Pol. J. Environ. Stud. Vol. 21, No. 4 (2012), 973-992

Original Research

Comparison of Extraction Techniques by Matrix Solid Phase Dispersion and Liquid-Liquid for Screening 150 Pesticides from Soil, and Determination by Gas Chromatography

Bożena Łozowicka*, Magdalena Jankowska, Ewa Rutkowska, Piotr Kaczyński, Izabela Hrynko

Plant Protection Institute – National Research Institute, Regional Experimental Station,
Laboratory of Pesticide Residues,
Chełmońskiego 22, 15-195 Białystok, Poland

Received: 5 May 2011
Accepted: 28 February 2012

Abstract

Interest in the determination and screening of pesticide residues in soil is caused by widespread use of chemical pesticides in agriculture, which increase soil contamination. Two extraction techniques, matrix solid phase dispersion (MSPD) and liquid-liquid extraction (LLE) of residual pesticides (acaricides, insecticides, herbicides, and fungicides) from soil were compared. Advantages and disadvantages of both approaches were discussed. Pesticides from different chemical classes (organohalogen, organophosphorus, carbamates, pyrethroids, strobilurines, triazoles) were quantified by GC with a dual system detection – electron capture (EC) and/or nitrogen-phosphorus (NP). The MSPD was validated by comparing it with conventional LLE. Recovery studies were carried out at three levels: 1) ranged between 0.005-0.05 mg/kg, 2) 0.05-0.5 mg/kg, and 3) 0.25-2.5 mg/kg and average recoveries obtained for these compounds ranged from 72.4 to 120% for MSPD and 70.6-120% for LLE with relative standard deviations (RSDs) below 20%. Both methods were linear over the range assayed, 0.005-2.5 mg/kg. The uncertainties of the analytical methods were lower than 25.6% and 30%, with and without recovery correction, respectively.

The rapid and practical MSPD technique has found a particular application in determining 147 pesticide residues of different physicochemical properties in soil with satisfactory validation parameters. The study estimated that MSPD has significant advantages over LLE because, coupled with simultaneous stage of purification, it allowed for a radical reduction time of analysis and its cost. MSPD fulfilled the requirements of multiresidue techniques. The method is reliable and can be useful for routine monitoring in soil.

Keywords: soil, pesticide residue, liquid-liquid extraction, matrix solid phase dispersion, gas chromatography

*e-mail: B.Lozowicka@iorpib.poznan.pl

Introduction

Pesticides are widely applied on agricultural crops to protect from disease, weeds, and insect damage. The widespread and inappropriate use of chemical pesticides in agriculture unfavorably affects the whole ecosystem by entering into the food chain and polluting the soil, air, ground, and surface water. Although organochlorine insecticides like aldrin, dieldrin, DDT, and its metabolite or lindane for instance have been banned years ago in many countries based on their mutagenic, carcinogenic, and endocrine disrupting properties, they still can be found in environmental samples due to their persistence and lipophilic properties [1-3]. Organophosphorous insecticides (like chlorpyrifos, chlorpyrifos-methyl, or chlorfenvinphos) and triazine herbicides (like atrazine, simazine, metribuzine) are the most commonly used pesticides around the world. They and their metabolites are detected in the environment, although several members of these classes have been banned for years [3]. Among the different groups of pesticides, herbicides are more likely to pollute soils. Phenylurea and urea herbicides (e.g. diuron, linuron, or metamitron) are in a sense emerging herbicides in recent years, but are already included on an EU list of priority substances containing some endocrine disruptors [4] and monitored in environmental samples [5].

As a consequence of the implementation of EU Directive 91/414 [6], all pesticides have to be subjected to an authorization procedure. The condition for issue of an authorization is that the pesticide or its residues do not exert unacceptable effects on human and animal health and not persistent in soil to extend accumulation and cause pollution problems.

Applied pesticides can degrade in soil surface, adsorb onto organic matter of soil, clay, or lixiviate, and can reach surface waters through superficial fluxes and leaching, contaminate groundwater by percolation, and disperse in atmosphere or accumulate as residues in food. These processes are highly dependent on the type of pesticide, soil, crop, climatic conditions, and application procedures, and thus the fate of pesticides is highly variable. Soil characteristics are important to pesticide movement. Clay soils have a high capacity to adsorb pesticides, whereas sandy soils and organic matter in the soil have a much lower capacity to adsorb these compounds.

The fate of pesticides in soil is controlled by the chemical, biological, and physical dynamics of this matrix [7]. Pesticides are degraded by chemical reaction (such as photolysis, hydrolysis, oxidation, and reduction) [8], and microbiological [9] processes. These processes take place on the surface of the soil or deeper soil layers. For instance, photolysis is important only for pesticides present on the surface of the soil and the rate of breakdown is influenced by the intensity and spectrum of sunlight, length of exposure, and the properties of the pesticide, whereas degradation by microorganisms such as fungi and bacteria is taking place at all soil depths, with varying rates due to changes in the density.

Knowledge of the fate and behavior of pesticides in agricultural soils is required for the assessment of environmental pollution and for the selection of remediation strategies.

Determination of pesticides in soil is a challenging task because of low concentrations of analytes, the great variety of pesticides covering a wide range of polarities, and the complex blend of substances. Various analytical techniques aimed at extracting, isolating, and determining pesticides and their transformation products in soil have been recently published [10-48]. Table 1 gives a summary of analytical methods used for the quantification of pesticides in soil. The steps involved are matrix preparation, extraction, clean-up, fractionation, and determination. Those analytical techniques usually require highly advanced equipment [20, 38, 40].

Sample preparation plays an important role in the field of pesticide residue analysis. Traditionally, Soxhlet extraction and ultrasound-assisted extraction are the conventional pretreatment methods to extract pesticides from soil and sediment samples [49]. However, these methods usually are laborious, time-consuming, and need large volumes of toxic organic solvents. Recently, research has been focused on those sample preparation methods that allow for the reduction of organic solvent consumption, the exclusion of sample component degradation, the elimination of additional sample clean-up, and pre-concentration steps before chromatographic analysis as well as the improvement of extraction efficiency, selectivity, and/or kinetics.

Matrix solid-phase dispersion (MSPD) is a simple and cheap sample preparation procedure involving simultaneous disruption and extraction of various solid and semi-solid materials [50-52]. It permits complete fractionation of the sample matrix components and has the ability to selectively isolate a single compound or several classes of compounds from the sample. MSPD involves direct mechanical blending of sample with a sorbent (mainly Florisil, C_{18}, alumina, or silica). In this process, the sorbent acts both as an abrasive material disrupting sample architecture and as a 'bound' solvent that assists in accomplishing sample disruption. The sample is dispersed over the surface of the bonded-phase support material, producing a unique mixed character phase for conducting target analyte isolation. After homogenization, blended mixture is transferred into an SPE barrel and subjected to elution with an appropriate eluent. Finally, the obtained eluate undergoes the analytical procedure [53].

MSPD has many advantages over the traditional techniques, such as the use of smaller amounts of organic solvent, lower solvent cost, and reduced toxic organic solvent. LLE technique is time-consuming, laborious, and requires large volumes of both sample and organic solvents, but still is the most popular.

The GC detection methods most commonly used for this purpose are nitrogen-phosphorus (NPD) [17, 20], flame ionization (FID) [19], electron-capture detection (ECD) [10, 11], and mass spectrometry (MS) [13-15]. In gas chromatography analysis, some applications simultaneously use

Table 1. Methods for analysis of pesticides and their metabolites in soil.

Pesticide group	Extraction	Clean up	Determination	Pesticides	[Ref]
Organochlorines	LSE methanol:water (4:1, v/v) 1 h LSE acetone:hexane 30 min	Partitioning chloroform Florisil	GC-EC	DDT, HCH, aldrin endosulfan	[10] [11]
Organochlorines	HS-SPME polydimethylsiloxane (PDMS, 100 mm) and divinylobenzene (PDMS, 65 mm)	-	GC-EC	aldrin, dieldrin, endrin, endosulfan, DDT, heptachlor, metoxychlor hexachlorocyclohexane	[12]
Organochlorines Carbamates	LPME acetone-water (stirring) hollow fiber (3 ml of toluene) SPME acetone:water polyacrylate, 85 mm	- -	GC-MS GC-MS	pentachlorobenzene, molinate, hexachlorobenzene, lindane, alachlor 2,5-dimethylphenol, 2,3,5-trimethylphenol, 1,2,3,4,5-terachlorobenzene	[13]
Organochlorines Organophosphorus	PLE acetonitrile:methanol (9:1, v/v)	SPE Ph+C_{18}+Al Diol+C_{18}+Al CN+Al	GC-MS	polycylic aromatic hydrocarbons and polychlorinated biphenyls (N-, P-, and Cl- containg pesticides)	[14]
Organochlorines Organophosphorus Carbamates Pyrethroids	MAE acetone:hexane (3:2, v/v) 120ºC, 20 min Soxhlet method acetone:hexane (3:2, v/v) for 8 h	- -	GC-MS	dimethoate, chloroneb, methomyl, oxamyl, toxaphen, DDT, monocrotophos, chlorpyrifos, diazinon, cypermethrin, lindane	[15]
Organochlorines	MAE hexane:acetone (1:2, v/v), 5 min Soxhlet and sonicator method	-	GC-EC	BHC, DDE and dieldrin	[16]
Organophosphorus	SFE 5% methanol in CO_2	diol-modified silica gel	GC-NP	fenpropimorh, pirimicarb, parathion-methyl, parathion-ethyl	[17]
Organophosphorus	MSPD soil+water+Florisil hexane-ethyl acetate	-	LC-UV	phenthoate and its enantiomeric ratio	[18]
Organophosphorus	LSE ethyl acetate	-	GC-FID	diazinon, malathion	[19]
Organophosphorus	LSE-buffered water	ligand-exchange, anion-exchange	GC-MS derivatisation	glyphosate and AMPA	[20]
Organophosphorus Triazine herbicides	SPME 10% methanol in water polydimethylsiloxane fiber (PDMS, 100 mm)	-	GC-EC GC-MS	carbophenothion, chlorpyrifos, methidathion, parathion-methyl, atrazine	[21]
Triazines	LSE metanol:water (3:1, v/v)	SPE C_{18}	GC-MS	46 pesticides (organophosphorus and organochlorine compounds, carbamates, anilides, anilines, and amides)	[22]
Triazines Carbamates Sulfonylureas			SFC-APCIMS (supercritical fluid chromatography interfaced with atmospheric pressure chemical ionization mass spectrometry)	ametryn, atrazine carbofuran chlorsulfuron, metsulfuron-methyl, and bensulfuron-methyl	[23]
Triazine herbicides	MAWE acetonitrile:water (80:20, v/v) acetonitrile 80ºC, 5 min	SPE Linchrolut EN cartridges	LC-UV	metribuzin and major conversion products	[24]
Triazine herbicides	SPME polyacrilate, 85 mm	-	GC-MS	propazine, terbuthylazine, sebuthylazine, ametryl, prometryn, terbutyn	[25]
Triazine herbicides	MAE	SPME CV-DVB, 65 mm (carbowaxdivinylbenzene)	GC-MS	simazine, atrazine, propazine, prometryn	[26]

Table 1. Continued.

