ISSN-0011-1643
*CCA-2642*
*Original Scientific Paper*

# Quantitative Determination of Pesticides in Soil by Thin-layer Chromatography and Video Densitometry

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Received November 20, 1998; revised May 20, 1999; accepted June 9, 1999

Reversed phase thin-layer chromatography (RP-TLC) in conjunction with video densitometry has been used for the quantitative determination of a six-component mixture of pesticides. Excellent separation of propham, chlorpropham, atrazine, diflubenzuron, tetramethrin and α-cypermethrin was achieved using the methanol/water solvent system (volume fraction of methanol, $\varphi$ = 80%). Video densitometric quantification was validated for linearity, precision and detection limit. All results were satisfactory according to the validation requirements. The method was tested for the determination of pesticides from spiked soil using ultrasonic extraction with various solvents.

*Key words:* thin-layer chromatography, video densitometry, pesticide, soil.

## INTRODUCTION

It is of primary interest to estimate the fate of pesticides in soils. The increasing number of environmentally significant pesticides requires development of analytical techniques that allow simultaneous detection and quantitative determination of different pesticides with minimum extraction and cleanup steps.

Although other chromatographic methods (HPLC, GC) are nowadays widely used for pesticides analysis, thin layer chromatography (TLC) has retained its status as a valid and simple method for quantitative and qualitative analysis of pesticides and their metabolites in environmental samples.[1–4]

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The preferred technique for quantitative TLC is *in situ* measurement of zones on the chromatographic plate. The classical approach involves *in situ* measurements of UV/VIS absorption, fluorescence or fluorescence quenching with a slit-scanning densitometer. The novel approach is video densitometry. The chromatograms, illuminated by visible or UV light, are captured by a sensitive color CCD (charge coupled device) camera. A special digitizing board (frame grabber) assists computerized conversion of images into data and rapid image processing *via* a PC system. Special software developed for quantitative evaluation of chromatograms supports on-screen manipulation of image documents, selection of scan locations, data extraction and integration.

Today, video technology is becoming more and more popular, and is accepted as a useful tool, not only for the documentation but also for the quantitative evaluation of TLC chromatograms.

The aim of this work was to develop an accurate and simple method for the quantitative determination of six pesticides from several different structural groups: carbamate (propham, chlorpropham), 1,3,5-triazine (atrazine), benzoylurea (diflubenzuron) and synthetic pyrethroid (tetramethrin and α-cypermethrin). The method combines simple and efficient ultrasonic solvent extraction of pesticides from soil, their separation on RP-18 TLC plates with rapid *in-situ* quantitation based on video-technology.

## EXPERIMENTAL

### *Materials*

*Soil.* – Soil was collected on the Medvednica mountain near Zagreb. It had not been treated with any agrochemicals for at least ten years before collecting. Its composition was: sand 61.45%, silt 20.75%, clay 11.80%, organic matter 6.44%.

*Standards.* – Pesticides atrazine, propham, chlorpropham, tetramethrin, α-cypermethrin (PLIVA, Zagreb, Croatia) and diflubenzuron (Chromos, Zagreb, Croatia) were at least 98% pure. Pesticides investigated and their characteristics are listed in Table I. Standard stock solution of the mixture of pesticides was prepared by dissolving accurate amounts of powdered samples in methanol.

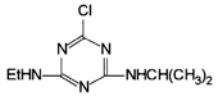
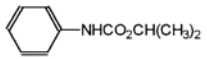
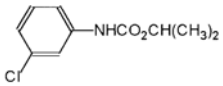
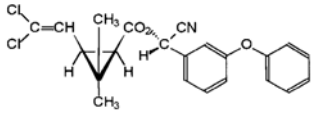
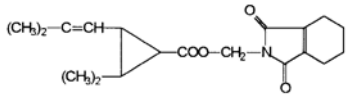
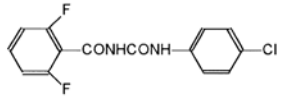
*Standard fortification mixture*. – Stock solution of the pesticide mixture was suitably diluted to give the mass concentrations of compounds as follows: α-cypermethrin 50 μg/mL, tetramethrin 100 μg/mL, diflubenzuron 10 μg/mL, chlorpropham 25 μg/mL, atrazine 20 μg/mL and propham 25 μg/mL.

