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Determination of metribuzin and major conversion products in soils by microwave-assisted water extraction followed by liquid chromatographic analysis of extracts

Emmanuil N. Papadakis, Euphemia Papadopoulou-Mourkidou*

Pesticide Science Laboratory, Aristotle University, P.O. Box 1678, 54006 Thessaloniki, Greece

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Abstract

A multiresidue method developed for the analysis of metribuzin and its major conversion products, deaminometribuzin (DA), diketometribuzin (DK) and deaminodiketometribuzin (DADK), in soils is presented. The method is based on microwave-assisted water extraction (MAWE) of soils using 10 m*M* phosphate buffer, pH 7 as extractant and analysis of aqueous extracts by HPLC–diode array detection. MAWE operational parameters were optimized with respect to extraction efficiency of the target compounds from soils with 1.5 and 3.5% organic matter content. Recoveries of all solutes above 80% were obtained from soils with 1.5% organic matter content; respective LOD and LOQ levels were determined at 5 and 10 μg/kg. In soils with organic matter content 3.5%, recoveries of all solutes were lower (<70%) and the respective LOD and LOQ values were determined at 10 and 50 μg/kg. However, recoveries of fresh and aged residues, the latter weathered under cold storage conditions, were not statistically different for both types of soils.

Keywords: Microwave-assisted water extraction (MAWE); Soil; Pesticides; Metribuzin; Deaminometribuzin; Diketometribuzin

1. Introduction

Metribuzin (MT) is a 1,2,4-triazine (triazinon) herbicide applied to soils for the control of annual grasses and broadleaf weeds in potatoes, tomatoes, asparagus, soybeans and sugarcane. For MT and other triazine herbicides in general there are concerns over the transfer of soil residues to rotating crops as well to surface and groundwater systems. Surface water contamination via runoff and subsurface losses and leaching of MT from crop land contaminating groundwater have been reported [1–5]. However, groundwater contamination by MT is still unclear since other studies showing the absence of MT in groundwater, even underneath sandy soils known to be vulnerable to pesticide leaching, have also been

*Corresponding author. Tel.: +30-310-471-478; fax: +30-310-471-478.

E-mail address: mourkidu@auth.gr (E. Papadopoulou-Mourkidou).

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reported [6,7]. MT is considered to be of short to moderate persistence in soils with DT_{50} values of 1–4 months. Its persistence is depended on soil pH and organic matter content both parameters affecting adsorption, leaching and microbial degradation rates [8–10].

Metribuzin is in use for many years, nevertheless, dedicated methods for the analysis of the parent compound and accompanied major conversion products in soils are very rare in international literature [11]; analytical methods reported so far have dealt with the analysis in soils of the parent compound only [12,13].

Simple, rapid and less labor intensive extraction techniques producing also minimum amounts of wastes of toxic solvents are needed in environmental analysis. Different instrumental sample extraction techniques have been developed in recent years and among these microwave-assisted extraction (MAE) is becoming particularly popular. During the last 2 years applications of MAE for the extraction of organic contaminants from environmental matrices increased rapidly [14–16], however, the main body of applications is devoted so far to the extraction of persistent hydrocarbons from marine sediments, soils and solid wastes. Applications of MAE for the analysis of only few chemical groups or individual pesticides have been reported so far [17–20]. The aims of this study were to (i) develop a simple and rapid method for the analysis of MT, DA, DK and DADK for use in environmental protection monitoring studies of field soils and other on or off farm contaminated sites, and (ii) investigate the feasibility of employing a microwave-assisted water extraction (MAWE) technique to minimize the amount of organic solvents needed. Analytical laboratories, especially those involved in national and contract monitoring studies of pesticides, produce annually high amounts of toxic wastes of organic solvents requiring the expenditure of a high percentage of economic resources for decontamination and disposal. Therefore, the availability of analytical methods for pesticide monitoring requiring the least amounts of organic solvents is highly desirable. Besides, analytical methods intended to be used for reasons of environmental protection (i.e. monitoring of pesticides in contaminated soils and other matrices) should be based on environmentally friendly techniques.

2. Experimental

2.1. Reagents and materials

Methanol, acetonitrile, K_2HPO_4, H_3PO_4, NaOH and $CaCl_2$ all of pro-analysis grade and SPE Lichrolut EN cartridges (200 mg) were purchased from Merck (Darmstadt, Germany). Water used as the mobile phase component and elsewhere was laboratory distilled water filtered through 0.45 μm acetate membrane filters (Millipore, USA) before any use. Analytical standards of metribuzin (MT), deaminated-metribuzin (DA), diketo-metribuzin (DK), and deaminated-diketo-metribuzin (DADK) were donated by Bayer (Monheim, Germany). Stock solutions of the above analytes at 50 μg/ml were made in methanol; mixed stock solution containing each analyte at 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10 ìg/ml were also prepared in methanol and these were used for the construction of calibration curves and the preparation of fortified soil samples (working standard solutions). Stock solutions of individual compounds were stored in aluminum/Teflon-lined capped vials at −23 °C; the working standards solutions were stored under refrigerated conditions (4–5 °C).

2.2. Apparatuses and respective operational parameters

The MSP 1000 laboratory microwave system (CEM, Matthews, NC, USA) equipped with 12 vessel carousel operated in the closed-vessel mode was used for the microwave-assisted extraction step. PTFE-lined extraction vessels were used and during operation both temperature and pressure were monitored in a single vessel; a sensor monitoring the solvent leaks in the interior of the microwave oven was also in use. The operational parameters of the microwave-assisted extraction (MAE) apparatus are shown in Table 1.