Pesticide group	Extraction	Clean up	Determination	Pesticides	[Ref]
Triazine herbicides	LSE acetone:hexane (2:1, v/v)		GC and HPLC with selective detectors	simazine, atrazine terbuthylazine, cyazine, ametryn, prometryn, atraton and their dealkylated products	[27] [28]
Triazine herbicides Chloroacetanilide herbicides	MAE acetonitrile, 80ºC, 5 min	-	GC-NP or GC-MS	atrazine, cyanazine, metribuzine, simazine, deethylatrazine, deisopropylatrazine acetochlor, alachlor, metolachlor	[29]
Chloroacetanilide herbicides	LSE acetonitrile:water (60:40, v/v) 0.5 h methanol:water and acetone	-	LC-MS SPE with PSA	acetochlor and metabolites acetochlor and propisochlor	[30] [31]
Acidic herbicides	LSE alkaline media	C_{18}	LC-MS	MCPA and its metabolites	[32]
Acidic herbicides	MAE phosphate buffer-methanol (50:50, v/v), 80ºC, 10 min	C_{18}	LC-UV	2,4-D; MCPA; 2,4,5-T; dichlorprop; 2,4-dichlorophenol; 2,4-DB; 2,4,5-trichlorophenol	[33]
Acidic herbicides	PLE	C_{18}-Hydra	LC-UV	benzatone; 2,4-D; triclopyr; 2,4,5-T; 2,4-Tp	[34]
Acidic herbicides	ASE methanol:water (80:20, v/v) solution in NaCl, 90ºC	GCB	LC-MS	arylphenoxypropionic herbicides	[35]
Phenylurea herbicides	MAE dichloromethane:methanol (90:10), 70ºC, 10 min	-	reversed phase LC-UV	linuron, monuron, monolinuron, isoproturon, metobromuron, diuron	[36]
Carbamates	LSE methanol	C_{18}	LC-Fluorescence (post column derivatisation)	N-methylcarbametes (oxamyl, dioxacarb, metlocarb, carbofuran, carbaryl, isoprocarb)	[37]
Carbamates	MAE methanol, 80ºC, 5 min SFE 5-10% methanol in CO_2	- -	LC-UV	propoxur, propham, methiocarb, chlorpropham	[38]
Carbamates Ureas	MAE	-	LC-UV	butylate, carbaryl, carbofuran, chlorpropham, ethiofencarb, linuron, metobromuron, and monolinuron	[39]
Dinitroanline	PLE acetonitrile:water (7:3, v/v), 120ºC	-	LC/MS/MS	trifluralin metabolites	[40]
Fungicides	LSE ethyl acetate	-	GC-NP GC-EC	quintozene, chlorothalonil, tolclofos-methyl, dichlofluanid, triadimefon, procymidone, myclobutanil, cyproconazol, oxadixil, ofurace, benalaxyl, nuarimol, fenarimol, pyrazophos	[41]
Various classes	LSE ethyl acetate	-	GC-MS		[42]
Acaricides Fungicides Herbicides Insecticides	LSE methanol:water (4:1, v/v), 20 min	SPE HLB Oasis cartridge	LC-MS	32 pesticides	[43]
Fungicides Insecticides	LSE water-acetonitrile mixture	Partitioning dichloromethane	GC-NP and GC-MS	25 pesticides	[44]
Various classes	MAE water:acetonitrile (1:1, v/v) and hexane		GC-EC	4 pesticides (trifluralin, metolachlor, chlorpyrifos, triadimefon)	[45]
Various classes	USE water:acetonitrile (1:2, v/v) QuEChRS acetonitrile PLE water:acetonitrile (1:2, v/v) 140ºC, 20 min.	- Bondesil-PSA -	GC-MS HPLC-MS/MS	24 pesticides (urea, phenylurea, and triazine herbicides, organochlorines, organophosphorus, carbamates pyrethroids, and other)	[46]
Various classes	SPE	C_8 and C_{18}	GC-NP	7 pesticides (molinate, atrazine, carbofuran, pirimicarb, prometryn, matahion and tetrachlorvinphos)	[47]
Various classes	LSE acetoritrile	-	HPLC-MS-MS	54 pesticides	[48]

Table 1. Continued.
Abbreviations:
ASE – accelerated solvent extraction
GCB – graphitized carbon black
GC-EC – gas chromatography with electron capture detector
GC-MS – gas chromatography coupled with mass spectrometry
GC-NP – gas chromatography with nitrogen phosphorus detector
HPLC – high-performance liquid chromatography
HS-SPME – head space solid-phase microextraction
LC-UV – liquid chromatography with ultraviolet detection
LC-MS – liquid chromatography coupled with mass spectrometry
LPME – liquid-phase microextraction
LSE – liquid-soild extraction
MAE – microwave-assisted extraction
MAWE – microwave-assisted water extraction
MSPD – matrix solid phase dispersion
PLE – pressurized liquid extraction
QuEChRS – Quick, Easy, Cheap, Effective, Rugged, and Safe extraction method
SFE – supercritical fluid extraction
SPE – solid-phase extraction
SPME – solid-phase microextraction
USE – ultrasonic solvent extraction

two GC detectors connected to two columns containing different stationary phase [54] or the same stationary phase [55]. Applications where the flux of one single column is divided between two different GC detectors or the splitter is placed after the injection port or the precolumn and sample runs in parallel onto two gas chromatographic columns of different polarities that may be used to analyze substances with different chemical structures while injecting the sample only once. In our work the determination was carried out with capillary gas chromatography using electron-capture (EC) and nitrogen-phosphorus (NP) detection as selective detection methods in parallel. The use of selective detection methods allowed much lower limits of detection (LODs) to be achieved. The low LODs obtained permit the flux to be divided into two different detector systems after the GC column in order to quantify pesticide residues of different natures. In the case of positive results we used a column with different polarity. Therefore, the parallel response was found to be a useful criterion for peak identification down to the limit of detection.

Among cited works, only a few of them relate to the determination of a wide spectrum of pesticides belonging to different chemical classes [46]. It is interesting that not many pesticides and all their possible metabolites have been monitored in soil. Therefore, there is a need for more studies in this field.

The goals of the present study were to develop and validate under ISO 17025 criteria a multi-residue screening method (MRM) to identify and quantify broad-spectrum pesticides (about 150 active substances) and their metabolites in soil by GC, and an autosampler with two selective detectors simultaneously: NPD and ECD. Two techniques: matrix solid phase dispersion (MSPD) and liquid-liquid extraction (LLE), were used to extract acaricides, insecticides, herbicides, and fungicides – widely used plant protection products belonging to different chemical classes, including: organohalogen, organophosphorus, carbamates, pyrethroids, strobilurines, and triazoles from soil. MSPD procedure has also employed the use some extra column adsorbents to obtain purification of extracts. Several parameters of the MSPD method (weight of sample, amount, and type of dispersant solid-phase and used for clean-up, extraction solvent) and for LLE (extraction solvent) were optimized.

MSPD technique for extraction pesticide residues in soil have been chosen because there are still few reports about their usefulness and on the other hand criticizing or comparing them with other techniques providing good results. Finally, the MSPD method was applied to the simultaneous quantification of 147 compounds in soil.

Every analytical result is associated with uncertainty. Therefore, the uncertainty of the result of a determination must be calculated and accompany its presentation. Moreover, an analytical result should be recorded not as one value, but according to the values of a continuous random variable, as a confidence interval, i.e. the interval likely to include the expected value. In recent years many laboratories have been under pressure to present uncertain data on the analytical results instead of just giving standard deviations due to most probable requirements for ISO standard 17025. In the method validation procedures, estimation of the uncertainty is one of the main focuses of interest due to its importance in showing data quality. Detailed analysis of uncertain sources can guide the analytical chemist about the critical stages of the method where uncertainty should be reduced. For most purposes, an expanded uncertainty (U) should be used. The expanded uncertainty provides an interval within which the value of the analyte is believed to lie within a higher level of confidence. According to the EURACHEM/CITAC document [56] the "bottom-up" approach can be used for estimation of combined standard uncertainty. This strategy splits the analytical process in single steps, estimating the individual contribution of each one to the uncertainty of the final results. Subsequently, it is

possible to decide which are the more significant and which are negligible (and therefore do not deserve special attention).

The identification uncertainty in the proposed method is very important. Mechanical and physical properties of the certain type of the soil may influence the uncertainty. Recoveries and LODs and LOQs might be different, depending on organic matter content [57].

Experimental Procedures

Chemicals and Reagents

All reagents used were residue analysis grade. Acetone, dichloromethane, diethyl ether, n-hexane and petroleum ether for pesticide residue analysis were provided by J.T. Baker (Deventer, Holland), as well as Florisil (60-100 mesh). Anhydrous sodium sulphate was purchased from Fluka (Seelze-Hannover, Germany). Silica gel (230-400 mesh) was obtained from Merck (Darmstadt, Germany). All sorbents were activated at 600ºC (very important). Certified Reference Material (CRM) was purchased from Tusnovics Instrument Poland Sp. z o.o. (Trading and Service Company, Poland).

Soil Samples

Blank soil samples were collected from the vincinty of Bialystok. The physico-chemical characteristics of soil are the following: textural class-loamy sand, organic matter 1.45%, pH 6.6, % silt 22.45 (0.002-0.05 mm), % sand 75.32 (0.05-2 mm), % clay 2.43 (< 0.002 mm).

Field loamy sand samples were collected from private customers from the Podlasie region (physico-chemical properties: organic matter content < 2%, pH 6-7.5, % silt 18-38, % sand 59–68, % clay 2–6.4).

Pesticide Standards

Pesticides (154) were obtained from Dr. Ehrenstorfer Laboratory (Germany). Standard solutions were prepared in acetone and stored at 4ºC (purity >95%). Multicompound standard working solutions (M10÷M18, whose composition is presented in Table 3) were prepared by dissolving appropriate amounts of each stock solution in n-hexane/acetone (9:1, v/v) mixture (concentration range 0.005-2.5 mg/ml).

Preparation of Spiked Soil Samples

Representative portions of residue-free/blank soil (the similar type of soil of field soil and certified soil material) (500 g) was air-dried at about 40ºC and then sieved through a mesh with a grain size of 2 mm. They were stored at room temperature until fortified.

Spiked samples were prepared by adding an appropriate volume of spiking solution to 2 g or 10 g of soil, depending on the procedure used. The spiked samples were left for 30 min.

Soil samples were extracted by two techniques, MSPD and LLE (according to the scheme presented in Fig. 1). The main purpose of this step was to calculate the average of the recovery percent of investigated pesticides by both extraction techniques.

Procedure 1 – LLE Extraction

To 10 g of soil sample 60 ml of dichloromethane/acetone /petroleum ether (1:1:1, v/v/v) was added and shaken for 1 h. Extract was filtered and 20 ml portion of dichloromethane/acetone/petroleum ether (1:1:1, v/v/v) was added and shaken 10 min. Extracts were combined into the same splitter and then 50 ml of petroleum ether was added. LLE extraction was carried out in two stages by the addition of appropriate portions of water (150 ml and 10 ml), each time discarding the aqueous layer. The combined organic layers were passed through a filter with 20 g anhydrous sodium sulphate.

Procedure 2 – MSPD Extraction

2 g of soil sample were put in a mortar with 4 g solid support (Florisil). All was manually blended using a pestle to produce a homogeneous mixture which was packed into a glass macro column with anhydrous sodium sulphate (5 g) and silica gel (2.5 g). The adsorbed analytes were eluted using 15 ml hexane/acetone (8:2, v/v) and 15 ml of hexane/acetone/diethyl ether (1:2:2, v/v/v). Stages in MSPD extraction procedure are shown in Fig. 2.

The extracts obtained from *Procedures 1* and *2* were evaporated to dryness using a rotary evaporator at about 40ºC and dried residue was dissolved in appropriate volume of hexane/acetone (9:1, v/v) (2 ml for *Procedure 1* and 10 ml for *Procedure 2*), and then transferred to 2 ml vials for further GC analysis.

GC Instrumentation

An Agilent 7890A gas chromatograph (Santa Clara, CA, USA) was equipped with an automatic split-splitless injector Model Agilent 7683B with a ^{63}Ni micro-electron capture detector (μEC) and nitrogen phosphorous detector (NP). The flux at the end of the GC column was divided into two branches by means of a "Y" press-tight connector connected at one end to the GC column and on the other to the two detectors (Fig. 3). Data acquisition and processing were performed using Chemstation (Hewlett-Packard, version B.04.01) software.

A DB-35 midpolarity column (35%-Phenyl)-methylpolysiloxane) with low bleed (30 m, 0.32 mm I.D., 0.5 μm film thickness) supplied by Agilent (Little Falls, DE, USA) was employed.

The operating conditions were as follows:

- for detectors – injector temperature: 210ºC; carrier gas: helium at a flow-rate of 1.9 ml/min; detector temperature: 300ºC EC and 310ºC NP; make-up gas: nitrogen at a flow-rate of 60 ml/min (EC) and 8 ml/min (NP), hydrogen 3.0 ml/min, air 60 ml/min

- for oven – initial temperature: 120ºC increase to 190ºC at 13ºC /min, then to 240ºC at 8ºC /min and finally to 295ºC at 16ºC /min and hold 20 min (EC and NP)

The 2 μL volume of final sample extract was injected at 210ºC in splitless mode (purge-off time 2 min). Total time of analysis: 35.07 min and equilibration time 2 min. Quantification was performed to compare the height of peaks obtained in samples with those found in matrix-matched calibration standards mixture (±0.005 min for positive match).