### *Soil Spiking and Extraction Procedure*

10 mL of standard fortification solution was added to 100 g of dried and sieved soil. Additional 50 mL of methanol was added to cover the soil particles. The slurry

TABLE I

Pesticides studied and their characteristics

| Pesticide | Chemical class | Structure | Water solubility | Half-life |
|---|---|---|---|---|
| Atrazine | triazine |  | 28 mg/L (20 °C) | > 60 days |
| Propham | carbamate |  | 250 mg/L (20 °C) | 15 days (16 °C) |
| Chlorpropham | carbamate |  | 89 mg/L (25 °C) | 65 days (15 °C) |
| α-cypermethrin | synthetic pyrethroid |  | 0.01 mg/L (25 °C) | 13 weeks in loamy soil |
| Tetramethrin | synthetic pyrethroid |  | 0.05 mg/L (20 °C, pH = 6.5) | 12 weeks |
| Diflubenzuron | benzoylurea |  | 0.08 mg/L (20 °C, pH = 5.5) | > 150 days (pH = 5–7)<br>42 days (pH = 9) |

was mixed for approximately 30 min and then left at room temperature for at least 24 hours to allow complete solvent evaporation.

Accurately weighted spiked soil (15 g) was added to 20 mL of solvent and sonicated for 15 min in an ultrasonic bath (frequency 25–40 kHz). Sonication was repeated with another 20 mL of solvent. The efficiency of ultrasonic extraction with acetone, ethylacetate, diethylether, benzene, hexane, dichlormethane, chloroform and acetonitrile was determined. Combined extracts were filtered through Whatman No. 40 filter paper and the filter cake was washed with acetone. The filtrate was evaporated on a rotary vacuum evaporator at 40 °C to dryness. The residue was dissolved in 0.5 mL of acetone. This solution was directly spotted on the TLC plate.

### *Thin-layer Chromatography*

TLC was performed on pre-coated 20 × 20 cm glass-backed RP-18 $F_{254}$s plates from E. Merck, Darmstadt, FRG. The samples were applied to the plates as bands by means of a TLC applicator AS30 (Desaga, Heidelberg, FRG); volume 10 μL, bandwidth 6 mm; distance between the middles of the bands 15 mm, distance from the edge 25 mm. The mobile phase used was methanol/water (volume fraction of methanol, $\varphi$ = 80%). Ascending development was performed at room temperature in a Camag double-trough chamber without previous saturation. The migration distance was 100 mm. After development, plates were dried in a dryer at 50 °C.

### *Quantitative* in-situ *Determination by Video Densitometry*

The image analyzing system used was the CAMAG Video Documentation System in conjunction with the Reprostar 3 (Camag, Muttenz, Switzerland). Chromatograms were captured under UV light ($\lambda$ = 254 nm) by means of a 3CCD color video camera HV-C20 (Hitachi, Japan) with an acquisition device which numerizes images to 768 × 576 pixels. Parameters were as follows: close-up lens +2 Dpt, zoom lens 15 mm, integration period-exposure time 1 frame (= 20 ms), frame accumulation-off mode, aperture (F-stop number) 2.8. Image acquisition, processing and archiving were controlled *via* Video Store 2, documentation software running under Windows 95. The extended version of Camag Video Scan software was used for the quantitative evaluation of TLC chromatograms.

## RESULTS AND DISCUSSION

Several preliminary experiments were conducted in order to choose a chromatographic system suitable for the quantitative determination of the given mixture. Stationary phases investigated included silica gel, RP-2, RP-8 and RP-18 precoated layers using various solvent systems. The best separation of the investigated compounds and symmetric spots were obtained on RP-18 plates. The optimal composition of solvent system was selected by computer-assisted optimization (windows diagrams). The mobile phase for reversed-phase TLC is usually a two-solvent mixture composed of polar organic solvent and water, as the weak, strength-adjusting carrier. Two selectivity-adjusting solvents (methanol and acetonitrile) were tested.