The liquid chromatographic (LC) analysis was carried out on a Spectra System, TSP (Thermo Separation Products, Austin, TX, USA) consisted of a P4000 tertiary solvent pump, a AS3000 autosampler equipped with a 20-μl loop and a UV6000LP diode array detector. Chromatography was carried out on a Nucleosil 100-5 C_{18}, 250×4.6 mm column (Macherey-Nagel, K.G. Duren, Ger-

Table 1
MAE operational conditions

Parameter	
Magnetron power (%)	100
Temperature (°C)	100
Pressure cutoff [p.s.i. (1 p.s.i.=6.894×10^3 Pa)]	90
Time to elapse to reach settings (s)	160
Extraction time (min)	10
Extractant volume (ml)	40
Sample weight (g)	10

many); a 7.5-mm Nucleosil guard column (Alltech, Darfield, USA) was always attached to the analytical column. Chromatographic data were monitored and processed by ChromQuest (TSP).

The mobile phase of the HPLC system consisted of a binary gradient mixture of 0.1% phosphoric acid (solvent A) and a 90:10 (v/v) mixture of acetonitrile and HPLC water (solvent B). The gradient composition is shown in Table 2. The mobile phase flow-rate was set at 1 ml/min and degassing was carried out on-line by use of TSP degasser. Injections of 20 μl were made by use of an TSP liquid autosampler. Acquisition for quantitative measurements was made at 294 (MT), 258 (DK and DADK) and 240 (DA) nm while detection and identification was based on UV spectra scanned in the 192–350 nm range and compared with respective library stored spectra. Quantitative measurements were made by use of external standard calibration curves.

2.3. Sample preparation

2.3.1. Microwave-assisted water extraction (MAWE)

Soils were air dried, mixed and sieved through a 2-mm sieve and 10±0.1 g portions were processed by MAE. Soil samples transferred into the MAE vessels were suspended into 40 ml of 10 m*M* phosphate buffer (pH 7). Each vessel was closed gas-tight and shaken vigorously by hand for 30 s; sets of 12 samples were processed according the operational program shown in Table 1. The vessels removed from the microwave oven and before opened were allowed to cool at 38–40 °C by use of an ice bath. Subsequently, samples were centrifuged for 5 min at 5000 rpm and 25-ml aliquots withdrawn from the supernatants were filtered through membrane filters. These filtrates were taken for direct HPLC analysis. In case of trace analysis the filtrates were diluted to 100 ml by addition of the appropriate volume of the buffer (pH 7) and were pre-concentrated by SPE (solid phase extraction) before analyzed by HPLC.

Table 2
HPLC gradient regime

Time (min)	% Solvent B[a]
0	22
20	100
25	100
30	22
35	22

[a] The composition of solvents A and B is given in Section 2.

2.3.2. Pre-concentration of extracts by SPE

SPE was carried out on Lichrolut EN cartridges (200 mg) pre-conditioned with 4 ml methanol followed by 4 ml of distilled water. Extracts were loaded on SPE cartridges at the rate of 5 ml/min by use of a vacuum operated pumping system. After sample loading and before air was drawing in to dry the cartridge, 20 ml of the phosphate buffer (pH 7) was added into the sample container and pumped onto the same cartridge. This measure was found necessary to avoid losses from sorption of analytes onto the surface of the sample container and connecting tubing; some very polar and ionized soil coextractives were also eliminated during this step. Cartridges were air dried for 15 min and solutes were eluted with 5 ml methanol followed by 2 ml of ethyl acetate. The combined eluates were concentrated to dryness by use of nitrogen stream and the residue re-dissolved in 200 μl of methanol was taken for HPLC analysis.

2.4. Validation of method

All method development and validation experiments were carried out by use of a typical Mediterranean sandy silt loam soil (pH 7.8) with 34, 13, 50 and 1.5% silt, clay, sand, and organic matter content, respectively. A batch of soil removed from the plough layer (0–10 cm) of a wheat field was air dried, mixed and sieved through a 2-mm sieve and 10±0.1 g portions were processed by MAE.

The method was validated with the analysis of fortified soil samples spiked at 1000, 500, 100, 50

and 10 μg/kg of soil dry weight by addition of appropriate volumes of the working standard solutions. Spiked samples were processed either 1 h after spiking (fresh residues) or after storage for 60 days at 3–5 °C (aged residues). Since MT is known to be sorbed onto the soil organic matter [8–10] and in the absence of natural soils rich in organic matter, the method was also validated by use of a soil artificially made to 3.5% in organic matter by addition of the appropriate amount of peat (10% w/w); the pH of this soil deceased to 6.6. Fortified samples were also prepared with the artificially enriched soils and the method was validated with the analysis of both fresh and aged residues. Spiked soils containing fresh and aged residues were also processed by a comparison extraction technique. Finally the proposed method was used for the analysis of soils from potato and asparagus fields where MT is commonly used.

2.5. Determination of the detection and quantification limits

The limit of detection (LOD, μg/kg) was determined as the lowest soil fortification level giving a response for each target analyte that can be recognized and tentatively identified by UV spectrum comparisons with respective data stored in spectral library. The limit of quantification (LOQ, μg/kg) was determined as the lowest fortification level that could be identified and quantified with adequate precision (relative standard deviation of mean recovery values (RSD) $<20\%$).

3. Results and discussion

3.1. General considerations

A series of preliminary experiments were conducted in selecting the optimum operation conditions of the microwave-assisted extraction step (Table 1). Among the parameters included in Table 1, the setting of magnetron power to 100% was selected arbitrarily. A sample weight of 10 ± 0.1 g was also selected without further consideration since this is the amount of sample commonly required in methods operated on modern instrumental analytical systems. The use of the water based extractant was also selected by default because one of the aims of this work was to exploit microwave-assisted water extraction (MAWE) as a viable technique for the analysis of pesticides in soils. Applications of MAWE for the analysis of imidazolinone herbicides and the analysis of the fungicide tebuconazole in plant tissues have been already reported [21,22]. Under these circumstances the parameters remained to be optimized were the extraction temperature and duration and the extractant volume. An upper limit of 90 p.s.i. (1 p.s.i.$=6.894\times10^3$ Pa) pressure cutoff was also set for precautionary reasons of operator safety even though the pressure monitored in MAE vessels during operation never exceeded 10 p.s.i..

