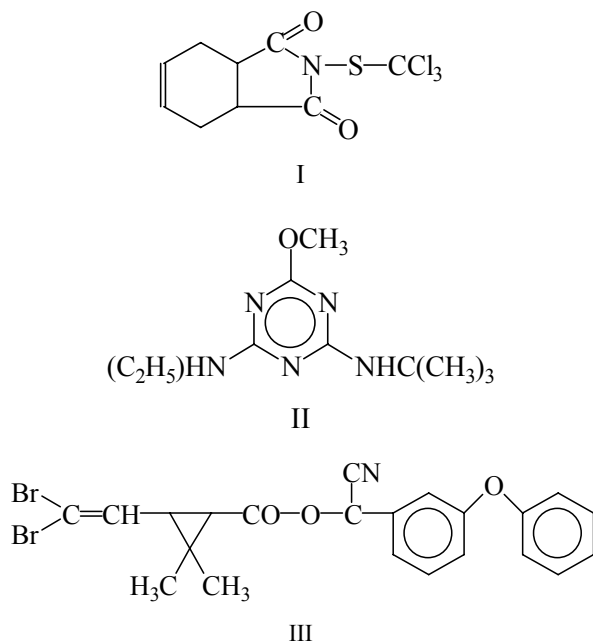


Fig. 1. Structural formula of captan (I), terbumeton (II) and deltamethrin (III)

The UV spectra (Fig. 2) of captan, terbumeton and deltamethrin in an acetonitrile/water mixture, volume ratio 80/20, show that they have an absorption maximum at 190, 195 and 217 nm, respectively. The wavelength of 210 nm is close to all these maximums, therefore measurements were performed at 210 nm. In addition, the UV spectra of compounds were used to check the peak purity and analyte peak identity.

The chromatogram (Fig. 3) shows the separation of captan, terbumeton and deltamethrin. For the best separation and symmetrical peak shape, several isocratic methods of acetonitrile/water (10 – 90 % acetonitrile) and several flow rates of mobile phase (0.5 – 1.5 ml/min) were evaluated. It was found that the best separation of investigated pesticides was achieved with mobile phase acetonitrile/water 80/20 (V/V) and flow rate 1.0 ml/min. Under these chromatographic conditions, the retention times were 3.37, 6.18 and 8.63 min for captan, terbumeton and deltamethrin, respectively.

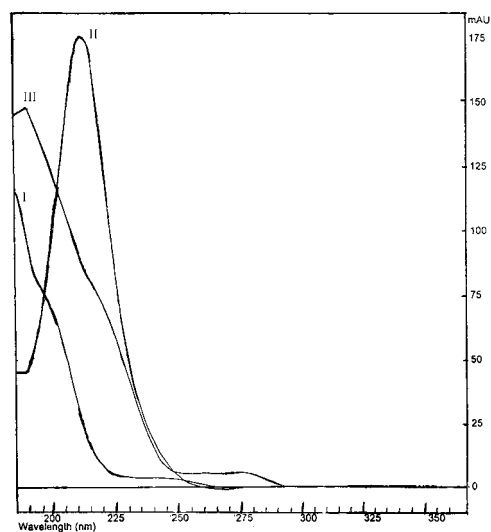


Fig. 2. UV spectra of captan (I), terbumeton (II) and deltamethrin (III) in acetonitrile/water 80/20 V/V