In the case of positive peaks of pesticides detected above LODs, the results were confirmed by analysis on the different polarity column. A fused silica capillary column, HP-5, with 5% phenyl methyl siloxane as nonpolar stationary phase (30 m, 0.32 mm I.D., 0.5 μm film thickness), was found ideal for conformational analysis under the following conditions:

- for detectors – injector temperature: 210ºC; carrier gas: helium at a flow-rate of 3.0 ml/min; detector temperature: 300ºC (EC and NP); make up gas: nitrogen at a flow-rate of 57 ml/min (EC) and 8 ml/min (NP), hydrogen 3.0 ml/min, air 60 ml/min;
- for oven – initial temperature: 120ºC increase to 190ºC at 16ºC /min, then to 230ºC at 8ºC /min and finally to 285ºC at 18ºC /min and hold 10 min (EC and NP).

Total time of analysis: 22.4 min.

Method Validation

Pesticide-free soil samples were used to validate the applied methods in accordance to EURACHEM/CITAC Guide [56]. Calibration standards were prepared in matrix solution (by adding respective spiking solutions to blank matrix of soil) to produce final concentrations between 0.005-2.5 mg/kg.

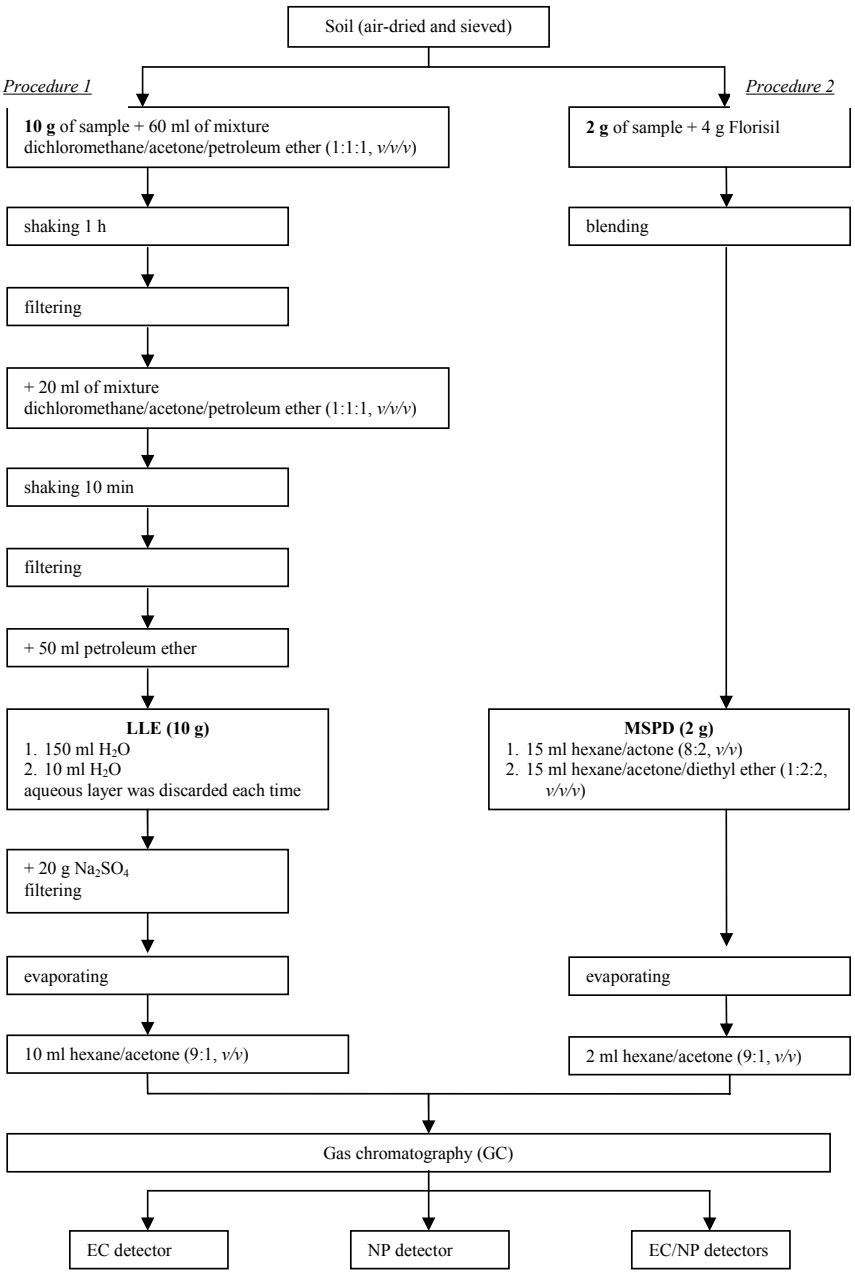

Fig. 1. Scheme of the LLE and MSPD extraction procedures for isolation and purification of pesticides from soil samples.

Recovery data was obtained at three different concentrations within the range in the matrix. Blank samples were spiked by the addition of appropriate volume of a mixture of standard pesticide solution, then samples were left for 1 h. The samples were then prepared according to the procedures described above. Method accuracy and precision were evaluated by performing recovery studies of each extraction technique. Three different levels have to be analyzed with five replicates for each level and these have to be performed on 5 distinct days in order to calculate the method repeatability, as the standard deviation (SD) of the recovery mean. The precision was expressed as the relative standard deviation RSD (%). The limit of quantification (LOQ) was assessed as the lowest concentration of a given pesticide giving a response with RSD lower than 20%.

Estimation of Uncertainty

The action that was taken during an uncertainty estimation of the analytical result was according to the Guide to the Expression of Uncertainty in Measurement [58]:

- it was defining the measuring procedure and determining the measured value
- developing a mathematical model to be used for calculating analytical results based on the measured parameters
- finding values for all possible parameters that can influence the final results, and estimating the associated standard uncertainties
- applying the law of propagation of uncertainty in order to calculate the combined standard uncertainty of the final results.

The combined standard uncertainty was determined by using ProNP3 (PROLAB) software.

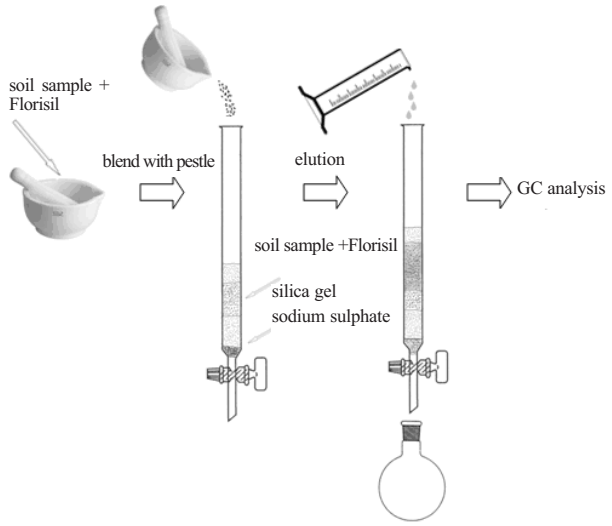

Fig. 2. Steps in soil sample extraction by MSPD and clean-up by column chromatography.

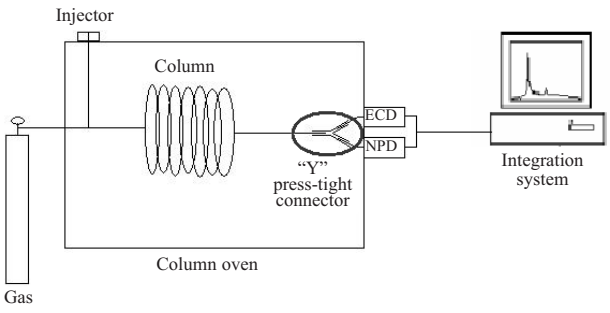

Fig. 3. GC with dual system EC/NP detectors scheme.

Results and Discussion

Matrix Effect

The possible matrix effect on the chromatographic response was studied. The system was evaluated both calibration standards made in pure solvent and matrix-matched calibration standards to evaluate if there were cases of signal suppression or augmentation.

When standards were prepared by spiking blank soil extract sample with known amounts of pesticides, higher peaks were accomplished for the same pesticide concentrations. Different responses were obtained with standard mixtures in solvent. There was an evident matrix effect that enhanced the chromatographic response of pesticides. Therefore, the quantification of pesticides was performed with fortified blank samples.

Optimization of Extraction Techniques

The studies were carried out with the varying of different parameters: sample weight, sorbents, extracting solvents, and extraction time. The conditions for the best extraction efficiency were used for the rest of the study (Table 2).

We carried out a simultaneous process of isolation of pesticides and purification steps by adsorption column chromatography before chromatographic analysis.

Preliminary studies were performed to evaluate MSPD efficiency for the effects of sample weight, dispersant and clean-up solid phase, amounts of sorbents, solvent or solvent mixture, and ratio and volume (Table 2) in extracting different groups of pesticide residues from soil samples. Analyte recoveries were calculated against the sample weight. The increase of sample weight up to 10 g did not affect the recoveries of compounds. Dispersion sorbents such as Florisil, silica gel, and basic alumina activated and deactivated (by the addition of water) were tested. The use of deactivated sorbents like 12% basic alumina and 5% silica gel sorbents gave recoveries below 40% (first activated at 130ºC and then deactivated by the addition of 12% and 5% of water). The increase of activation temperature of sorbents to 600ºC was necessary to increase the recoveries of pesticides. The optimum extraction conditions with high recovery were conducted with 2 g soil samples and 4 g of Florisil (activated at 600ºC) as a sorbent with simultaneous stage of clean-up due to the presence of interfering peaks from the matrix. Using clean-up adsorbent at the bottom of the column mini-

Table 2. Optimum extraction factors.

Factor	MSPD extraction		LLE extraction	
	Experimental conditions	Optimum conditions	Experimental conditions	Optimum conditions
Sample weight	2-10 g	2 g	5-20 g	10 g
Solvent (ratio, volume)	acetonitrile 10-50 ml acetone/methanol (2:1, 9:1, v/v) 25 ml hexane/diethyl ether (1:1, 1:2, 2:1, v/v) 50-100 ml hexane/acetone (8:2, v/v) 10-50 ml hexane/acetone/diethyl ether (1:1:1, 1:2:2, 2:2:1, v/v/v) 10-50 ml	hexane/acetone (8:2, v/v) 15 ml hexane/acetone/diethyl ether (1:2:2, v/v/v) 15 ml	acetonitrile 50-150 ml methanol:water (4:1, v/v) 50 ml acetone/dichlormethane (1:1, 2:1, v/v) 50-100 ml dichlormethane/petroleum ether (1:1, 2:2, v/v) 50-100 ml dichloromethane/acetone/petroleum ether (1:1:1, 2:1:1, 1:1:2, v/v/v) 50-100 ml	dichloromethane/acetone/petroleum ether (1:1:1, v/v/v) 60 ml + additional 20 ml portion of mixture
Extraction time	Factors not subjected to optimization	-	0.5-2 h	1 h and 10 min
Dispersal phase - activated (high temperature) - deactivated (addition of appropriate volume of water)	Florisil, silica gel, basic alumina (130-600ºC, 5-15%)	Florisil activated at 600ºC	Factor not subjected to optimization	-
Cleaning sorbent	anhydrous sodium sulphate, silica gel, Florisil (activated at 600ºC) C_{18} (500 mg or 1 g)	silica gel, activated at 600ºC	anhydrous sodium sulphate, silica gel, Florisil (all activated at 600ºC)	-

mized such interference. Purification of the extract was tested with several adsorbents like neutral alumina, silica, Florisil, or their combinations. Using 2 g Florisil as the dispersion phase and 2.5 g silica gel as the cleaning adsorbent and anhydrous sodium sulphate gave the best recoveries. Acetone, acetonitrile, hexane, diethyl ether, and its mixtures in different ratios were tested. The extraction solvent was 15 ml of hexane/acetone (8:2, v/v) and hexane/acetone/diethyl ether (1:2:2, v/v/v).