Since all the sought analytes are relatively polar compounds (water solubility >1.0 g/l) for reasons of comparison the suitability of polar organic solvents (methanol and acetonitrile) and mixtures of these solvents, in addition to water based extractants (10 m*M* phosphate buffer of pH 7 and 0.01 *M* $CaCl_2$), were also evaluated in preliminary MAE based experiments (Fig. 1). These preliminary experiments were carried out with soils spiked at 100 μg/kg level, the extractant to matrix ratio set at 20:10 (v:g) and the MAE operated at 80 °C for 5 min. Under these conditions recoveries of DA (deaminometribuzin) and DADK (deaminodiketometribuzin) higher than 80% were obtained with all extractants whereas the recoveries of MT and DK (diketometribuzin) were variable depending upon the extractant (Fig. 1). As it was expected the recoveries of MT, the most lipophilic compound among the target analytes, were the highest (around 100%) when soils were extracted with methanol or acetonitrile and the mixture of these solvents and decreased when water based extractants were used instead. For DK the highest recovery (70%) was obtained with the phosphate buffer, however, comparable recoveries were also obtained when the 0.01 *M* calcium chloride or methanol were used instead. A 0.01 *M* calcium chloride solution was also included in this preliminary evaluation because this solution is the most commonly used in pesticide adsorption/desorption studies in soils. A 10 m*M* phosphate buffer of pH 7 instead of pure distilled water was used as extractant to assist the dissolution of soil organic matter [23].

The data presented in Fig. 1 were considered very encouraging to further investigate and optimize the

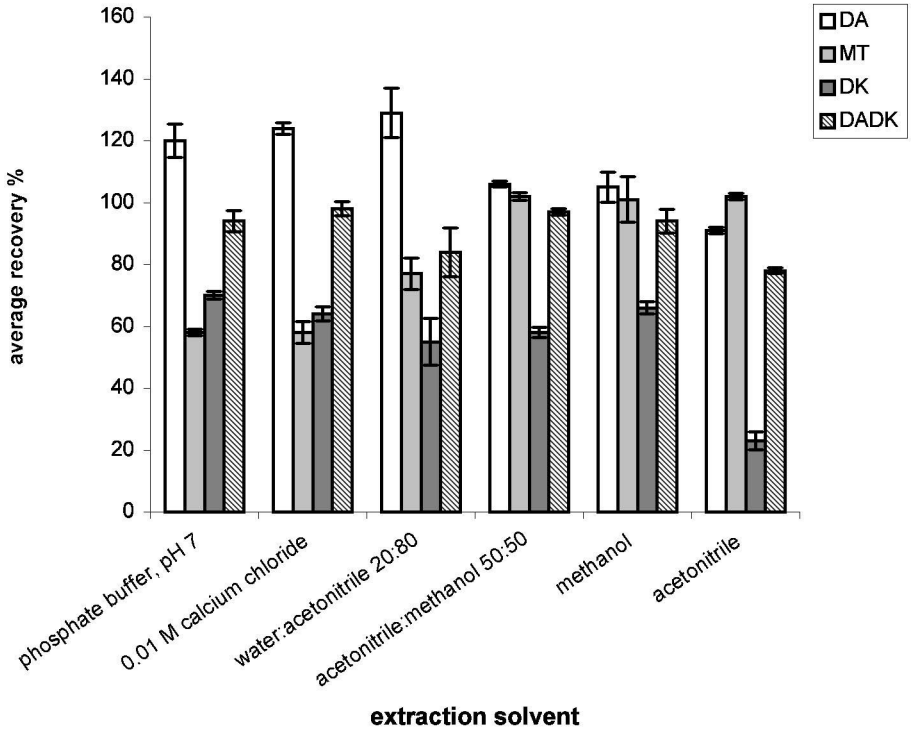

Fig. 1. Mean % recoveries ($n=3$) of solutes from spikes soils (100 μg/kg) processed by MAE for 10 min at 80 °C with 20 ml of the following extractants: 10 m*M* phosphate buffer, pH 7; 0.01 *M* calcium chloride solution; water–acetonitrile (20:80, v/v); acetonitrile–methanol (50:50, v/v); methanol; acetonitrile. Extracts were analyzed as described in Section 2. Error bars represent standard error of the mean values.

parameters affecting the microwave-assisted water extraction (MAWE) of the target analytes from soils using the 10 m*M* phosphate buffer (pH 7) as the extraction solvent, even though methanol appeared to be as equally effective and even better especially for metribuzin.

Therefore, the MAWE technique was further optimized with respect to % recoveries of solutes by selecting the appropriate extraction duration and respective temperature and the extractant volume. Preliminary experiments carried out at 80 °C for 5, 10, 15 and 20 min, respectively, showed that the solute recoveries increased as the extraction duration increased from 5 to 20 min (data are not shown), however, the recovery increases were not significant beyond the 10-min duration and therefore this time setting was selected.