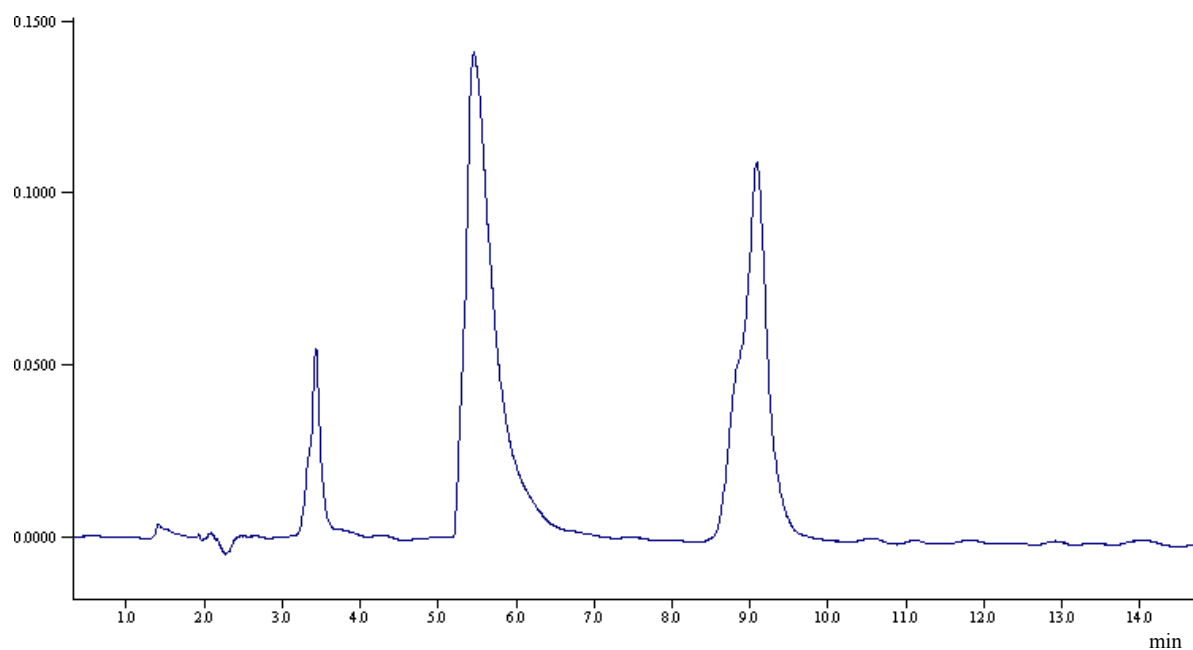


Fig. 3. HPLC/DAD (210 nm) chromatogram of standard mixture of captan (3.37 min, 380 ng), terbumeton (6.18 min, 370 ng) and deltamethrin (8.63 min, 390 ng)

The values calculated for the retention factors (k), separation factors (α) and resolution (R) between adjacent peaks are given in Table 1. It can be seen that these chromatographic conditions give high efficiency of separation for investigated pesticides.

Table 1

Retention factors (k), separation factors (α) and resolution (R) for investigated pesticides

| Compound | t_R (min) | k | α | R |
|--------------|-------------|------|----------|------|
| Captan | 3.36 | 1.07 | 2.62 | 6.0 |
| Terbumeton | 6.17 | 2.80 | 1.54 | 3.50 |
| Deltamethrin | 8.63 | 4.31 | – | – |
| Methanol | 1.63 | – | – | – |

The sensitivity of the method for each compound was determined by construction of a calibration curve in the low concentration region (0.15 – 0.75 $\mu\text{g/ml}$, i.e. 3 – 15 ng injected in the column). The limit of detection was calculated as three times the ratio between the SD and the slope of the low concentration curve ($\text{LOD} = 3 \cdot \text{SD} / \text{slope}$) and the limit of quantification as ten times the same ratio ($\text{LOQ} = 10 \cdot \text{SD} / \text{slope}$) [19]. The obtained results are listed in Table 2. Under these chromatographic conditions terbumeton shows the best sensitivity.

Table 2

Limit of detection (LOD) and limit of quantification (LOQ) for investigated pesticides

| Compound | Captan | Terbumeton | Deltamethrin |
|-----------------|----------------------|------------------------|-----------------------|
| Reg. Equation * | $y = 243.2x + 679.4$ | $y = 1541.5x - 2356.9$ | $y = 1201.5x - 744.7$ |
| SD | 1413.4 | 3986.1 | 5382.9 |
| LOD (ng) | 17.4 | 7.7 | 13.4 |
| LOQ (ng) | 58.1 | 25.8 | 44.8 |

* y – peak area

x – mass of pesticide (ng)

Calibration graphs were constructed by plotting the peak area as a function of the injected amount of the active ingredient. Statistical data, i.e. regression equation, relative standard deviation (RSD) and the value of the multiple correlation coefficients (R^2) are listed in Table 3. The data

show very good linearity for all compounds in the investigated area.