In preliminary tests with LLE, the influence of solvent and extraction time was tested. LLE was carried out with the parameters given in Table 2. Samples were extracted under different conditions, as shown in the results to obtain the optimal LLE conditions with this procedure. Analyte recoveries were calculated against extraction volume at different dichloromethane/acetone/petroleum ether ratios. Analyte recoveries were increased when the volume was increased to 80 ml. Further increase of the extraction volume resulted in no significant improvement of analyte recoveries. During experiments pesticides were satisfactorily recovered from 10 g soil sample by 60 ml of mixture dichloromethane/acetone/petroleum ether (1:1:1, v/v/v) and an additional 20 ml portion of this mixture for 10 min shaking. Analyte recoveries increased when the extraction time was 1 h, but a further increase of the extraction time to 2 h provided slightly smaller values. Therefore 1 h extraction time was selected for this procedure. No clean-up step was necessary.

However, the MSPD extraction offers an important savings in time, reduces the sample amount, and requires less solvent for efficient isolation of analyzed compounds in comparison with the classical multiresidue methods. The consumed solvent's volumes were 15 ml with the MSPD method, and 130 ml with LLE.

Method Validation

Recoveries and relative standard deviation (RSD) are listed in Table 3. The procedures involving LLE and MSPD extractions were validated for soil samples fortified at three levels: 1) ranging between 0.005-0.05 mg/kg, 2) 0.05-0.5 mg/kg, and 3) 0.25-2.5 mg/kg.

The linearity of the methods was tested over the range 0.005-2.5 mg/kg. Procedures showed a satisfactory linear behavior in the tested range, with correlation coefficients ≥ 0.997. Table 3 summarizes several parameters of the two analytical methodologies. Calibration curves were obtained from matrix-matching calibration solutions. The lowest concentration level in the calibration curve is established as a practical determination limit. All compounds exhibited good linearity in the studied range. Determination coefficients (the square of the correlation coefficients) found were higher than 0.980 in all cases.

Detection limits of pesticide residues (LOD) of all tested pesticide residues extracted by MSPD technique compared with LLE extraction and analyzed by GC-EC and GC-NP were determined to evaluate the efficiency of both extraction methods. The average LOD ranged from 0.001 to 0.020 and from 0.005 to 0.040 mg/kg for MSPD and LLE, respectively.

Table 3. Method performance comparison.

No.	Multicompound standard solutions	Active substance	Retention time t_R [min]				Concentration range [mg/kg]		MSPD* (Procedure 2)			LLE** (Procedure 1)		
			DB-35		HP-5									
			EC	NP	EC	NP			Mean recovery [%] (n=3)	RSD [%]	U [%]	Mean recovery [%] (n=3)	RSD [%]	U [%]
1	M14	dichlorvos**	5.291	5.288	-	-	0.030	1.500	-	-	-	*61.9*	8.0	29.5
2	M13	cymoxanil*	6.250	6.245	3.073	3.071	0.050	2.500	*50.1*	4.8	17.8	-	-	-
3	M15	dichlobenil*	6.856	6.854	3.470	3.742	0.010	0.500	91.5	9.5	19.7	-	-	-
4	M11	propham		7.428		4.114	0.050	2.500	92.4	8.6	15.4	72.7	6.7	23.5
5	M15	metacriphos	8.086	8.078		4.425	0.010	0.500	100.0	11.4	19.4	*59.4*	8.8	22.6
6	M16	trifluralin	8.754	8.746	5.840	5.846	0.010	0.500	106.4	5.7	12.6	89.2	7.0	24.3
7	M14	DEET		9.157		5.158	0.050	2.500	89.5	5.7	12.1	94.0	8.7	19.8
8	M13	heptenophos		9.479		5.009	0.015	0.750	82.7	6.6	13.6	*52.7*	6.1	19.4
9	M18	tecnazene	9.618		5.213		0.005	0.250	92.3	0.3	14.6	*66.4*	3.7	23.4
10	M16	propachlor	9.772	9.764	5.441	5.432	0.040	2.000	106.2	1.7	18.9	77.1	1.2	25.4
11	M15	ethoprophos	9.805	9.796		5.443	0.010	0.500	105.0	5.8	14.9	*54.7*	8.5	23.5
12	M11	chlorpropham		9.764		5.519	0.050	2.500	84.4	5.7	15.6	*48.4*	4.7	29.6
13	M13	propoxur		9.960		5.391	0.050	2.500	108.0	0.4	17.6	*51.4*	1.8	17.4
14	M11	diphenylamine		10.128		5.354	0.050	2.500	76.2	12.4	14.6	*62.8*	7.1	18.5
15	M15	phorate	10.645	10.634	5.690	5.961	0.010	0.500	95.3	4.6	18.6	98.1	0.9	28.7
16	M18	HCB	10.780		6.375		0.005	0.250	101.6	4.2	18.5	79.4	1.6	19.5
17	M18	alpha-HCH	11.087		6.131		0.005	0.250	105.2	2.5	13.8	75.2	4.5	24.5
18	M16	propyzamide	11.243	11.234	6.598	6.600	0.030	1.500	108.2	3.6	17.6	77.8	6.1	26.5
19	M13	diazinon	11.560	11.548	6.822	6.826	0.010	0.500	114.0	4.2	18.8	74.0	9.3	26.5
20	M16	atrazine		11.566		6.531	0.010	0.500	110.5	4.2	10.6	74.9	10.7	27.4
21	M17	simazine*		11.711		6.303	0.010	0.500	93.0	6.4	14.6	-	-	-
22	M18	dichloran	11.790	11.784	6.267		0.005	0.250	104.7	4.9	18.6	76.5	5.4	23.1
23	M17	quintozene	11.820		6.895		0.005	0.250	95.0	10.2	14.5	76.5	7.6	23.8
24	M18	gamma-HCH (lindane)	12.099		6.774		0.010	0.500	104.8	1.3	17.9	79.8	2.0	20.0
25	M14	pyrimethanil		12.104		6.977	0.020	1.000	104.8	14.3	16.3	79.0	9.8	26.5
26	M11	carbofuran		12.190		6.289	0.050	2.500	107.3	7.5	15.6	103.0	5.6	16.8
27	M18	beta-HCH	12.203		6.585		0.005	0.250	103.6	4.9	15.6	83.4	5.4	25.4
28	M13	dimethoate*	12.305	12.295	6.420	6.424	0.010	0.500	104.6	10.5	20.1	-	-	-
29	M10	formothion*	12.315	12.304	7.499	7.504	0.020	1.000	84.0	2.1	19.5	-	-	-
30	M11	fenpropimorph		12.808		8.499	0.050	2.500	105.1	4.6	20.3	*63.6*	4.3	24.9
31	M17	vinclozolin	12.881	12.871	7.677		0.005	0.250	99.2	3.7	16.8	79.9	4.2	25.6
32	M15	acetochlor	12.905	12.895	7.856	7.858	0.050	2.500	90.9	4.6	23.6	82.6	2.4	22.2
33	M14	chlorothalonil	12.921	12.911	7.098		0.010	0.500	93.7	9.2	16.5	82.5	5.1	19.7
34	M13	pirimicarb		12.978		7.301	0.020	1.000	97.0	13.2	21.1	*59.1*	8.2	25.6
35	M18	heptachlor	13.089		8.086		0.005	0.250	105.5	3.4	16.9	93.9	8.3	26.1
36	M13	chlorpyrifos-methyl	13.364	13.352	7.920	7.925	0.010	0.500	102.2	4.5	15.9	76.2	18.6	17.6
37	M16	prometrine		13.394		8.074	0.010	0.500	110.7	10.2	17.6	74.3	8.1	23.1

Table 3. Continued.

No.	Multicompound standard solutions	Active substance	Retention time t_R [min]				Concentration range [mg/kg]		MSPD*			LLE**		
			DB-35		HP-5				(Procedure 2)			(Procedure 1)		
			EC	NP	EC	NP			Mean recovery [%] (n=3)	RSD [%]	U [%]	Mean recovery [%] (n=3)	RSD [%]	U [%]
38	M12	fenchlorphos	13.448	13.437	8.197	8.202	0.010	0.500	104.9	2.9	17.6	*66.7*	7.6	27.6
39	M16	parathion-methyl	13.573	13.562	7.748	7.752	0.010	0.500	103.8	1.2	22.1	*65.1*	1.3	26.5
40	M10	metalaxyl		13.630		7.961	0.030	1.500	75.6	10.2	15.9	*51.1*	2.6	27.7
41	M13	pirimiphos-methyl		13.662		8.26	0.010	0.500	111.0	8.5	16.8	*69.3*	6.4	26.3
42	M14	tolclofos-methyl	13.666	13.655	7.800	7.805	0.010	0.500	97.6	0.6	17.5	78.2	9.8	22.4
43	M17	metribuzin	13.705	13.694	7.481	7.482	0.005	0.250	87.5	4.9	15.6	*60.5*	0.5	26.7
44	M18	aldrine	13.856		8.763		0.005	0.250	106.9	7.4	15.3	76.9	5.8	20.0
45	M12	tetraconazole	13.953	13.941	8.738	8.74	0.010	0.500	100.0	2.6	19.6	*51.4*	4.0	28.1
46	M17	triadimefon	13.991	13.978	8.673	8.675	0.010	0.500	89.6	6.7	16.8	74.2	3.4	21.6
47	M10	malathion	14.013	14.001	8.573	8.578	0.020	1.000	101.5	12.1	23.1	81.0	15.3	24.7
48	M14	chlorpyrifos	14.060	14.049	8.838	8.843	0.010	0.500	99.2	1.1	18.4	80.3	8.8	23.6
49	M16	fenitrothion	14.065	14.052	8.424	8.429	0.010	0.500	106.6	1.8	18.2	71.8	7.6	20.1
50	M11	carbaryl		14.160		7.732	0.050	2.500	104.4	8.7	21.6	113.0	4.1	24.3
51	M17	parathion-ethyl	14.197	14.184	8.681	8.685	0.007	0.350	100.9	7.7	21.5	72.0	1.5	27.4
52	M18	fipronil	14.207	14.196	9.619	9.662	0.005	0.250	104.0	2.0	17.8	83.7	7.2	23.5
53	M13	pirimiphos-ethyl		14.233		9.058	0.020	1.000	*122.0*	4.7	17.8	78.9	7.1	25.6
54	M14	dichlofluanid	14.345	14.334	8.387	8.390	0.010	0.500	90.8	4.6	21.6	77.7	3.3	24.5
55	M11	dicofol**	14.424		8.622		0.010	0.500	-	-	-	120.0	1.2	28.9
56	M11	fenthion		14.493		8.801	0.020	1.000	111.6	6.2	21.6	*63.0*	2.9	27.1
57	M16	pendimethalin	14.625	14.607	9.473	9.476	0.010	0.500	105.2	1.6	15.6	71.4	2.3	18.9
58	M12	bromophos methyl	14.652	14.639	8.909	8.915	0.010	0.500	109.0	4.7	17.6	80.4	12.4	27.5
59	M15	izofenphos methyl	14.659	14.646	9.134	9.131	0.010	0.500	109.0	11.6	22.3	*61.8*	9.2	24.6
60	M17	isofenphos		14.777		9.664	0.005	0.250	105.0	14.4	18.2	74.5	6.5	27.2
61	M14	cyprodinil		14.791		9.352	0.020	1.000	98.2	11.4	14.6	76.2	2.7	20.3
62	M12	penconazole	14.807	14.794	9.286	9.288	0.010	0.500	86.1	7.8	17.6	*46.1*	3.7	28.7
63	M13	triadimenol	14.846 15.002	14.833 14.989	9.408 9.543	9.109 9.544	0.050	2.500	116.5	1.2	18.9	76.1	7.2	25.1
64	M16	chlorfenvinphos	15.010	14.996	9.637	9.641	0.010	0.500	107.1	0.4	19.6	87.4	4.8	22.6
65	M10	mecarbam	15.032	15.018	9.474	9.479	0.010	0.500	93.3	5.7	20.4	*68.7*	5.0	21.0
66	M18	heptachlor-epoxide	15.074		9.619		0.005	0.250	106.0	2.8	19.7	75.7	5.7	24.0
67	M12	bromophos ethyl	15.124	15.110	10.019	10.024	0.010	0.500	119.2	2.5	16.8	72.0	2.6	24.8
68	M14	procymidone	15.164	15.151	9.616	9.618	0.040	2.000	97.1	1.3	14.6	84.2	10.9	21.0
69	M17	tolylfluanid	15.190	15.176	9.380	9.383	0.030	1.500	94.6	0.6	13.6	95.2	18.4	19.1
70	M10	metazachlor	15.222	15.209	9.171	9.173	0.050	2.500	89.0	7.3	18.6	90.9	3.7	22.8
71	M11	quinalphos	15.256	15.241	9.533	9.534	0.010	0.500	116.6	4.3	18.6	*66.7*	2.7	22.3
72	M15	paclobutrazol	15.376	15.361	9.872	9.871	0.050	2.500	*59.3*	2.3	16.8	108.7	6.6	24.7
73	M14	fluazifop-p-butyl		15.557		10.763	0.050	2.500	96.0	9.5	17.6	80.7	15.1	21.3
74	M17	alpha-endosulfan	15.653		9.962		0.005	0.250	103.4	3.0	16.5	77.6	1.9	23.5

Table 3. Continued.