Given that the main mechanisms of sorption in soils, at least for metribuzin, are attributed to sorption sites onto the soil organic matter (OM), it was expected that the soil OM content to affect not only the sorption but also the desorption efficiency of the selected extractant. Therefore, all further optimization experiments were carried out with the typical soil (1.5% OM content) as well with the soil batch artificially enriched to 3.5% OM by addition of peat. The effect of the extraction temperature on the extraction efficiency, as depicted by the % mean recoveries, when MAE was operated at 80, 100 and 120 °C, respectively, is shown in Fig. 2. When the typical soil with 1.5% OM was used, % recoveries were higher at 100 than either 80 or 120 °C for all solutes except for DADK; the % recoveries of DADK decreased as the extraction temperature increased from 80 to 120 °C, indicating a higher thermal instability of DADK compared to the rest of the analytes. For the soils enriched in OM, the effect of the extraction temperature was compound depen-

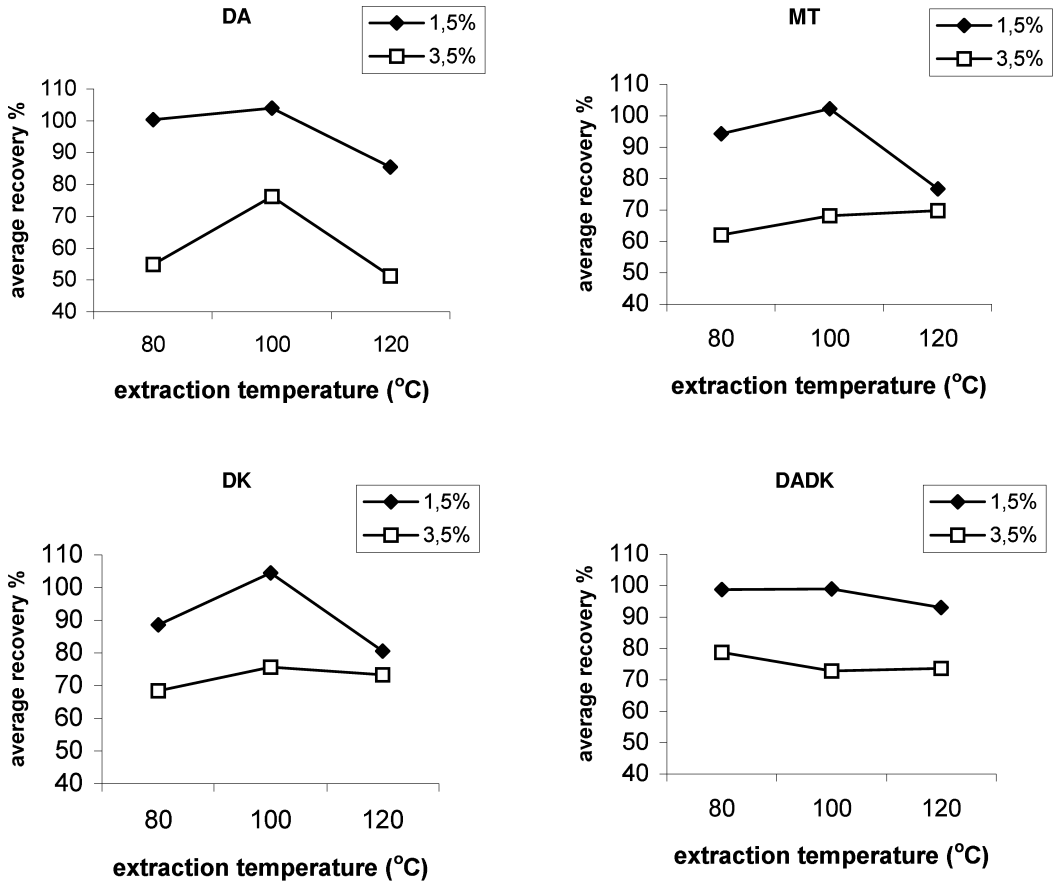

Fig. 2. Mean % recoveries of target solutes from soils with 1.5 and 3.5% OM content, respectively, fortified at 1000 μg/kg and processed by MAWE for 10 min at different temperature settings. The extractant volume was set at 40 and 60 ml for soils with 1.5 and 3.5% OM content, respectively. All other conditions were set as described in Section 2.

dent (Fig. 2). For DADK, which appeared to be the most thermally sensitive compound, as discussed above, the recoveries decreased as the extraction temperature increased whereas for DA and DK the recoveries were higher at 100 than 80 or 120 °C. For MT, recoveries increased as the extraction temperature increased from 80 to 120 °C. This compound is probably the most strongly sorbed onto the organic matter and as such is most thermally protected by the presence of increased OM content given the fact that this compound indicated thermal instability at 120 °C in soils with lower OM content. On the basis of the data presented in Fig. 2 which indicate a different degree of thermal sensitivity for each solute, the thermal stability dependent also on the soil OM content, and to compensate for the above differences the 100 °C extraction temperature was selected.

The effect of the extractant volume on the extraction efficiency of the MAE, as defined above, is shown in Fig. 3. For the soil with 1.5% OM, the recoveries of DK, MT and DADK increased as the extractant volume increased from 20 to 40 ml and decreased with further increase to 50 ml while for DA the respective recovery values increased as the extractant volume increased up to 50 ml. The increase of recoveries as the extractant volume is decreased has been also reported elsewhere [15] and was attributed to a better swirling of samples by the microwave energy at lower extractant volumes.

