

Multiresidue Analysis of Pesticides in Soil by Liquid-Solid Extraction Procedure

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SUMMARY

A multiresidue method for simultaneous determination of four pesticides (diazinon, acetochlor, aldrin and carbofuran) belonging to different pesticide groups, extracted from soil samples, is described. The method presented is based on liquid-solid extraction (LSE) and determination of pesticides, i.e. the pesticides were extracted by methanol-acetone mixture, purified on florisil column and eluted by ethyl acetate-acetone mixture. Optimization of the main parameters affecting the LSE procedure, such as the choice of purification sorbent, as well as the elution solvent and its volume, were investigated in details and optimized. Also, validation of the proposed method was done.

Gas chromatography-mass spectrometry (GC-MS) was used for detection and quantification of the pesticides studied. Relative standard deviation (RSD) and recovery values for multiple analysis of soil samples fortified with 30 µg/kg of each pesticide were below 8% and higher than 89%, respectively. Limits of detection (LOD) for all the compounds studied were less than 4 µg/kg.

Keywords: Pesticides; Soil; Residues; Analytical methods

INTRODUCTION

The safety of food production depends on a number of factors, including the history of persistent toxic chemicals applied. As pesticides are the most widespread agrochemicals, their monitoring in plant material and soil in which it had grown is frequently performed. Considering the movement and transformation processes in which pesticides participate after being introduced into the environment (Đurović, 2011), as well as their potential persistence and effects they can produce, it seems that soil contamination

over a long period of time is the biggest problem in terms of food safety.

Detailed investigation of pesticide residues began in mid-20th century in reaction to early uses of organic pesticides. Numerous methods of analysis were developed, mostly using the conventional liquid-liquid extraction (LLE) or Soxhlet extraction. Although these extraction procedures may be considered conventional, no small number of publications dealing with their applications in pesticide residues determination in samples of different origin can be found in recent years (Kreuzig et al., 2000; Doong and Liao, 2001; Bavcon et al., 2003;

Caballo-Lopez and Luque de Castro, 2003; Kodaka et al., 2003; Monkiedje et al., 2003; Navarro et al., 2003; Nawab et al., 2003; Stipicevic et al., 2003; Sassman et al., 2004; Barra et al., 2005; Wanga et al., 2008). On the other side, as these methods are part of a standard procedure, many laboratories currently use them for routine analysis.

Considering all of this and the fact that most of these methods are based on determination of pesticides belonging to the same chemical group, the aim of this study was to develop a multiresidue LSE method for simultaneous determination of pesticides that have distinct chemical structures and belong to different pesticide groups. The main parameters affecting LSE procedures, such as the choice of purification sorbent, as well as elution solvent and its volume, were investigated and optimized. Additionally, the most important analytical parameters, such as linearity, limit of detection (LOD), precision and confidence of the presented method were determined under the optimized LSE procedure.

MATERIAL AND METHODS

Reagents and materials

The pesticides chosen for this study were: diazinon, acetochlor, aldrine and carbofuran (Dr Ehrenstorfer, Germany). The main physico-chemical properties of the pesticides studied are presented in table 1. Stock solutions (1 g/L) of each pesticide standard were prepared by dissolving the weighed amount in acetone (J. T. Baker, Deventer, Holland). Working standard mixed solutions (10 mg/L and 1 mg/L of each compound) were prepared weakly by diluting individual stock solutions with acetone. All solvents used in the study (hexane, acetone, methanol and ethyl acetate) were purchased

from J. T. Baker (Deventer, Holland), as well as anhydrous sodium sulfate (99.0% purity). Florisil (60-100 mesh) was purchased from Serva (Germany), and silica gel (0.2-0.5 mm) and aluminum oxide (neutral) from Kemika (Croatia). Before use, sodium sulfate was dried for 24 h at 130°C, silica gel for 2 h at 110°C (SILICA), and florisil for 4 h at 600°C (FL, 600), 2 h at 300°C (FL, 300), and 18 h at 130°C (FL, 130). Aluminum oxide (Al₂O₃) was used without previous treatment or purification.

An uncontaminated soil sample originating from Kikinda was used in the study. The main physico-chemical properties of the soil were: pH (H₂O)=8.39; organic matter content=3.17%; sand content=73.96%; silt content =22.60%; clay content=3.44%. The soil was air dried and sieved (2 mm) before using.

Extraction was performed in polypropylene centrifuge tubes with caps (50 ml) (Sarstedt, Germany). Filter papers 1PS, 150 mm diameter (Watman Int. Ltd., Maidstone, UK) and a centrifuge (UZ 4, Iskra, Slovenia) were used in the soil extraction procedure.