No.	Multicompound standard solutions	Active substance	Retention time t_R [min]				Concentration range [mg/kg]		MSPD*			LLE**		
			DB-35		HP-5				(Procedure 2)			(Procedure 1)		
			EC	NP	EC	NP			Mean recovery [%] (n=3)	RSD [%]	U [%]	Mean recovery [%] (n=3)	RSD [%]	U [%]
75	M11	tetrachlorvinphos	15.717	15.702	10.129	10.135	0.020	1.000	88.1	8.5	17.6	94.8	0.9	26.2
76	M15	hexaconazole	15.750	15.734	10.252		0.010	0.500	77.7	6.5	16.8	*69.1*	2.2	19.4
77	M10	iprovalicarb		15.775 15.919		10.344 10.505	0.050	2.500	78.7	7.9	14.5	*52.1*	2.3	21.3
78	M16	picoxystrobin	15.776	15.762	10.287	10.289	0.050	2.500	109.5	1.1	12.3	80.7	3.6	19.2
79	M18	p,p'DDE	15.869		10.517		0.005	0.250	103.4	9.5	18.9	76.3	5.4	26.3
80	M15	profenfos	16.003	15.988	10.351	10.355	0.010	0.500	80.3	10.9	19.5	71.6	9.1	26.5
81	M16	captan*	16.003		9.527		0.020	1.000	94.3	12.3	19.8	-	-	-
82	M13	buprofezin		16.022		10.723	0.030	1.500	102.0	7.9	15.9	*69.9*	3.8	26.5
83	M11	mepanipyrim		16.028		9.903	0.030	1.500	100.3	12.6	21.5	*55.6*	6.7	24.5
84	M16	napropamide		16.045		10.379	0.030	1.500	111.6	5.8	18.4	72.4	5.3	23.5
85	M16	folpet*	16.086		9.396		0.020	1.000	100.5	4.9	25.3	-	-	-
86	M12	flusilazole		16.095		10.542	0.010	0.500	92.2	2.4	21.3	*45.7*	6.4	26.7
87	M17	methidathion	16.107	16.092	9.822	9.827	0.008	0.400	102.2	13.2	17.6	98.8	7.6	19.2
88	M14	flutriafol**		16.166		10.136	0.050	2.500	-	-	-	82.7	2.8	23.5
89	M13	bupirimate	16.219	16.204	10.612	10.613	0.020	1.000	105.0	8.4	17.4	87.2	2.6	23.4
90	M18	dieldrin	16.258		10.635		0.005	0.250	103.1	1.2	23.5	80.0	9.8	24.6
91	M17	myclobutanyl	16.377	16.363	10.685	10.687	0.030	1.500	82.9	7.2	13.7	75.0	5.1	21.6
92	M15	hexythiazox	16.381	16.367	9.771	9.773	0.100	5.000	110.1	5.8	20.1	*59.0*	6.8	26.8
93	M10	kresoxim-methyl	16.602	16.588	10.763	10.764	0.020	1.000	94.2	6.9	11.9	70.6	11.2	22.6
94	M12	cyproconazole		16.713 16.787		10.753	0.020	1.000	*45.2*	10.8	19.7	*46.3*	3.6	29.1
95	M17	nitrofen	16.763		10.681		0.005	0.250	96.4	6.8	13.1	*56.9*	8.5	28.6
96	M15	diniconazole	16.814	16.797	11.009	11.011	0.010	0.500	105.3	1.2	16.9	*66.9*	8.5	21.5
97	M14	fludioxonil	17.025	17.010		10.521	0.030	1.500	97.0	7.1	19.5	73.1	4.3	27.4
98	M12	p,p'DDD	17.033		11.200		0.010	0.500	100.2	12.3	23.1	*47.9*	8.7	21.4
99	M17	endrin	17.037		10.985		0.007	0.350	96.1	6.4	18.5	86.5	4.7	25.6
100	M11	ethion	17.049	17.033	11.309	11.277	0.010	0.500	98.2	10.3	11.6	71.4	5.2	18.6
101	M16	azaconazole	17.062	17.048	10.597	10.599	0.020	1.000	*44.0*	1.7	25.6	79.3	12.9	29.8
102	M18	o,p'DDT	17.088		11.265		0.006	0.300	102.3	5.4	17.6	95.3	4.6	26.3
103	M17	beta-endosulfan	17.413		10.903		0.005	0.250	101.4	4.8	22.4	79.9	7.8	24.6
104	M13	trifloxystrobin	17.456	17.440	11.523	11.525	0.020	1.000	101.0	4.6	17.6	*68.4*	5.7	27.3
105	M12	propiconazole	17.721 17.829	17.705 17.814	11.515 11.607	11.517 11.608	0.020	1.000	106.4	13.7	18.6	*47.9*	7.3	25.3
106	M18	p,p'DDT*	17.810		11.767		0.006	0.300	97.9	6.8	23.4	-	-	-
107	M15	quinoxyfen	17.826	17.812	11.534		0.020	1.000	86.2	10.2	12.3	79.2	4.7	18.1
108	M17	benalaxyl		17.954		11.404	0.050	2.500	94.4	5.7	21.5	75.0	6.9	23.5
109	M10	acrinathrin	18.470 18.789		12.921 13.088		0.050	2.500	116.2	3.3	17.6	*54.0*	8.6	24.6
110	M17	bifenthrin	18.017		12.404		0.010	0.500	102.1	8.3	18.7	84.0	8.6	26.3

Table 3. Continued.

No.	Multicompound standard solutions	Active substance	Retention time t_R [min]				Concentration range [mg/kg]		MSPD* (Procedure 2)			LLE** (Procedure 1)		
			DB-35		HP-5									
			EC	NP	EC	NP			Mean recovery [%] (n=3)	RSD [%]	U [%]	Mean recovery [%] (n=3)	RSD [%]	U [%]
111	M14	fenhexamid	18.085	18.071	11.780		0.050	2.500	72.4	6.3	19.5	71.4	8.2	27.1
112	M12	tebuconazole		18.135		11.776	0.010	0.500	*47.1*	6.1	22.3	*52.2*	3.1	28.6
113	M16	triazophos		18.270		11.329	0.010	0.500	114.9	2.5	19.3	73.2	12.4	28.1
114	M13	oxadixyl	18.410	18.395	11.291	11.291	0.050	2.500	*56.1*	5.1	18.7	*66.0*	5.0	26.2
115	M17	endosulfan-sulfate	18.556		11.523		0.010	0.500	93.9	8.5	17.5	78.6	15.6	20.1
116	M14	iprodione	18.661	18.647	11.042		0.050	2.500	91.7	3.0	19.5	*65.2*	1.6	26.3
117	M17	fenpropathrin	18.927	18.911	12.498	12.5	0.010	0.500	98.0	2.8	19.9	71.0	9.7	26.1
118	M11	tebufenpyrad	18.930	18.911		12.547	0.040	2.000	95.8	11.3	23.5	*55.2*	2.6	26.3
119	M16	bromopropylate	18.971		12.199		0.020	1.000	111.0	5.5	22.3	94.6	6.4	27.2
120	M15	epoxiconazole	19.116	19.100	12.03		0.010	0.500	91.1	0.2	18.5	*45.1*	2.6	26.5
121	M10	lenacil*		19.275		11.696	0.050	2.500	78.0	8.7	17.6	-	-	-
122	M17	lambda-cyhalothrin	19.322 19.675	19.308 19.656	12.973 13.127		0.020	1.000	104.6	14.0	24.1	76.2	11.8	25.6
123	M15	fenazaquin		19.587		12.484	0.030	1.500	96.9	8.9	16.7	*51.4*	6.3	25.5
124	M10	metconazole**		19.967		12.652	0.050	2.500	-	-	-	*41.5*	2.8	29.0
125	M18	methoxychlor (DMDT)*	19.954		12.458		0.010	0.500	88.3	11.3	18.3	-	-	-
126	M14	dimoxystrobin	19.731	19.715	12.356	12.36	0.020	1.000	93.0	0.2	17.5	75.0	0.4	19.8
127	M12	bromuconazole	19.938 21.043	19.924 21.026	12.214 12.525	12.217 12.526	0.020	1.000	95.5	10.4	21.6	*49.3*	3.8	23.4
128	M16	pyriproxyfen		20.815		12.702	0.050	2.500	113.6	6.9	19.5	79.5	10.3	26.5
129	M16	tetradifon	20.885		12.554		0.010	0.500	119.9	0.7	16.8	84.8	10.9	27.6
130	M13	phosalone	21.068	21.051	12.92	12.925	0.020	1.000	106.0	5.8	19.5	110.0	9.9	24.3
131	M15	phosmet	21.125	21.109	12.27	12.275	0.020	1.000	109.2	8.7	20.6	74.8	7.3	26.7
132	M16	fenamidon	21.170	21.153	12.417	12.419	0.020	1.000	113.3	1.4	16.3	*47.4*	5.4	23.4
133	M15	pyrazophos	21.556	21.539		13.191	0.010	0.500	101.0	8.8	16.8	108.7	4.6	17.6
134	M14	acetamiprid*	22.255	22.239	12.244	12.387	0.020	1.000	*51.1*	5.8	21.7	-	-	-
135	M10	permethrin**	22.477 22.771		13.584 13.693		0.050	2.500	-	-	-	110.9	9.1	21.6
136	M12	bitertanol	23.009	22.987		13.534	0.030	1.500	107.0	3.6	16.5	*50.5*	2.1	29.7
137	M13	fenarimol	22.748	22.729	13.353	13.356	0.015	0.750	96.8	4.6	18.5	79.9	13.7	28.4
138	M13	azinphos-methyl	23.115	23.099	12.939	12.943	0.050	2.500	100.3	8.1	19.5	*61.1*	7.5	28.3
139	M15	pyridaben	23.247	23.231	13.779	13.806	0.040	2.000	96.0	5.9	14.6	93.8	2.5	19.4
140	M11	pyraclostrobin	23.314	23.307	15.826	15.821	0.100	5.000	94.1	6.9	17.4	83.1	8.5	20.3
141	M16	prochloraz**	23.627	23.607	13.931	13.932	0.050	2.500	-	-	-	90.4	11.0	16.5
142	M10	beta-cyfluthrin	23.667 23.825 24.133		14.054 14.138 14.263		0.050	2.500	*123.3*	3.0	20.5	*62.5*	2.0	30.0
143	M11	cyfluthrin	23.670 23.821 24.131		14.386 14.477 14.606		0.050	2.500	90.8	7.6	17.8	*52.8*	8.1	26.7
144	M13	azinphos-ethyl	23.993	23.978	13.442	13.448	0.050	2.500	104.1	11.2	17.8	75.0	10.8	25.6

Table 3. Continued.