For soils with 3.5% OM, the recoveries of DK and

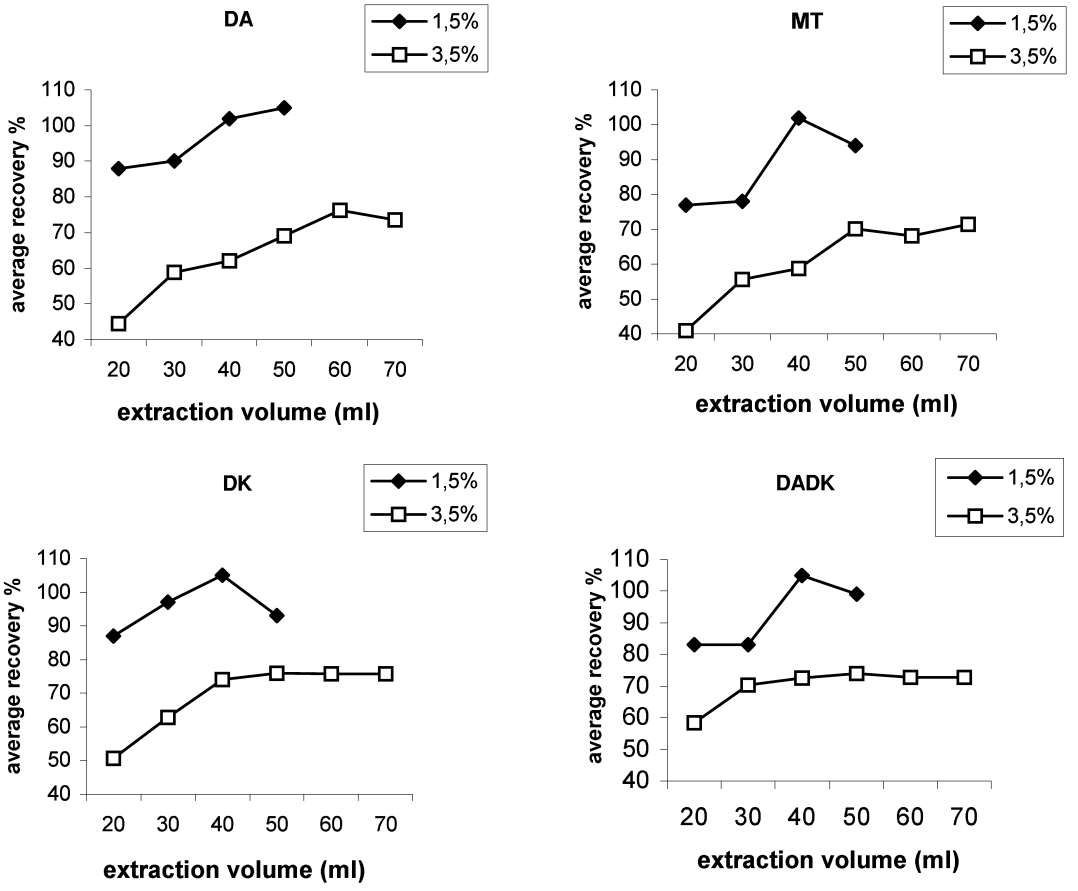

Fig. 3. Mean % recoveries of target solutes from soils with 1.5 and 3.5% OM content, respectively, fortified at 1000 μg/kg and processed by MAWE at 100 °C for 10 min and the extractant volume ranging from 20 to 70 ml. All other conditions were set as described in Section 2.

DADK increased as the extractant volume increased from 20 to 40 ml reaching a plateau without significant increases as the extractant volume increased from 40 to 70 ml. For MT and DA, the recoveries increased as the extractant volume increased up to 50 and 60 ml, respectively; the recoveries of both compounds were not increased significantly with further increase of the extractant volume up to 70 ml. Thus, to ensure for the accurate determination of all solutes and in both types of soils the extractant volume was set at 40 ml.

3.2. Optimization of the SPE pre-concentration step of the aqueous extracts

The conditions for the SPE pre-concentration step of the aqueous extracts were selected after preliminary experiments carried out to determine the breakthrough volumes of the sought analytes on Lichrolut EN, 200 mg cartridges. All solutes were found to have breakthrough volumes >200 ml. For all solutes, except for MT, the recovery values were approximately the same when the sample volumes ranged from 25 to 200 ml; the recoveries of MT, which is the most lipophilic solute among the target analytes, increased as the sample volume increased from 25 to 100 ml. This inconsistency reported also for other lipophilic compounds [24] is probably due to sorption of MT onto the sample container and transfer lines of the SPE system. Thus the sample volume, before loaded onto the cartridge, was adjusted to 100 ml and the use of additional 20 ml of buffer (pH 7)

to rinse the sample container and connecting tubing of the SPE improved recoveries. For the elution of sought analytes from the SPE cartridges the use of 2 ml of ethyl acetate in addition to 5 ml of methanol was found necessary to efficiently desorb MT from the cartridge sorbent material.

Besides Lichrolut EN other commercially available and equivalent sorbents were also evaluated, however, among these Lichrolut EN was selected because cartridges packed with Lichrolut EN were found to be re-usable (each cartridge could be used for 10–15 samples) reducing thus tremendously the cost of the overall method.

3.3. Liquid chromatographic analysis, LOD and LOQ levels and linearity

The chromatographic conditions were selected as such to analyze crude soil extracts with no cleanup. Under the selected conditions (mobile phase composition and analytical column) all sought analytes were separated from co-extractives and were eluted in the form of symmetrical peaks (the peak symmetry ranging from 0.85 to 1.0). Sample chromatographic data are presented in Fig. 4. For soils with OM 1.5% the LOD and LOQ (μg/kg) levels were determined at 5 and 10 μg/kg, respectively, while for soils with 3.5% OM content the respective levels were set at 10 and 50 μg/kg. For soils with higher OM content the method should be re-evaluated. In the absence of a natural soil high in OM content soils artificially enriched in OM were prepared by addition of 15, 20 and 30% of peat (w/w). However, data derived from these experiments were unreliable (decreased method precision) due to difficulties in dispersing the peat mass homogeneously into the soil matrix and the high water holding capacity of peat.