Instrumentation

A gas chromatograph-mass spectrometer (GC/MS) (CP-3800/Saturn 2200, Varian, Australia) with 30 m x 0.25 mm x 0.25 µm VF-5ms column (Varian) was used. The GC was programmed as follows: initial temperature was 120°C, then increased to 170°C at 8°C/min and held for 4.5 minutes, increased to 280°C at 9°C/min and held for 5.5 minutes. Helium was used as the carrier gas and its flow rate was 1.1 ml/min.

The ion trap mass spectrometer was operated in the electron impact/selected ion monitoring (EI/SIM) mode. The ion trap and transferline temperatures were set to 220°C and 250°C, respectively. One specific pesticide ion was selected for detection and quantification, while a second one was used for confirmation. The ions

Table 1. The main physico-chemical properties of pesticides studied (PesticideManual, 2000-2001; <http://sitem.herts.ac.uk/aeru/footprint/en/>, 2012)

Pesticide	Chemical class	M _r ^a (g/mol)	S _w ^b (mg/L)	Log K _{ow} ^c	H ^d (Pam ³ /mol)
Carbofuran	Carbamate	221.3	320.0	1.52	5.00 x 10 ⁻⁵
Diazinon	Organophosphorus	304.3	60.0	3.30	6.09 x 10 ⁻²
Acetochlor	Chloroacetamide	269.8	223.0	4.14	3.83 x 10 ⁻¹
Aldrine	Organochlorine	364.9	0.027	6.5	6.97 x 10 ⁻³

^aMolecular weight; ^bwater solubility; ^cpartition coefficient between n-octanol and water (as the log value); ^dHenry's constant

inspected (m/z) were as follows: 223 (146) for acetochlor, 179 (199) for diazinon, 263 (293) for aldrine and 164 (149) for carbofuran.

Soil extraction optimization

The main parameters affecting the LSE procedure, such as the choice of purification sorbent, as well as elution solvent and its volume, were investigated and optimized. As our previous studies (Đurović et al., 2010a, 2010b) of multiresidue determination of pesticides by solid phase microextraction (SPME) showed that two successive extractions with methanol:acetone=1:1 mixture (MET-AC) were the best choice among different solvents tested (water, hexane, acetonitrile, acetone, methanol, methanol:acetone=1:1 and methanol:acetone:hexane=2:2:1), the same extraction mixture was chosen in this study, too.

LSE optimization was done using the following procedure: soil samples (10 g) were weighed in 50-mL centrifuge tubes and fortified with mixed pesticides at 30 $\mu\text{g}/\text{kg}$ of each pesticide studied. The spiked samples were homogenized for 15 min using a rotary stirrer and left for 24 hours prior to further analysis. After addition of 2 g of Na_2SO_4 (anh), the samples were homogenized twice with 15 mL of MET-AC for 30 min on the rotary stirrer and then centrifuged for 5 min at 4000 rpm. The extract was filtered and evaporated to dryness at 40°C using a rotary evaporator. The residues were redissolved in 5 mL of solvent (ethyl acetate-acetone mixture (4:1, v/v) (EA-AC) or acetone-hexane mixture (1:4, v/v) (AC-HEX)) and 2 mL of the obtained solution was passed through a glass column previously filled with 1 g of Na_2SO_4 (anh.) and 5 g of sorbent (florisil, silica gel or aluminum oxide). The sorbent was entered into the column with 30 mL of appropriate solvent (EA-AC or AC-HEX). Pesticides were eluted from the column by 30 mL of EA-AC or AC-HEX. Eluate was evaporated to dryness, the residues were redissolved in 2 mL of acetone, and 1 μL of the obtained solution was transferred into a vial for GC-MS analyses.

RESULTS AND DISCUSSION

The main parameters affecting the LSE determination of selected pesticides in soil were investigated and optimized. Optimization was done by a well-structured step-by-step approach including the choice of a most suitable purification sorbent as well as elution solvent and its volume.

Selection of sorbent and elution solvent

As most pesticides included in this study have non-polar or intermediate polar character (Table 1), it was suitable to use polar adsorbents for purification of the obtained soil extracts. Since silica gel, aluminum oxide and florisil (Mg deposited on silica gel) have an expressive polar character, these materials were included as sorbents in the study. Additionally, it is well known that the kind of sorbent activation could significantly affect purification efficiency (Zweig, 1978). Therefore, different ways of sorbent activation were included in our study, as presented in the section Materials and Methods/Reagents and materials.