No.	Multicompound standard solutions	Active substance	Retention time t_R [min]				Concentration range [mg/kg]		MSPD* (Procedure 2)			LLE** (Procedure 1)		
			DB-35		HP-5									
			EC	NP	EC	NP			Mean recovery [%] (n=3)	RSD [%]	U [%]	Mean recovery [%] (n=3)	RSD [%]	U [%]
145	M15	coumaphos	24.498	24.482	13.863	13.871	0.020	1.000	104.0	3.9	16.8	79.0	9.6	28.7
146	M12	fluquinconazole	24.822	24.803	13.827	13.829	0.020	1.000	101.3	3.3	25.6	*63.2*	1.2	28.4
147	M16	alpha-cypermethrin	25.086 25.675		14.740 14.950		0.040	2.000	120.0	2.7	14.7	*61.8*	4.7	27.1
148	M14	zeta-cypermethrin	25.092 25.310 25.668		14.486 14.589 14.696		0.050	2.500	92.8	2.4	20.1	80.1	0.9	19.6
149	M10	cypermethrin	25.100 25.326 25.675		14.757 14.864 14.976		0.050	2.500	90.9	5.7	19.2	*67.1*	1.9	28.6
150	M12	fenbuconazole	26.643	26.631	14.199	14.198	0.030	1.500	96.0	7.3	17.9	*51.9*	8.4	28.6
151	M15	boscalid	29.121		14.658		0.020	1.000	78.0	4.6	16.8	*47.1*	7.4	22.4
152	M13	esfenvalerate	29.260 30.248		15.580 15.868		0.040	2.000	74.4	14.8	19.1	*49.5*	6.0	24.8
153	M10	fenvalerate	29.278 30.265		16.039 16.351		0.040	2.000	118.8	2.5	18.9	*46.2*	5.1	29.8
154	M10	indoxacarb**	32.819		17.142		0.050	2.500	-	-	-	*69.8*	9.8	24.6

*active substance isolated only by MSPD
** active substance isolated only by LLE
U – combined standard uncertainty determined for the lowest validation levels
italics – active substance with mean recovery 40-130%

The different aspects explained above for estimating the standard uncertainties have been applied to the multiresidue analytical method. A methodology for calculating the uncertainty of results on the basis of in-house validation data has been applied to a pesticide multiresidue method. Uncertainty sources have been identified and standard uncertainty established. The uncertainty of MSPD method for all compounds was lower by a few percentage points in comparison to the uncertainty of the LLE method. An increase in the uncertainty in reducing level of concentration of the active substance in the sample was observed. However, depending on the concentration and the physicochemical parameters of the determined active substance, the combined standard uncertainty ranged 10-30% (Table 3).

Comparison of Extraction Techniques

The percentage mean recoveries obtained for most pesticides were satisfactory and ranged from 70 to 120% for all the pesticides studied. Mean recoveries at three spiked levels varied from 72.4 to 120% for MSPD with several exceptions (acetamiprid, azaconazole, cymoxanil, metconazole, oxadixyl, paclobutrazol, tebuconazole (<70%) and beta-cyfluthrin, pirimiphos-ethyl (>120%) and for LLE 70.6-120% with 55 exceptions (mean recoveries <70%). However, other validation parameters were satisfactory (RSDs <20%). As seen from Table 3, LLE gave precision with RSD ranging from 0.4-18.6% for over 1 h extraction. In comparison, the precision of MSPD were below 15% and ranged from 0.2 to 14.8%.

The evaluated extraction procedures allowed for determination of 7 acaricides, 55 fungicides, 18 herbicides, and 74 insecticides. Captan, cymoxanil, folpet (fungicides) and dichlobenil, lenacil, simazine (herbicides) and acetamiprid, dimethoathe, formothion, metoxychlor (DMDT), and p,p'DDT (insecticides) were isolated only by MSPD extraction. On the other hand dicofol (acaricide) and flutriafol, metconazole, prochloraz (fungicides) and dichlorvos, indoxacarb, and permethrin (insecticides) were extracted only by LLE (Table 3).

The MSPD extraction technique fulfilled requirements of multiresidue method and enabled isolation of 147 pesticides from 154 analyzed with good validation parameters. This technique proved to be a good alternative for LLE, because numerous disadvantages of LLE have been noticed (Table 4).

The proposed instrumental method allows the determination of pesticides in soil by GC using two selective detectors functioning simultaneously. In the present work we used configuration with a "Y" piece at the end of the GC column in order to divide the flux at the end of the GC column into two branches of equal flow (Fig. 3) (one to the NPD and the other to the ECD), thus allowing pesticides of different nature to be quantified in the same run: 119 pesticides were detected by the ECD, whereas 117 were ana-

Table 4. Comparison of extraction techniques.

Extraction technique	Advantages	Disadvantages
LLE	· Well-known procedure · Wide experience in the extraction field	· Long extraction time · Large consumption of toxic and inflammable solvents (dichloromethane) – problems with evaporation · Multiple extractions · Laborious, time-consuming, expensive · Filtration required after extraction · From 144 extracted pesticides 55 (38%) with acceptable mean recovery values (<70%) 89 pesticides extracted with satisfactory mean recovery values
MSPD	· Simple and fast extraction (time reduction) · Small amount of organic solvent · Environmentally safe extraction · Economical and convenient to perform · Coupled with simultaneous stage of purification · No filtration required · Extraction of 147 pesticides, of which 138 have satisfactory mean recovery values · Lower limits of detection (LODs)	· The use of anhydrous sorbents activated at high temperatures

lyzed by NPD, although ECD and NPD also provided a discernible signal for 82 of them.

In the case of coeluting pesticides, the application of a dual detection system allows their determination. For example, the co-eluted peaks of pyrimethanil and gamma-HCH were observed. However, this was not a problem since pyrimethanil was only detected with NPD and gamma-HCH only with ECD (Table 3). In that situation also a capillary column with different polarity in the same detection system is used.

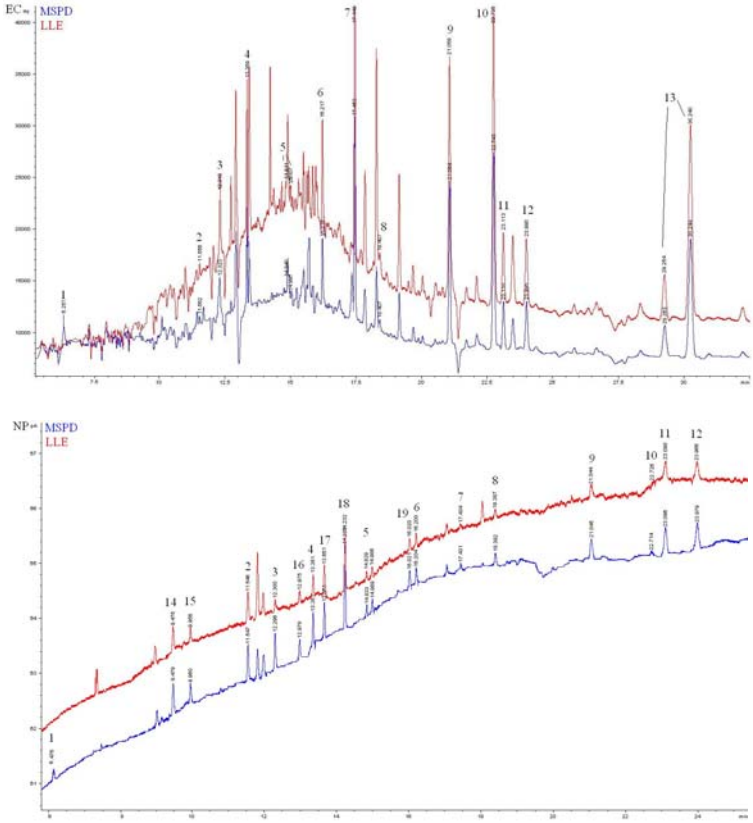

Fig. 4. EC and NP chromatograms of fortified soil samples after MSPD extraction and LLE extraction: 12 *pesticides on both detectors* − 1. cymoxanil (0.025 mg/kg); 2. diazinon (0.005 mg/kg); 3. dimethoate (0.05 mg/kg); 4. chlorpyrifos methyl (0.005 mg/kg); 5. triadimenol (isomers) (0.025 mg/kg); 6. bupirymate (0.01 mg/kg); 7. tifloxystrobin (0.2 mg/kg); 8. oxadixil (0.025 mg/kg); 9. fozalone (0.01 mg/kg); 10. fenarimol (0.15 mg/kg); 11. azinphos-methyl (0.025 mg/kg); 12. azinphos-ethyl (0.025 mg/kg); *1 pesticide only on EC* − 13. esfenvalerate (isomers) (0.02 mg/kg); *6 pesticides only on NP* − 14. heptophos (0.0075 mg/kg); 15. propoxur (0.025 mg/kg); 16. pirimicarb (0.01 mg/kg); 17. pirimiphos-methyl (0.005 mg/kg); 18. pirimiphos-ethyl (0.01 mg/kg); 19. burpofezin (0.015 mg/kg) (DB-35 column).

Fig. 4 present EC and NP chromatograms of the spiked soil samples extracted by LLE and MSPD. As can be observed, e.g. cymoxanil, diazinon, dimethoate, chlorpyrifos methyl, triadimenol, bupirymate, tifloxystrobin, oxadixil, fozalone, fenarimol, and azinphos-methyl and azinphos-ethyl gave clear signals with both detectors, while heptophos, propoxur, pirimicarb, pirimiphos-methyl, pirimiphos-ethyl, and burpofezin gave signals only with NPD and esfenvalerate (isomers) only with the ECD. Cymoxanil was extracted only by MSPD and its peaks can be observed only on a chromatogram representing MSPD extraction on Fig. 4.

A blank trace of both MSPD and LLE extractions also is shown (Fig. 5). Quantification of simazine was unable by LLE extraction because the peak of simazine (t_R= 11.711 min) eluted at the same retention time as peak from the soil matrix (t_R=11.715 min). Extract obtained from MSPD was free of interfering peaks at this time. It is very important when compound identification is possible only on a single detector. In this way the presence or absence of the compound can be confirmed.

Quality Control Procedure

A quality control procedure was established for ensuring that results obtained are under statistical control. This procedure consisted of incorporating to each batch of samples a blank extract, matrix-matching calibration solutions, and three spiked samples. Results were considered when the analysis of blank extracts showed that neither contamination nor degradation of sample had occurred, the recovery factors of spiked soil samples were between 70 and 120%, and the calibration plots fit to lines with determination coefficients higher than 0.95. In addition to the in-house quality assurance program, in 2000-10 the Laboratory successfully participated in 20 rounds of proficiency testing schemes organized and run by the Food Analysis Performance Assessment Scheme (FAPAS; Central Science Laboratory in York), by the European Commission (at the beginning by the University of Uppsala and then by the University of Almeria), and by using certified reference material (CRM) (Fig. 6).