The complete procedure was described previously.[5] The best separation was achieved using methanol/water solvent system with volume fraction of methanol, $\varphi$ = 80%. The $R_F$ values were as follows: α-cypemethrin 0.12, tetramethrin 0.18, diflubenzuron 0.28, chlorpropham 0.37, atrazine 0.43 and propham 0.51. Quantitative evaluation of TLC plates was performed by video densitometry, which is an alternative method to the classical evaluation by reflection/transmission slit-scanners. Due to the sequential nature of line scanning, quantitation with the slit-scanning densitometer is currently a limiting factor in planar analysis.[6] The main advantage of video densitometry is the visual control of scanning. This technique offers very fast and efficient data acquisition because of its multichannel scanning capability. Some investigations show a lower sensitivity and precision of video densitometry in comparison with the classical slit-scanning technique.[7,8] Our results show that the relative standard deviation of video densitometry is slightly higher than the RSD of slit-scanning densitometry, but within a reasonable range (2–5%) and the linearity is almost identical for both methods.[9,10] Sensitivity depends on the substances tested. Video densitometry shows a limitation in the determination of substances absorbing only in extremely short-wave light, while with substances that absorb UV in the 254 nm region (excitation maximum of the fluorescence indicator embedded in the layer) the results are comparable to those obtained with the slit-scanning densitometer.

The maximum absorption wavelengths of the tested pesticides were: atrazine 220 nm, propham 230 nm, chlorpropham 230 nm, tetramethrin 225 nm, α-cypermethrin 220 nm and diflubenzuron 260 nm and they can be detected by video technology *via* the fluorescence quenching at 254 nm.

To get more information about the possibilities of video densitometric determination of pesticides, the reproducibility of repeated measurements, sensitivity and linearity of peak area measurements were determined.

Repeatability, expressed as the relative standard deviation (RSD / %) of peak area measurement, was determined by applying ten bands of equal standard solution on one plate. The results are given in Table II. The RSD values were considerably high (from 3.9 to 5.2%). By closer examination of these results, it can be seen that the peak areas of bands No. 1 and 10 are different from the peak areas of bands 2–9. The principal source of error in video densitometry is inhomogeneous illumination of the TLC plate. Due to the position of UV lamps, the inhomogeneous illumination is especially marked on the vertical edges of a large chromatographic plate (20 × 20 cm). Therefore, we calculated the RSD for eight middle bands and for six middle bands (Table III). The results showed that significantly better results could be obtained by applying the samples far from edges, at least 4 cm (bands

TABLE II

Repeatability of video densitometric determination of pesticides on RP-18 plate (20 × 20 cm)

| Band | Peak area/pixels | | | | | |
|---|---|---|---|---|---|---|
| | α-cypermethrin | tetramethrin | diflubenzuron | chlorpropham | atrazine | propham |
| 1 | 2859 | 2541 | 2380 | 1956 | 3856 | 1683 |
| 2 | 2509 | 2398 | 2353 | 1659 | 3295 | 1599 |
| 3 | 2707 | 2434 | 2207 | 1924 | 3425 | 1494 |
| 4 | 2663 | 2512 | 2351 | 1811 | 3319 | 1544 |
| 5 | 2675 | 2476 | 2215 | 1849 | 3460 | 1558 |
| 6 | 2605 | 2344 | 2185 | 1772 | 3382 | 1487 |
| 7 | 2607 | 2289 | 2302 | 1860 | 3374 | 1588 |
| 8 | 2549 | 2439 | 2224 | 1729 | 3289 | 1488 |
| 9 | 2640 | 2486 | 2225 | 1856 | 3659 | 1596 |
| 10 | 2931 | 2734 | 2445 | 1697 | 3448 | 1699 |
| mean | 2674.5 | 2465.3 | 2288.7 | 1811.3 | 3450.7 | 1573.6 |
| RSD/% | 4.90 | 4.92 | 3.91 | 4.69 | 5.17 | 4.80 |

TABLE III

Comparison of repeatability of video densitometric determination depending on the position of bands on RP-18 plate (20 × 20 cm)

| Pesticide | RSD-1 / % (tracks 1–10) | RSD-2 / % (tracks 2–9) | RSD-3 / % (tracks 3–8) |
|---|---|---|---|
| α-cypermethrin | 4.90 | 2.52 | 2.19 |
| tetramethrin | 4.92 | 3.12 | 3.26 |
| diflubenzuron | 3.91 | 2.98 | 2.87 |
| chlorpropham | 4.69 | 2.68 | 2.70 |
| atrazine | 5.17 | 3.50 | 2.89 |
| propham | 4.80 | 3.17 | 2.91 |

2–9). In this case, the RSD values ranged from 2.5 to 3.5%, which is acceptable concerning the international requirements for quantitative TLC analysis. Figure 1. shows the image of that chromatographic plate captured with the Camag Documentation System using a CCD camera.