External standard calibration curves were used for

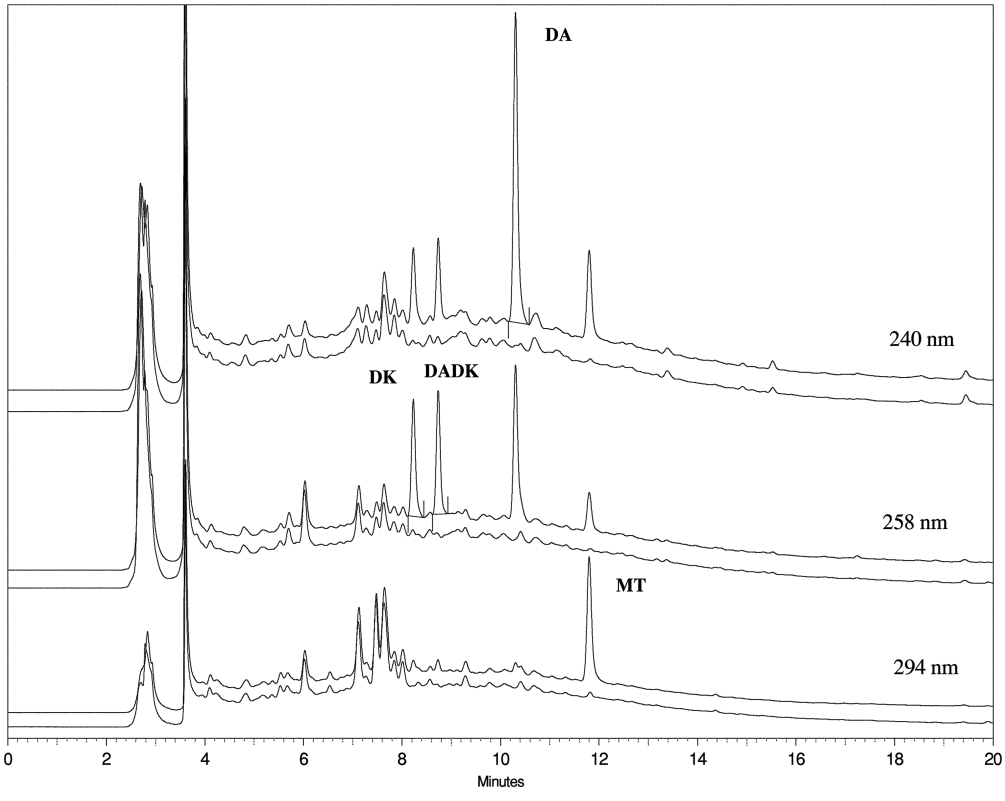

Fig. 4. Sample chromatograms from the analysis of a spiked soil sample and a respective control processed as described in Section 2.

the solute quantification and these were linear in the concentration range of 1.0–300 ng with respective correlation coefficients being better than 0.999.

3.4. Method validation

The method was validated by analyzing spiked samples fortified in the 10–1000 μg/kg range. Two series of soil samples with 1.5 and 3.5% OM, respectively, were fortified and samples containing fresh (1 h) and aged (60 days) residues were analyzed. After spiking, samples were allowed to stand for 1 h at ambient temperature, and then were either processed (fresh residues) or stored under refrigerated conditions for the designated time periods (aged residues). Samples were stored under cold conditions to limit losses due to microbial and/or chemical degradation and evaporation of solutes. Sample data from these validation studies are shown in Figs. 5 and 6 for the soils with 1.5 and 3.5% of OM content, respectively. For soils with 1.5% OM content, the % mean recoveries of all solutes were >80% in the fortification range of 50–1000 μg/kg; however, lower recoveries (>65%) were obtained at the LOQ level (10 μg/kg). The relative standard deviation values (RSDs) of all % mean recovery values (n=6) were <10% at the