CRM was used to verify the accuracy of the procedure and for the quantitative determination of organochlorine

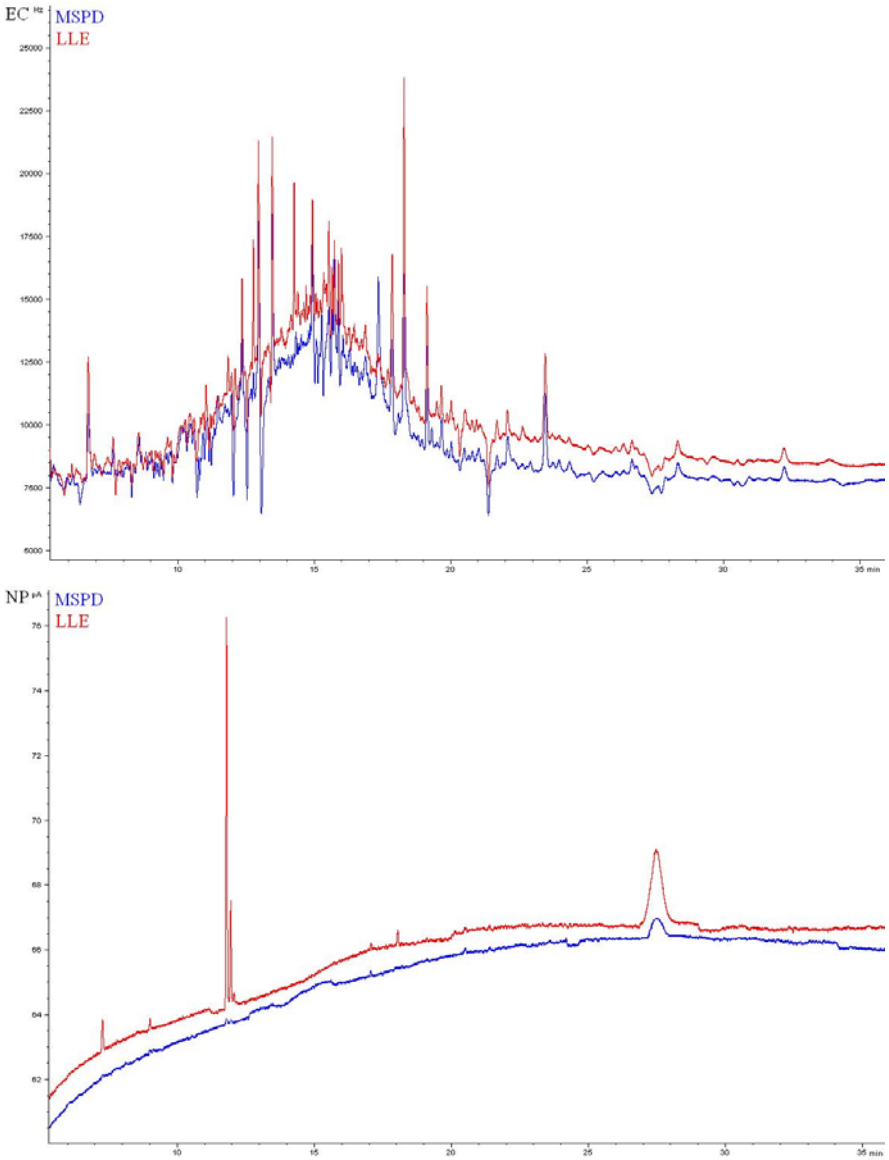

Fig. 5. EC and NP chromatograms of blank soil sample from both extraction procedures MSPD and LLE (DB-35 column).

Table 5. Comparison of concentration values for five pesticides in CRM (ERm – CC007) soil sample.

Organochlorine pesticide	Certified value ± uncertainty [mg/kg]	U [%]	Obtained value ± uncertainty [mg/kg]	U [%]	Obtained concentration range min-max [mg/kg]
alfa-HCH	**0.0320**±0.00086	2.7	0.0320±0.00080	2.5	0.0312–0.0328
beta-HCH	**0.3860**±0.07604	19.7	0.3761±0.01843	4.9	0.3577–0.3945
p,p'DDE	**0.0563**±0.00152	2.7	0.0578±0.00549	9.5	0.0523–0.0633
o,p'DDT	**0.0357**±0.00099	2.8	0.0350±0.00189	5.4	0.0331–0.0369
p,p'DDT	**0.1535**±0.01089	7.1	0.1527±0.01038	6.8	0.1423–0.1631

pesticides in soil by evaluated procedure by gas chromatography. Certified values of CRM were compared with the values obtained from the analysis of soil samples using MSPD (Table 5). The obtained values of soil sample carried through the whole extraction procedure, taking into account uncertainties, are consistent with the certified values as shown in Table 5.

Application to Field Samples

MSPD procedure was applied to over 10 soil samples. In three samples were detected p,p'DDT and metabolite of DDT: p,p'DDE. Typical GC/EC chromatograms of the blank soil sample (unfortified), selected standard mixture and field soil sample (containing p,p'DDE and p,p'DDT) extracted using MSPD technique presents Fig. 7 (A, B, C), respectively. The MSPD method proposed for analysis of pesticides in soil provided clean blank extracts and therefore no clean-up step was necessary.

Conclusions

A rapid and efficient multiresidue procedure has been developed for determining pesticides in soil samples. The results of this study show that the MSPD procedure has significant advantages over classical LLE. The method based on solid phase dispersion of soil samples is fast and easy to perform and allows for analysis of different groups of pesticides. The proposed method was less time consuming, and cheaper in terms of consumables and equipment required in comparison to LLE. It was possible to prepare extracts for

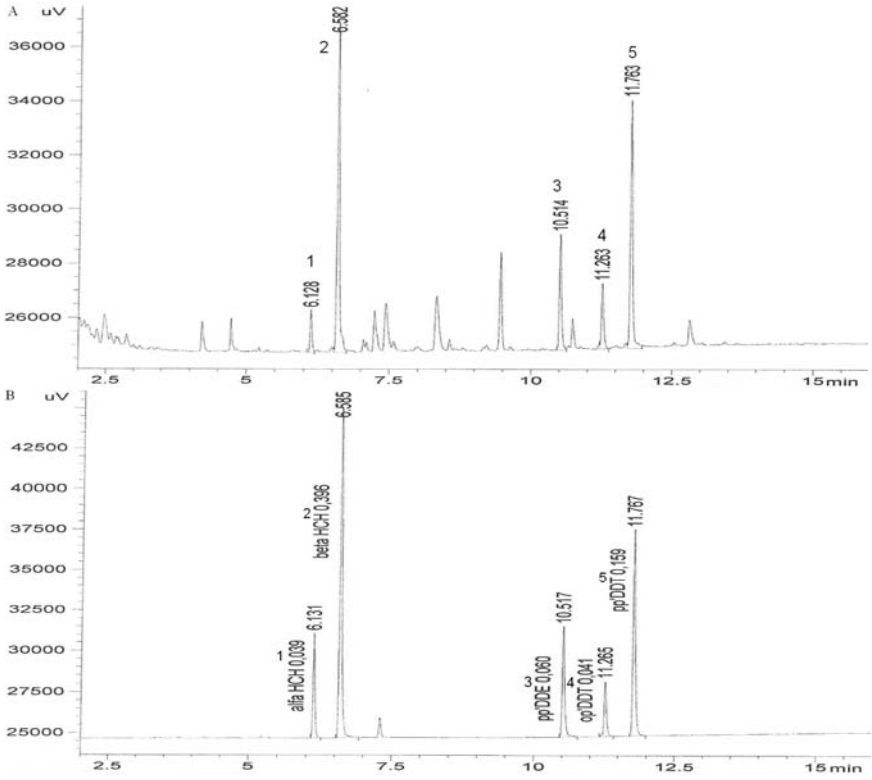

Fig. 6. GC chromatogram of A: CRM soil sample − 1. alfa-HCH; 2. beta-HCH; 3. p,p'DDE; 4. o,p'DDT; 5. p,p'DDT and B: standard mixture − 1. alfa-HCH (0.005 mg/kg); 2. beta-HCH (0.01 mg/kg); 3. p,p'DDE (0.005 mg/kg); 4. o,p'DDT (0.005 mg/kg); 5. p,p'DDT (0.005 mg/kg) (HP-5 column).

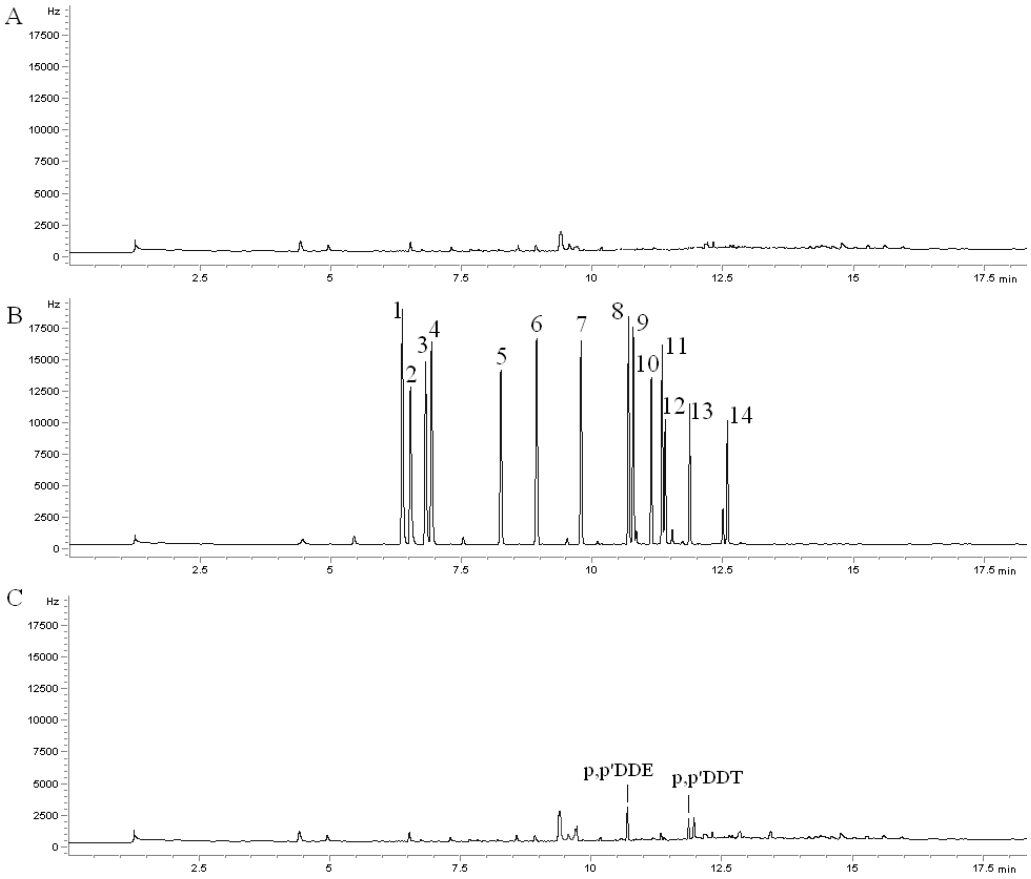

Fig. 7. Chromatograms of: A: blank soil sample, B: selected standards mixture − 1. alpha-HCH; 2. HCB; 3. beta-HCH; 4. gamma-HCH; 5. heptachlor; 6. aldrine; 7. heptachlor-epoxide; 8. p,p'DDE; 9. dieldrine; 10. endrin; 11. p,p'DDD; 12. o,p'DDT; 13. p,p'DDT; 14. metoxychlor (DMDT) and C: field soil sample with detected p,p'DDE 0.007±0.0006 mg/kg (t_R=10.519 min) and p,p'DDT 0.009±0.0006 mg/kg (t_R=11.768 min) (HP-5 column).

a batch of 10 samples in 30 min using MSPD. The LLE extraction procedure would require over 3 h to prepare a similar batch of samples. In addition, the MSPD uses smaller volumes of solvents like acetone and n-hexane, reducing human exposure to toxic solvents and environmental impact of the analytical procedure, compared with LLE extraction, where a larger volume of dichloromethane is used. The remarkable advantage of the presented method is that the isolation and purification are combined into one stage, the main source of errors involved in most analytical methods are avoided. The only inconvenience of MSPD extraction is the use of anhydrous sorbents activated at high temperatures.

Nevertheless, the MSPD method was more sensitive due to the cleaner extracts produced (lower limits of detection) compared to extracts produced by the LLE extraction method. The MSPD method successfully recovered 90% (138) of the active substances with mean recoveries in the recommended range of 70-120% (with good validation parameters) in comparison with LLE that could recover only 57% (88) of pesticides. MSPD fulfilled the requirements of multiresidue method for determination of pesticide residues in soil.