Table 3

Statistical data of calibration curves of investigated pesticides

| Compound | Regression equation * | RSD (%) | R^2 |
|--------------|-----------------------------|---------|--------|
| Captan | $y = 1.374641 \cdot 10^2 x$ | 5.459 | 0.9971 |
| Terbumeton | $y = 8.832499 \cdot 10^2 x$ | 4.776 | 0.9995 |
| Deltamethrin | $y = 4.325856 \cdot 10^2 x$ | 4.849 | 0.9972 |

* y – peak area

x – mass of pesticide (ng)

The results obtained for intra day precision [20, 21] of retention time and the peak area for all investigated compounds are listed in Table 4. A very good repeatability of the retention time for all compounds was achieved. The repeatability of the peak area for terbumeton and deltamethrin was good, but for captan was some worse.

Table 4

Intra day precision of retention time and peak area of captan, terbumeton and deltamethrin

| n | Captan (180 ng) | | Terbumeton (130 ng) | | Deltamethrin (190 ng) | |
|-------|-----------------|---------|---------------------|---------|-----------------------|---------|
| | t_R (min) | Area | t_R (min) | Area | t_R (min) | Area |
| 1 | 3.37 | 38402 | 6.19 | 91678 | 8.61 | 100746 |
| 2 | 3.37 | 39503 | 6.18 | 91375 | 8.60 | 99756 |
| 3 | 3.38 | 41503 | 6.16 | 91905 | 8.65 | 97559 |
| 4 | 3.36 | 39501 | 6.17 | 90503 | 8.65 | 98732 |
| 5 | 3.36 | 37901 | 6.18 | 89741 | 8.64 | 99101 |
| 6 | 3.37 | 39887 | 6.19 | 91883 | 8.63 | 99178 |
| 7 | 3.36 | 37665 | 6.18 | 92847 | 8.65 | 97665 |
| Mean | 3.37 | 39194.6 | 6.18 | 91418.8 | 8.63 | 98962.4 |
| SD | 0.007 | 1331.6 | 0.01 | 1017.7 | 0.02 | 1124.2 |
| RSD % | 0.22 | 3.40 | 0.17 | 1.11 | 0.24 | 1.14 |

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Резиме

ОПТИМИЗАЦИЈА НА HPLC УСЛОВИ ЗА ЕДНОВРЕМЕНО ОПРЕДЕЛУВАЊЕ НА КАПТАН, ТЕРБУМЕТОН И ДЕЛТАМЕТРИН

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Клучни зборови: високо ефикасна течна хроматографија; пестициди; каптан; терbumeton; делтаметрин

Со примена на високо ефикасна течна хроматографија е развиен метод за едновремено определување на пестицидите каптан, терbumeton и делтаметрин. Најдобро разделување е постигнато со примена на аналитичка колона Licrosorb RP 18, 200 × 4,6 mm, 5 μm, со изократно елуирање со мобилна фаза ацетонитрил/вода (80/20, V/V), проток на мобилната фаза 1,0 ml/min, UV детекција на 210 nm. Конструирани се калибрациони криви во подрачје од 20 – 400 ng маса од пестицид,

инјектирана во хроматографската колона, кои покажуваат линеарна зависност во испитуваното подрачје. Вредностите на коефициентите на корелација за сите калибрациони криви беа повисоки од 0,9971. Осетливоста на методот беше определена со пресметување на границата на детекција и границата на определување за секој пестицид. Беше определена дневната прецизност на ретенционото време и површината на пикот за секој пестицид.