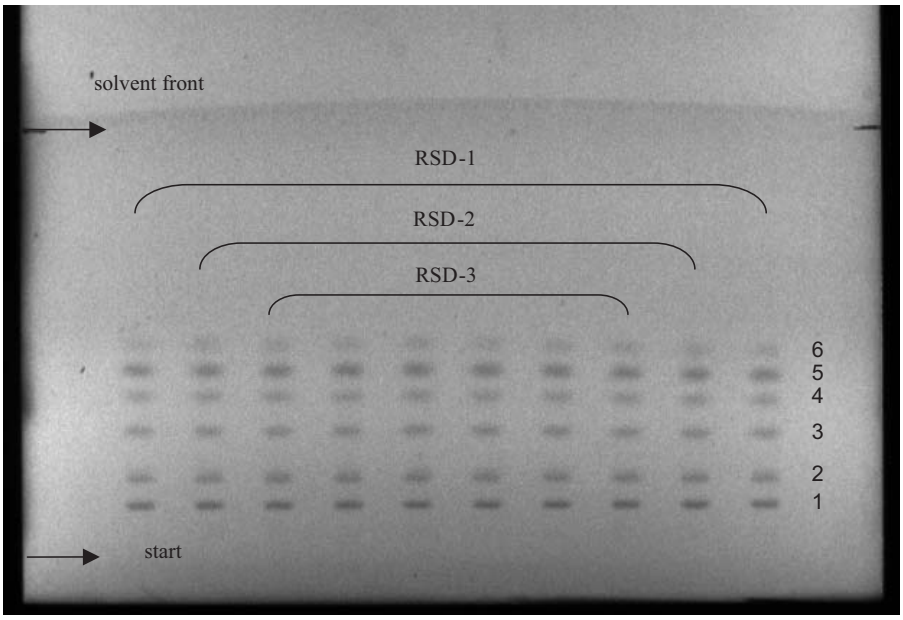



Figure 1. CCD image of the chromatographic plate used to test repeatability; 1) α-cipermethrin, 2) tetramethrin, 3) diflubenzuron, 4) chlorpropham, 5) atrazine, 6) propham.

In accordance with ICH guidelines, the video densitometric determination of pesticides was validated for linearity and detection limit. The results are summarized in Table IV. Mandel's test of linearity was used for determi-

TABLE IV

Linearity of quantitative determination of pesticides by RP-TLC and video densitometry

| Pesticide | Limit of detection (ng/spot) | Linear functional correlation (μg/spot) | Linear regression of peak area measurement | Correlation coefficient $r$ |
|---|---|---|---|---|
| α-cypermethrin | 200 | 0.50–25.0 | $A = 880\ c + 141$ | 0.990 |
| tetramethrin | 450 | 1.0–50.0 | $A = 500\ c - 161$ | 0.992 |
| diflubenzuron | 30 | 0.05–2.0 | $A = 12969\ c + 176$ | 0.994 |
| chlorpropham | 150 | 0.25–10.0 | $A = 1767\ c + 235$ | 0.991 |
| atrazine | 80 | 0.25–10.0 | $A = 4587\ c + 691$ | 0.994 |
| propham | 150 | 0.25–10.0 | $A = 2506\ c + 926$ | 0.993 |

nation of the calibration function. The second order polynomial function showed no significantly better fit, so the calibration data were fitted with a linear function. The linearity was good ($r > 0.99$) in a wide concentration range. The detection limit was determined as a minimal mass of pesticide giving the chromatographic peak that can be automatically measured. Minimal integration parameters were limited, as standard values, to 5 pixels for peak width, 100 pixels for peak height and 300 pixels for peak area. The obtained detection limits (30–450 ng/spot) make the quantitative analysis of selected pesticides feasible by taking a larger sample of soil for extraction (20–50 g) and by applying a larger volume of extract (50 μL) on the chromatographic plate.