On the other side, considering the characteristics of the sorbents used and the nonpolar or moderately polar nature of the studied pesticides, it was most appropriate to use nonpolar solvents or solvents of moderate polarity as elution solvent. In the presented study, two combinations of such solvents were tested (EA-AC and AC-HEX).

The results obtained for the combination of these three sets of experimental parameters (sorbent types, different ways of sorbent activation and different elution solvents used) are presented in figure 1. The recovery values of most of the selected pesticides and experimental parameters are shown to be higher than 70%, and therefore they may be considered as acceptable in terms of method confidence (Taverniers, et al., 2004). As shown in figure 1, somewhat lower recoveries were obtained for: carbofuran and the combination of FL, 130 as sorbent and AC-HEX as elution solvent, and SILICA and AC-HEX; diazinon and the combination of Al_2O_3 and EA-AC; and aldrine with combined FL, 130 and AC-HEX, and SILICA and Al_2O_3 with both elution solvents. An explanation of these results could be the exceptional complexity of the soil matrix and the fact that the samples were fortified with pesticides and left to rest for 24 h prior to analysis (intending to better simulate real-life conditions).

However, comparing the efficiency of sorbents, we concluded that florisil efficacy was highest for all pesticides studied, regardless of its activation mode or type of elution solvent used. And finally, comparing the results obtained for florisil as a sorbent, and both ways of its activation, and the type of elution solvents used, the best efficiency was achieved with the combination of FL, 130 and EA-AC. Therefore this combination of sorbent and solvent was chosen for further work.

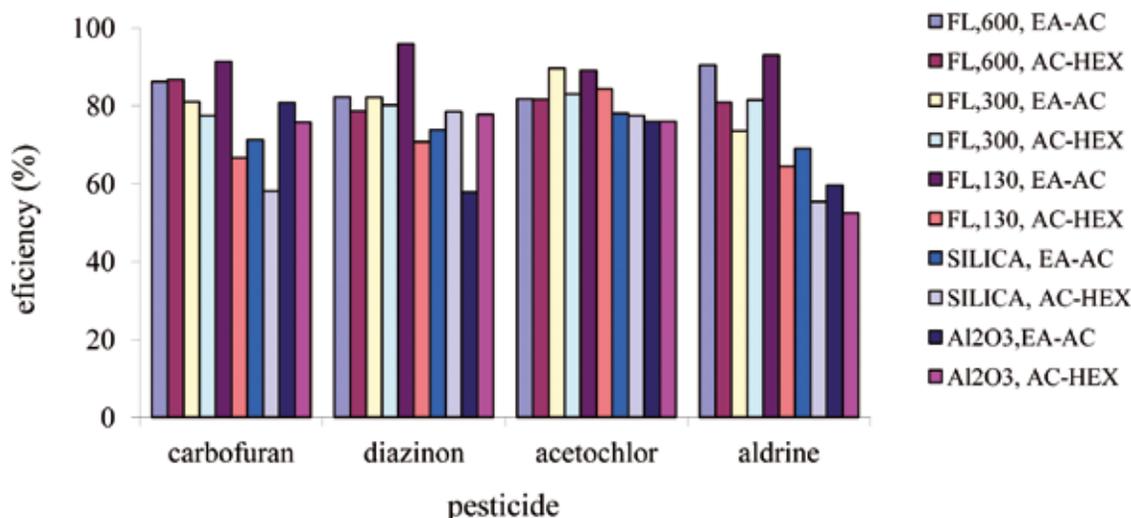


Figure 1. Dependence of method efficiency on sorbent types, different ways of sorbent activation and different elution solvents

Selection of optimum elution solvent volume

After selecting the most suitable purification sorbent and elution solvent, the next step was to determine the optimal elution solvent volume. In order to do that, after methanol-acetone extraction of the spiked soil samples and purification of the obtained extract on FL, 130 column, the volume of EA-AC as elution solvent was varied in the range of 20 to 35 mL. As figure 2 shows for all pesticides under study, the best method efficiency was achieved using 30 mL of EA-AC as elution solvent. Therefore, this volume was chosen as optimal for further work.

Method validation

The optimized LSE method, based on pesticides extraction by methanol-acetone mixture, purification on florisol column and elution by ethyl acetate-acetone mixture was validated. The most important analytical parameters, such as linearity, limit of detection (LOD), precision and confidence of the presented method were tested and determined.

Linearity was tested over the concentration range from 10 to 300 $\mu\text{g}/\text{kg}$ for all pesticides studied. The obtained arrangements and correlation coefficients (R) are presented in table 2. The acquired correlation coefficients exceeded 0.99 for all compounds, indicating good linearity.