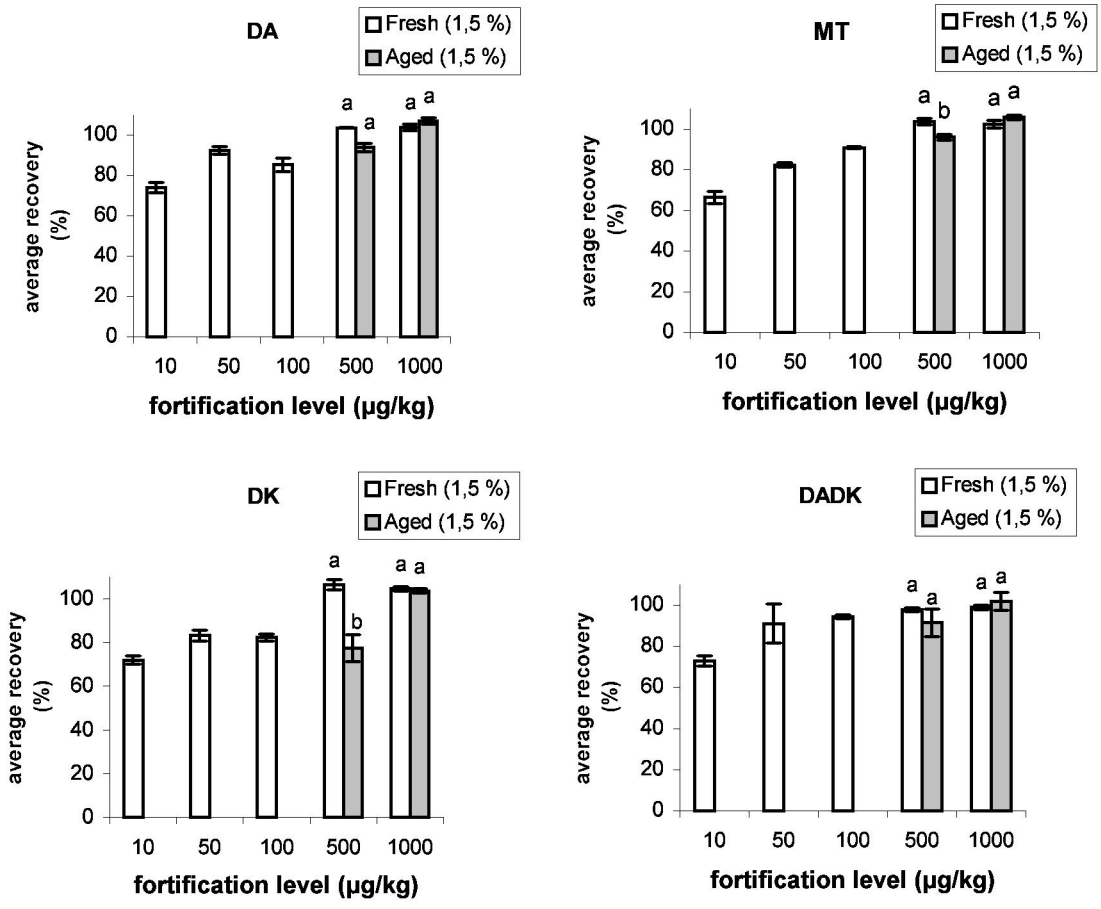

Fig. 5. Mean % recoveries of target solutes of fresh and aged residues from soils with 1.5% OM content spiked at the 10–1000 μg/kg range processed as described in Section 2. Bars with the same head letter are not significantly different (least significant difference test at α=0.05). Error bars represent standard error of the mean values.

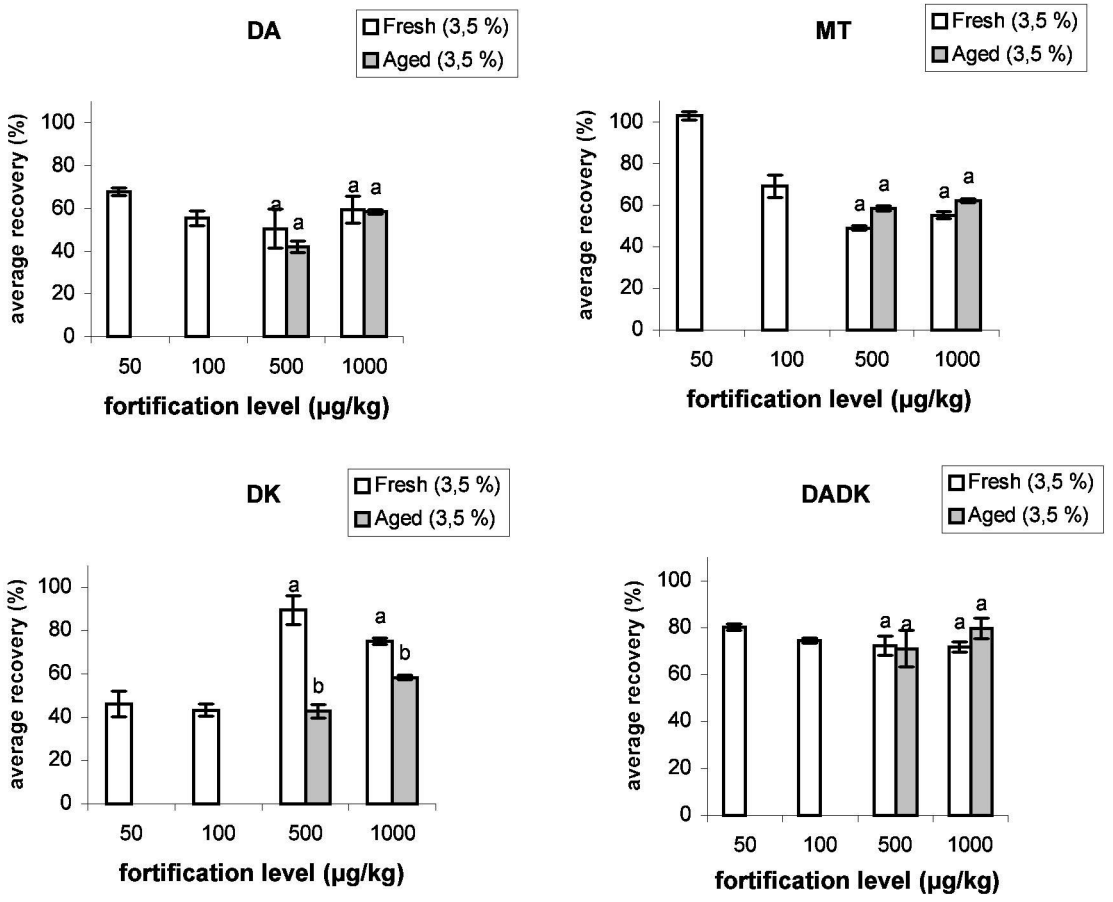

Fig. 6. Mean % recoveries of target solutes of fresh and aged residues from soils with 3.5% OM content spiked at the 50–1000 μg/kg range processed as described in Section 2. Bars with the same head letter are not significantly different (least significant difference test at $\alpha=0.05$). Error bars represent standard error of the mean values.

50–1000 μg/kg fortification levels and <20% at the 10 μg/kg level (data are not shown).

The % mean recoveries of all solutes of fresh residues were not statistically different from the respective values of the aged residues (Fig. 5). There was only one exception with soils fortified at the 500 μg/kg level; in the latter soils the recoveries of MT and DK were significantly lower in soils with aged residues.

For soils with 3.5% OM content (Fig. 6) the % mean recoveries of all solutes were lower than the respective values shown for soils with 1.5% OM content (Fig. 5). However, the recoveries of fresh and aged residues were not statistically different for all solutes except for DK; the recoveries of aged residues of DK were significantly lower than the respective values of fresh residues (Fig. 6). For these soils containing 3.5% OM the % mean recoveries of DADK were >70, of MT and DA >50% and for DK >40%. However, in spite of the lower recoveries of solutes from these soils the respective RSDs of the mean recovery values were in the same range as for the soils with 1.5% OM content (data are not shown). All the above differences of mean recovery values were evaluated by the least significant difference test at $\alpha=0.05$.