References

1. TOR A., EMIN AYDIN M., OZCAN S. Ultrasonic solvent extraction of organochlorinepesticides from soil. Anal. Chim. Acta **559**, 173, **2006**.
2. CONCHA-GRANA E., TURNES-CAROU M.I., MUNIATEGUI-LORENZO S., LOPEZ-MAHIA P., PRADA-RODRIGUEZ D., FERNANDEZ-FERNANDEZ E. Evaluation of HCH isomers and metabolites in soils, leachates, river water and sediments of a highly contaminated area. Chemosphere **64**, 588, **2006**.
3. GONCALVES C., ALPENDURADA M.F. Assessment of pesticide contamination in soil samples from an intensive horticulture area, using ultrasonic extraction and gas chromatography–mass spectrometry. Talanta **65**, 1179, **2005**.
4. Commission of the European Communities. Directive 2000/60/EC, EU Water Framework Directive, Brussels, Belgium, **2000**.
5. BELMONTE VEGA A., GARRIDO FRENICH A., MARTINEZ VIDAL J.L. Monitoring of pesticides in agricultural water and soil samples from Andalusia by liquid chromatography coupled to mass spectrometry. Anal. Chim. Acta **538**, 117, **2005**.
6. Commission of the European Communities. Council Directive 91/414/EEC concerning the placing of plant pro-

tection products on the market. Official Journal of the European Communities No.L 230, **1991**.
7. SPARKS R. Environmental Soil Chemistry. Elsevier, Amsterdam, **2003**.
8. BAVCON M., TREBSE P., ZUPANCIC-KRALJ L. Investigations of the determination and transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionization detector. Chemosphere **50**, 595, **2003**.
9. NAVARRO S., VELA N., GARCIA C., NAVARRO G. An overview on the environmental behavior of pesticide residues in soils. J. Agric. Food Chem. **51**, 7359, **2004**.
10. NAWAB A., ALEEM A., MALIK A. Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by *Pseudomonas* strains. Bioresour. Technol. **88**, 41, **2003**.
11. GHADIRI H., ROSE C.W. Degradation of endosulfan in a clay soil from cotton farms of western Queensland. J. Environ. Manage. **62**, 155, **2001**.
12. DOONG R.A., LIAO P.L. Determination of organochlorine pesticides and their metabolites in soil samples using headspace solid-phase microextraction. J. Chromatogr. A **918**, 177, **2001**.
13. HOU L., KEE LEE H. Determination of pesticides in soil by liquid-phase microextraction and gas chromatography-mass spectrometry. J. Chromatogr. A **1038**, 37, **2004**.
14. DĄBROWSKA H., DĄBROWSKI Ł., BIZIUK M., GACA J., NAMIEŚNIK J. Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in a single run. J. Chromatogr. A **1003**, 29, **2003**.
15. EL-SAEID M.H., AL-WABEL M.I., ABDEL-NASSER G., AL-TURKI A.M., AL- GHAMDI A.G. One-step extraction of multiresidue pesticides in soil by microwave-assisted extraction technique. J. App. Sci. **10**, 1775, **2010**.
16. SANGHI R., KANNAMKUMARATH S.S. Comparison of extraction methods by Soxhlet, sonicator and microwave in the screening of pesticide residues from solid matrices. J. Anal. Chem. **59**, (11), 1032, **2004**.
17. KREUZIG R., KOINECKE A., BAHADIR M. Use of supercritical fluid extraction in the analysis of pesticides in soil. J. Biochem. Biophys. Methods **43**, 403, **2000**.
18. LI Z.Y., ZHANG Z.C., ZHOU Q.L., GAO R.Y., WANG Q.S. Fast and precise determination of phenthoate and its enantiomeric ratio in soil by the matrix solid-phase dispersion method and liquid chromatography. J. Chromatogr. A **977**, 17, **2002**.
19. BAVCON M., TREBSE P., ZUPANCIC-KRALJ L. Investigations of the determination and transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionization detector. Chemosphere **50**, 595, **2003**.
20. BÖRJESSON E., TORSTENSSON L. New methods for determination of glyphosate and (aminomethyl)phosphonic acid in water and soil. J. Chromatogr. A **886**, 207, **2000**.
21. BOUAID A., RAMOS L., GONZALEZ M.J., FERNÁNDEZ P., CÁMARA C. Solid-phase microextraction method for the determination of atrazine and four organophosphorus pesticides in soil samples by gas chromatography. J. Chromatogr. A **939**, 13, **2001**.
22. BAO M.L., PANTAMI F., BARBIERI K., BURRINI D., GRIFFINI O. Multi-residue pesticide analysis in soil by solid-phase disk extraction and gas chromatography/ion-trap mass spectrometry Int. J. Env. Anal. Chem. **64**, (4), 233, **1996**.
23. DOST K., JONES D.C., AUERBACH R., DAVIDSON G. Determination of pesticides in soil samples by supercritical-fluid chromatography-atmospheric pressure chemical ionization mass spectrometric detection. Analyst **125**, (10), 1751, **2000**.
24. PAPADAKIS E.N., PAPADOPOULOU-MOURKIDOU E. Determination of metribuzin and major conversion products in soils by microwave-assisted water extraction followed by liquid chromatographic analysis of extracts. J. Chromatogr. A **962**, 9, **2002**.
25. ZAMBONIN C.G., PALMISANO F. Determination of triazines in soil leachates by solid-phase microextraction coupled to gas chromatography-mass spectrometry. J. Chromatogr. A **874**, 247, **2000**.
26. HOGENDOORN E.A., HULS R., DIJKMAN E., HOOGERBRUGGE R. Microwave assisted solvent extraction and coupled-column reversed-phase liquid chromatography with UV detection Use of an analytical restricted-access-medium column for the efficient multi-residue analysis of acidic pesticides in soils J. Chromatogr. A **938**, 23, **2001**.
27. BARCHAŃSKA H., BARANOWSKA I. Procedures for analysis of atrazine and simazine in environmental matrices. Rev. Env. Cont. Toxicol. **200**, 53, **2009**.
28. STIPICEVIC S., FINGLER S., ZUPANCIC-KRALJ L., 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. J. Sep. Sci. **26**, 1237, **2003**.
29. VRYZAS Z., PAPADOPOULOU-MOURKIDOU E. Determination of triazine and chloroacetanilide herbicides in soils by microwave-assisted extraction (MAE) coupled to gas chromatographic analysis with either GC-NP or GC–MS. J. Agric. Food Chem. **50**, 5026, **2002**.
30. DAGNAC T., JEANNOT R., MOUVET C., BARAN N. Determination of oxanilic and sulfonic acid metabolites of acetochlor in soils by liquid chromatography-electrospray ionization mass spectrometry. J. Chromatogr. A **957**, 69, **2002**.
31. HU J.-Y., ZHEN Z.-H., DENG Z.-B. Simultaneous determination of acetochlor and propisochlor residues in corn and soil by solid phase extraction and gas chromatography with electron capture detection. Bull Environ. Contim. Toxicol. **86**, 95, **2011**.
32. POZO O., PITARCH E., SANCHO J.V., HERNANDEZ F. Determination of the herbicide 4-chloro-2-methylphenoxyacetic acid and its main metabolite, 4-chloro-2-methylphenol in water and soil by liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A **923**, 75, **2001**.
33. PATSIAS J., PAPADAKIS E.N., PAPADOPOULOU-MOURKIDOU E. Analysis of phenoxyalkanoic acid herbicides and their phenolic conversion products in soil by microwave assisted solvent extraction and subsequent analysis of extracts by on-line solid-phase extraction-liquid chromatography. J. Chromatogr. A **959**, 153, **2002**.
34. LUQUE-GARCÍA J.L., DE CASTRO M.D.L. Coupling continuous subcritical water extraction, filtration, preconcentration, chromatographic separation and UV detection for the determination of chlorophenoxy acid herbicides in soils. J. Chromatogr. A **959**, 25, **2002**.
35. MARCHESE S., PERRET D., GENTILI A., CURINI R., MARINO A. Development of a method based on accelerated solvent extraction and liquid chromatography/mass spectrometry for determination of arylphenoxypropionic herbicides in soil. Rapid Commun. Mass Spectrom. **15**, 393, **2001**.

36. MOLINS C., HOGENDOORN E.A., DIJKMAN E., HEUSINKVELD H.A.G., BAUMANN R.A. Determination of linuron and related compounds in soil by microwave-assisted solvent extraction and reversed-phase liquid chromatography with UV detection. J. Chromatogr. A **869**, 487, **2000**.
37. CABALLO-LÓPEZ A., 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. J. Chromatogr. A **998**, 51, **2003**.
38. SUN L., LEE H.K. Optimization of microwave-assisted extraction and supercritical fluid extraction of carbamate pesticides in soil by experimental design methodology. J. Chromatogr. A **1014**, 165, **2003**.
39. PAIGA P., MORAIS S., CORREIA M., ALVES A., DELERUE-MATOS C. A multiresidue method for the analysis of carbamate and urea pesticides from soils by microwave-assisted extraction and liquid chromatography with photodiode array detection. Anal. Lett. **41**, (10), 1751, **2008**.
40. LERCH R.N., FERRER I., THURMAN E.M., ZABLOTOWICZ R.M. Identification of trifluralin metabolites in soil using ion-trap LC/MS/MS. Am. Chem. Soc. **17**, 291, **2003**.
41. SANCHEZ-BRUNETE C., MIGUEL E., TADEO J.L. Multiresidue analysis of fungicides in soil by sonication-assisted extraction in small columns and gas chromatography. J. Chromatogr. A **976**, 319, **2002**.
42. SANCHEZ-BRUNETE C., ALBERO B., TADEO J.L. Multiresidue determination of pesticides in soil by gas chromatography-mass spectrometry detection. J. Agric. Food Chem. **52**, (6), 1445, **2004**.
43. BELMONTE VEGA A., GARRIDO FRENICH A., MARTINEZ VIDAL J.L. Monitoring of pesticides in agricultural water and soil samples from Andalusia by liquid chromatography coupled to mass spectrometry. Anal. Chim. Acta **538**, 117, **2005**.
44. FENOLL J., HELLÍN P., MARÍN C., MARTÍNEZ C.M., FLORES P. Multiresidue analysis of pesticides in soil by gas chromatography with nitrogen-phosphorus detection and gas chromatography mass spectrometry. J. Agric. Food Chem. **53**, (20), 7661, **2005**.
45. FUENTES E., BAEZ M.E., REYES D. Microwave-assisted extraction through an aqueous medium and simultaneous cleanup by partition on hexane for determining pesticides in agricultural soils by gas chromatography: A critical study. Anal. Chim. Acta **578**, 122, **2006**.
46. LESUER C., GARTNER M., MENTLEM A., FUERHACKER M. Comparison of four extraction methods for the analysis of 24 pesticides in soil samples with gas chromatography-mass spectrometry and liquid chromatography-ion trap-mass spectrometry. Talanta **75**, 284, **2008**.
47. REDONDO M. J., RUIZ M. J., BOLUDA R., FONT G. Determination of pesticides in soil samples by solid phase extraction disks. Chromatographia **36**, 187, **1993**.
48. FENOLL J., HELLÍN P., MARTÍNEZ C.M., FLORES P. Multiresidue analysis of pesticides in soil by high-performance liquid chromatography with tandem mass spectrometry. J. AOAC Int. **92**, (5), 1566, **2009**.
49. BABIC S., PETROVIC M., KASTELAN-MACAN M. Ultrasonic solvent extraction of pesticides from soil. J Chromatogr. A **823**, 3, **1998**.
50. BAKER S.A. Applications of matrix solid-phase dispersion in food analysis, J. Chromatogr. A **880**, 63, **2000**.
51. BLANCO E., CASAIS M.C., MEJUTO M.C., CELA R. Approaches for the simultaneous extraction of tetrabromobisphenol A, tetrachlorobisphenol A, and related phenolic compounds from sewage sludge and sediment samples based on matrix solid-phase dispersion. Anal. Chem. **78**, 2772, **2006**.
52. KRISTENSON E.M., BRINKMAN U.A.Th., RAMOS L. Recent advances in matrix solidphase dispersion. Trends Anal. Chem. **25**, 96, **2006**.
53. KADENCZKI L., ARPAD Z., GARDI I., AMBRUS A., GYORFI, L., REESE, G. Column extraction of residues of several pesticides from fruits and vegetables: A simple multiresidue analysis method. J. AOAC Int. **75**, 53, **1992**.
54. TSE H., COMBA M., ALAEE M. Method for the determination of organophosphate insecticides in water, sediment and biota. Chemosphere, **54**, 41, **2004**.
55. MORELLO M., PREVIALE L., QUAGLINO P.J. Gas chromatographic system equipped with a mass detector and a selective nitrogen-phosphorous detector operating simultaneously in the analysis of pesticide residues. J. Chromatogr. A, **740**, 263, **1996**.
56. EURACHEM GUIDE. The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics, December **1998**.
57. RASHID A, NAWAZ S, BARKER H, AHMAD I, ASHRAF M. Development of a simple extraction and clean-up procedure for determination of organochlorine pesticides in soil using gas chromatography-tandem mass spectrometry. J. Chromatogr. A **1217**, (17), 2933, **2010**.
58. GUIDE TO THE EXPRESSION OF UNCERTAINTY IN MEASUREMENT (GUM), International Organization for Standardization, Geneva, **1993**.