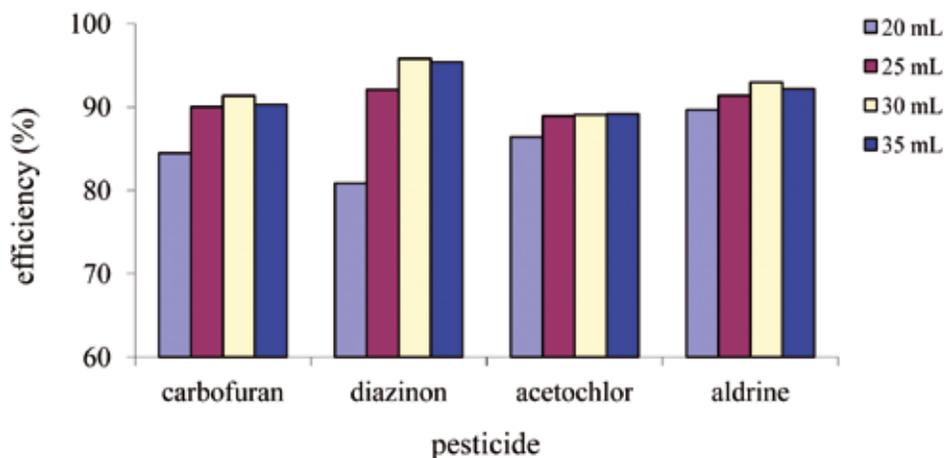


Figure 2. Dependence of method efficiency on the volume of elution mixture of ethyl acetate-acetone (4:1, v/v)

Table 2. Linearity ranges ($\mu\text{g}/\text{kg}$), correlation coefficients (R), limits of detection (LODs, $\mu\text{g}/\text{kg}$), relative standard deviations (RSDs, %) and recoveries (%) for pesticides studied

Pesticide	Concentration range ($\mu\text{g}/\text{kg}$)	R	Recovery (%)	RSD (%)	LOD ($\mu\text{g}/\text{kg}$)
Carbofuran	10-300	0.992	91.30	6.07	1.19
Diazinon	10-300	0.994	95.79	6.02	1.52
Acetochlor	10-300	0.995	89.04	7.55	0.73
Aldrine	10-300	0.997	92.94	5.08	3.95

Confidence and precision of the proposed LSE method were determined by performing four consecutive measurements of soil samples fortified with up to 30 $\mu\text{g}/\text{kg}$ of all pesticides studied. The recovery and RSD values are presented in table 2. The recovery values exceeded 89% for all pesticides studied, indicating that the proposed method can be used for efficient determination of the selected pesticides from complex matrix samples such as soil. As RSD values for all compounds under study were below 8%, the proposed method is satisfactory in terms of precision, too.

The limit of detection (LOD) was determined to be $3.29 \times s_B$ (where s_B is the blank standard deviation), according to IUPAC recommendations (Currie, 1999). As table 2 shows, the obtained LODs for all pesticides studied were less than 4 $\mu\text{g}/\text{kg}$, indicating that the sensitivity of the proposed method is good enough for soil sample analysis.