The method reported here was also validated with the analysis of field soils collected from the plough layer (0–10 cm) of two fields cultivated with potatoes and asparagus, respectively, known to have

received MT treatments. In none of these soils residues of MT and accompanied degradation products were found at levels above the respective LODs. Therefore, under these circumstances, the method was further validated by analyzing batches of spiked soils (500 μg/kg) containing fresh and cold aged residues (aged for 2 months at 3–5 °C) by a comparison technique based on flask-shaking extraction (FSE). In FSE samples of 10 g of soils were shaken for 24 h with 40 ml of methanol:water mixture (80:20, v/v) by use of a closed planar mechanical shaker operated under ambient conditions. Samples were centrifuged for 5 min at 5000 rpm and 25-ml aliquots from the supernatants, evaporated to remove most of methanol content and diluted to 100 ml by addition of distilled water, were pre-concentrated by SPE as previously described. The data from this exercise are shown in Table 3. The accuracy of both methods is acceptable as depicted by the mean recovery values of all solutes being >80%. For each method and solute the recoveries of fresh and aged residues were not statistically different. However, for all solutes except for DK the recoveries of both fresh and aged residues were significantly higher for samples processed by FSE than MAWE; the recovery values of DK in samples processed by either MAWE or FSE were not statistically different. The precision of both methods was equally good judging from the low RSDs of the mean recovery values being <4% for MAWE-based method and <6% for the FSE-based method. In this exercise the differences among the mean recovery values were evaluated by the Duncan test at $\alpha=0.05$. Certainly, in spite of the slightly higher recovery values given by the FSE, the MAWE technique is superior in terms of rapidity (10 min vs. 24 h extraction period), does not require the use of an organic solvent and is comparable to FSE in terms of accuracy and slightly superior in terms of precision.

The lower solute recoveries obtained by MAWE from soils enriched with peat (Figs. 2–6), at least for MT, are in agreement with data reported on the absorption/desorption behavior of MT on humic acids isolated from peat [25]. The absorption isotherms of MT on peat humic acids showed high affinity of MT to the substrate, the substrate allowing easy penetration of solute molecules and as a consequence the desorption process was difficult and slow, the non-desorbed amount of MT (79% of the adsorbed amount) considered of either being chemically bound onto humic acids or degraded. MT was also reported to adsorb irreversibly onto rot-wood lignin and only 62% of the applied amount was leached over a period of 24 h [26]. The bioavailability (availability to plants and microbes) of MT in soils was also highly dependent on the soil OM content, decreased as the soil OM was increased [8].

Given that the recoveries of all solutes were highly decreased as the OM content of soils increased. whereas generally there were not significant differences between the recoveries of fresh and cold aged residues, irrespective of soil organic matter content, it can be deduced that 20–50% of the amount of target analytes spiked to soils containing 10% peat is irreversibly bound to humic acids of peat. Therefore, the addition of peat to soil could serve as an effective bioremediation tool in on/off farm highly contaminated sites to reduce the risk of surface and groundwater contamination not only from MT but also from its major conversion products.

Table 3
Comparative data of % mean recoveries and respective relative standard deviations (RSDs) of target solutes from spiked[a] soils processed by microwave-assisted water extraction (MAWE) and flask shaking extraction (FSE) techniques

Compound	MAWE (%)		FSE (%)	
	Fresh residues	Aged residues	Fresh residues	Aged residues
DA	88.5 (1)[a]	86.6 (1)[a]	97.4 (0.4)[b]	93.9 (5)[ab]
MT	91.6 (0.6)[ab]	90.5 (0.3)[a]	96.5 (1)[bc]	99.7 (4)[c]
DK	80.3 (5)[a]	81.1 (4)[a]	82.1 (0.6)[a]	85.2 (2)[a]
DADK	84.3 (1)[a]	88.8 (0.4)[a]	99.7 (0.1)[b]	103.2 (6)[b]

Values in the same row followed by different letters are statistically different (comparisons of means by the Duncan test at $\alpha=0.05$).

[a] The typical Mediterranean SSL soil with 1.5% organic matter content was spiked at 500 μg/kg and residues were analyzed 4 h (fresh residues) and 60 (aged residues) days after spiking; the samples were stored refrigerated during aging.

In spite of the lower recovery values of solutes from soils enriched in peat, the overall performance of the MAWE based method reported here is comparable to a method reported recently for the analysis of MT and its conversion products in soils [11]. In the latter method, pressurized liquid extraction was employed using as extractant a methanol–water mixture (75:25, v/v) at 60 °C; recoveries of 75% were reported for MT, DA and DADK and 50% for DK for soils with OM content 3.9% (2.3% organic carbon content); however, recoveries of <60% were also reported for all solutes when subsoils with OM 0.039% (0.023% organic carbon) were processed.

4. Conclusions

A method based on MAWE coupled to HPLC–DAD was developed for the analysis of MT, DA, DK and DADK in soils. Recoveries higher than 80% were obtained for all solutes of both fresh and aged residues of soils with 1.5% OM content. However, recoveries from soils with higher OM content are expected to be different. Studies using a soil artificially enriched in organic matter by addition of peat (10% w/w) showed reduced recoveries (<70%), however, the data derived from this soil cannot be considered as indicative of the method performance with soils rich in organic matter because, as reported in the literature [25], MT has high affinity for peat humic acids leading to possibly irreversible binding and degradation.

The MAWE technique, in terms of accuracy and precision, was found to be comparable to the conventional flask-shaking technique employing as extractant a methanol–water mixture.

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