As a next step, the efficiency of ultrasonic extraction was checked by recovery experiments. The ultrasonic solvent extraction is more rapid than the conventional shake-flask or Soxhlet extraction methods, and the solvent consumption is significantly lower. In TLC, since layers are not reused, it is possible to spot cruder samples than could be injected into an HPLC column. Therefore, purification or cleanup procedures for TLC are generally simple. In this work, the concentrated extract was directly spotted on chromatographic plate. Pesticides were extracted from soil by various organic solvents having a broad range of polarity. The efficiency of extraction was compared by recovery experiments. The results are summarized in Table V. The extraction with acetone gave the highest recovery rates for most pesticides. The recovery was generally higher than 95%, except for tetramethrin with the recovery of only 83%. Scanning profiles of the standard mixture, the extract from the spiked soil and the blind extract are shown in Figure 2. Soil extract (chromatogram B) contains some ballast components that either remain at the start position or migrate with solvent without affecting detection of analyte zones.

## CONCLUSIONS

The optimized chromatographic system (RP-18 precoated layer and methanol/water solvent system with volume fraction of methanol, $\varphi = 80\%$ was suitable for separation of the six-component pesticide mixture. Application of the video densitometer enables accurate and precise quantitation of separated compounds in TLC plates. In comparison with the classical slit-scanning densitometry, video densitometry has a slightly lower sensitivity and precision in the determination of selected pesticides.[9,10] However, the results of video densitometry are acceptable (RSD < 3.5%, good linearity $r >$ 0.99) from the analytical point of view. Therefore, this method can be successfully applied to the quantitative determination of pesticides from soil. Ultrasonic solvent extraction offers high selectivity combined with single-step sample preparation.

TABLE V

Recoveries of pesticides by ultrasonic extraction with various organic solvents

| Solvent | Sample recovery / % ($n$ = 5)[a] | | | | | |
|---|---|---|---|---|---|---|
| | α-cypermethrin | tetramethrin | diflubenzuron | chlorpropham | atrazine | propham |
| Acetone | 97.2 ± 4.4 | 83.4 ± 4.2 | 92.8 ± 4.9 | 106.3 ± 7.9 | 103.5 ± 2.8 | 95.3 ± 7.1 |
| Ethylacetate | 82.4 ± 3.8 | ND[b] | 96.5 ± 3.7 | 94.6 ± 5.7 | 101.2 ± 5.5 | 97.7 ± 6.1 |
| Hexane | 66.7 ± 5.6 | 78.0 ± 4.5 | 63.0 ± 6.6 | 60.1 ± 4.8 | 66.9 ± 6.2 | 36.4 ± 4.8 |
| Chloroform | 83.2 ± 3.5 | 82.5 ± 4.1 | 90.2 ± 3.7 | 62.1 ± 4.9 | 77.2 ± 7.4 | 49.4 ± 1.5 |
| Benzene | 77.0 ± 3.4 | 42.0 ± 4.8 | 90.1 ± 3.6 | 72.2 ± 5.6 | 64.2 ± 5.1 | 27.2 ± 3.5 |
| Acetonitrile | 69.4 ± 4.9 | ND | 79.1 ± 3.1 | ND | 71.2 ± 4.4 | 45.3 ± 4.6 |
| Dichloromethan | 76.8 ± 4.3 | 78.2 ± 5.7 | 91.0 ± 4.1 | 81.3 ± 6.3 | 89.2 ± 3.9 | 50.3 ± 6.5 |
| Diethyl ether | 75.9 ± 4.2 | 70.5 ± 5.8 | 80.5 ± 5.9 | 67.3 ± 5.3 | 78.0 ± 4.5 | 21.4 ± 6.1 |

[a]$n$, number of samples.
[b]ND, not determined (decomposed).