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REFERENCES

- Barra, R., Popp, P., Quiroz, R., Bauer, C., Cid, H. and Von Tümppling, W.*: Persistent toxic substances in soils and waters along an altitudinal gradient in the Laja River Basin, Central Southern Chile. *Chemosphere*, 58(7): 905-915, 2005.
- Bavcon, M., Trebše, P. and Zupančić-Kralj, L.*: Investigations of the determination and transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionisation detector. *Chemosphere*, 50(5): 595-601, 2003.
- Caballo-López, A. and Luque de Castro, M.D.*: Continuous ultrasound-assisted extraction coupled to on line filtration–solid-phase extraction–column liquid chromatography–post column derivatisation–fluorescence detection for the determination of *N*-methylcarbamates in soil and food. *Journal of Chromatography A*, 998(1-2): 51-59, 2003.
- Currie, L.A.*: Detection and quantification limits: origins and historical overview. *Analytica Chimica Acta*, 391(2): 127-134, 1999.
- Doong, R.A. and Liao, P.L.*: Determination of organochlorine pesticides and their metabolites in soil samples using headspace solid-phase microextraction. *Journal of Chromatography A*, 918(1): 177-188, 2001.
- Durović, R.*: Procesi koji određuju sudbinu pesticida u zemljištu. *Pesticidi i fitomedicina*, 26(1): 9-22, 2011.
- Durović, R., Gajić Umiljendić, J., Cupać, S. and Ignjatović, Lj.*: Solid phase microextraction as an efficient method for characterization of the interaction of pesticides with different soil types. *Journal of the Brazilian Chemical Society*, 21(6): 985-994, 2010a.
- Durović, R., Đorđević, T., Šantrić, Lj., Gašić, S. and Ignjatović, Lj.*: Headspace solid phase microextraction method for determination of triazine and organophosphorus pesticides in soil. *Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes*, 45(7): 626-632, 2010b.
- Kodaka, R., Sugano, T., Katagi, T. and Takimoto, Y.*: Clay-catalyzed nitration of a carbamate fungicide diethofencarb. *Journal of Agricultural and Food Chemistry*, 51(25): 7730-7737, 2003.
- Kreuzig, R., Koinecke, A. and Bahadır, M.*: Use of supercritical fluid extraction in the analysis of pesticides in soil. *Journal of Biochemical and Biophysical Methods*, 43(1-3): 403-409, 2000.
- Monkiedje, A., Spiteller, M. and Bester, K.*: Degradation of racemic and enantiopure metalaxyl in tropical and temperate soils. *Environmental Science & Technology*, 37(4): 707-712, 2003.
- Navarro, S., Vela, N., García, C. and Navarro, G.*: Persistence of simazine and terbuthylazine in a semiarid soil after organic amendment with urban sewage sludge. *Journal of Agricultural and Food Chemistry*, 51(25): 7359-7365, 2003.
- Nawab, A., Aleem, A. and Malik, A.*: Determination of organochlorine pesticides in agricultural soil with special reference to γ -HCH degradation by *Pseudomonas* strains. *Bioresource Technology*, 88(1): 41-46, 2003.
- Pesticide Manual*: British Crop Protection Council – C D S Tomlin (12th ed.), 2000-2001.
- PPDB*: Pesticide Properties DataBase, University of Hertfordshire. <http://sitem.herts.ac.uk/aeru/footprint/en/> Accession date: February 02, 2012.

Sassman, S.A., Lee, L.S., Bischoff, M. and Turco, R.F.: Assessing *N,N'*-dibutylurea (DBU) formation in soils after application of *n*-butylisocyanate and benlate fungicides. *Journal of Agricultural and Food Chemistry*, 52(4): 747-754, 2004.

Stipicevic, S., Fingler, S., Zupancic-Kralj, L. and Drevenkar, V.: Comparison of gas and high performance liquid chromatography with selective detection for determination of triazine herbicides and their degradation products extracted ultrasonically from soil. *Journal of Separation Science*, 26(14): 1237-1246, 2003.

Taverniers, I., De Loose, M. and Van Bockstaele, E.: Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends in Analytical Chemistry*, 23(8): 535-552, 2004.

Wanga, X., Zhaob, X., Liub, X., Lib, Y., Fub, L., Hub, J. and Huang, C.: Homogeneous liquid-liquid extraction combined with gas chromatography-electron capture detector for the determination of three pesticide residues in soils. *Analytica Chimica Acta*, 620(1-2): 162-169, 2008.

Zweig, G.: *New and Updated Methods.* Academic Press, Inc., London, UK, 1978.

Metoda tečno-čvrste ekstrakcije za određivanje pesticida u uzorcima zemljišta

REZIME

U radu je prikazana metoda tečno-čvrste ekstrakcije (LSE) za istovremeno određivanje pesticida (diazinon, acetohlor, aldrin i karbofuran) u zemljištu, koji na osnovu svoje strukture pripadaju različitim hemijskim grupama. Metoda se zasniva na metanolsko-acetonskoj ekstrakciji uzoraka zemljišta, koja je praćena prečišćavanjem na florisilu i eluiranju sa smešom etil-acetata i acetona. Izvršena je optimizacija glavnih eksperimentalnih parametara koji mogu da utiču na efikasnost LSE metode, kao što su izbor sorbenta za prečišćavanje zemljišnog ekstrakta pesticida, izbor adekvatnog eluenta i njegove optimalne zapremine. Takođe, izvršena je i validacija optimizovane metode.

Detekcija i kvantifikacija ispitivanih pesticida su izvršeni metodom gasno-masene spektrometrije (GC-MS). Vrednosti relativnih standardnih devijacija (RSD) i prinosa određivanja pesticida u uzorcima zemljišta obogaćenim do koncentracija od 30 µg/kg svakog jedinjenja su ispod 8%, odnosno iznad 89%, respektivno, dok su granice detekcije (LOD) niže od 4 µg/kg.

Ključne reči: Pesticidi; zemljište; ostaci; analitičke metode