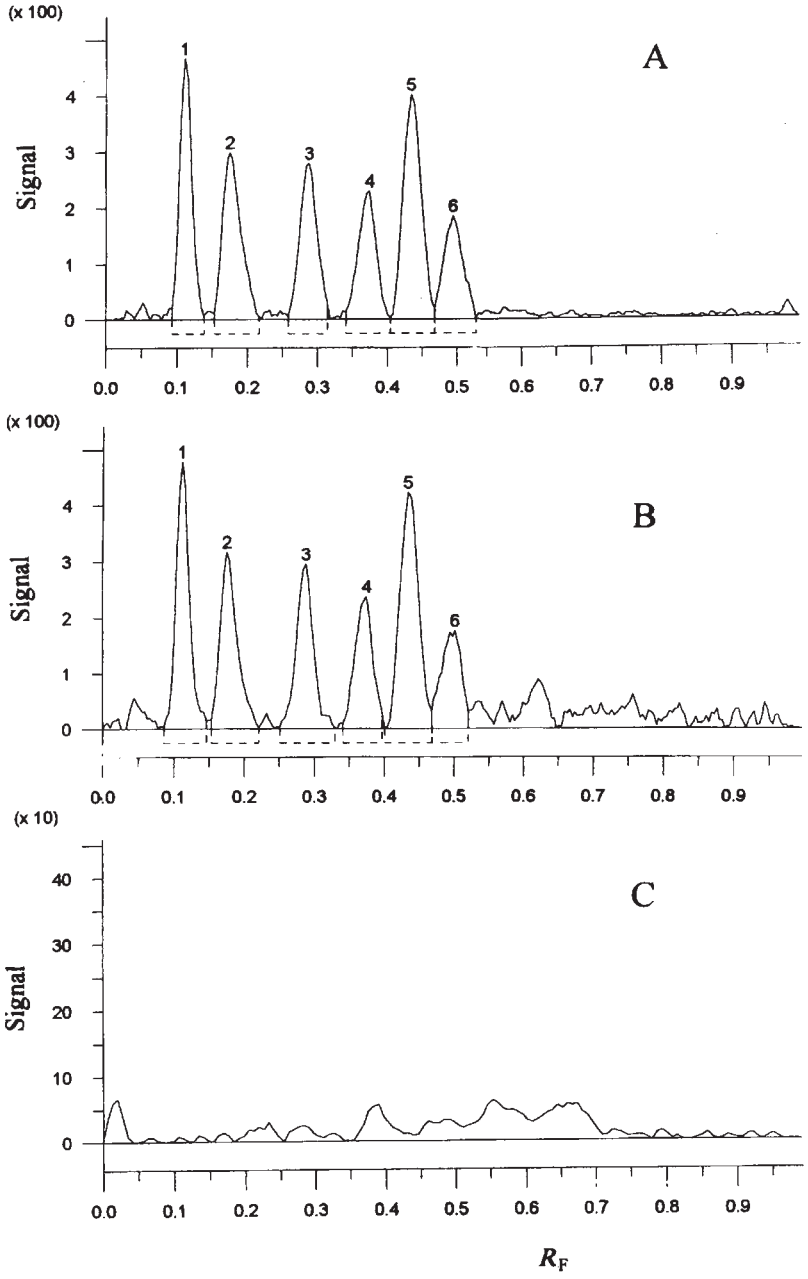



Figure 2. Chromatogram of pesticide mixture obtained by the video densitometer: A – standard mixture, B – extract from spiked soil, C – blind extract; 1) α-cypermethrin, 2) tetramethrin, 3) diflubenzuron, 4) chlorpropham, 5) atrazine, 6) propham.

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## SAŽETAK

### Kvantitativno određivanje pesticida u tlu tankoslojnom kromatografijom i videodenzitometrijom

*Mira Petrović, Sandra Babić i Marija Kaštelan-Macan*

Sastojci smjese pesticida kvantitativno su određeni primjenom tankoslojne kromatografije obrnutih faza i videodenzitometrije. Uporabom pokretne faze metanol/voda (volumni udio metanola, $\varphi$ = 80%) postignuto je vrlo dobro razdvajanje sljedećih pesticida: profama, klorprofama, atrazina, diflubenzurona, tetrametrina i $\alpha$-cipermetrina. Procijenjena je linearnost, preciznost i točnost kvantitativnog određivanja primjenom videodenzitometra. Svi su dobiveni rezultati bili zadovoljavajući, pa je metoda primijenjena za određivanje pesticida iz tla nakon ultrazvučne ekstrakcije.