Microwave-assisted solvent extraction of the herbicide methabenzthiazuron from soils and some soil natural organic and inorganic constituents. Influence of environmental factors on its extractability

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A microwave-assisted solvent extraction (MASE) method for the determination of methabenzthiazuron (MBT) in soil samples by HPLC-DAD (diode array detection) was evaluated. Spiked soil samples having different physico-chemical properties, and selected soil-derived matrices with diverse MBT adsorption capacity, characterized by their Freundlich equation K_f values, were used to verify the method applicability to a broad range of different soils. The spiking procedure was considered a crucial point to reproduce as closely as possible the solute–soil adsorption taking place in the natural environment. Ageing effects, where the compound could diffuse into inaccessible locations within the soil matrix in view of its great stability, were considered of particular concern. In spite of the heterogeneous physico-chemical properties of soils under study, recoveries were greater than 90%. Performance of the MASE procedure correlated highly with the adsorption capacity of soil-derived matrices: the lowest recoveries were for illite (67–73%), among the mineral surfaces, and for a humic acid (67–72%), among the organic fractions. Intra-assay variation for each type of sample soil range from 0.40 to 3.89% (RSD). Limits of detection and quantification were 0.047 and 0.15 μg g^{-1}, respectively. Analyte residence time was not a very significant factor on the extractability.

Introduction

Methabenzthiazuron (MBT) [1-(1,3-benzothiazol-2-yl)-1,3 dimethylurea], a selective herbicide used to control grasses and broad leaved weeds,[1] has a great soil pollution capacity. Partial recoveries of residues, which become difficult to extract by the conventional solvent extraction analytical approach have been attributed to a fraction tightly bound to soil components. It is slowly degraded both in the laboratory and in the field,[2–5] the stability being attributed to its rapid binding to the soil colloidal complex and more specifically through the incorporation into relatively stable humus components as non-extractable bound residues.[4] In a [^{14}C] MBT-treated soil aged for 6 months, the remaining ^{14}C activity was equivalent to 50–60% of the MBT originally applied and the total amount of ^{14}C extracted by using H_2O/acetone/chloroform accounted for 70% of the total ^{14}C in soil samples; however, over 90% was identified as the parent MBT.[2] Adsorption experiments in calcareous soils with low organic matter have also demonstrated a relationship with clay and smectite contents as well as with surface area. After an incubation period of 42 days, no degradation was observed and leaching experiments with clay and silt loam soil columns revealed that more than 96% of the MBT applied was retained within the upper layer.[6] On the other hand, adsorption studies on five non-allophanic and one allophanic agricultural Chilean soils showed K_f values from the Freundlich equation ranging from 5.3 to 82.1 cm^3 g^{-1} and linear regression analysis between K_f and organic matter content of non-allophanic soils gave a high correlation coefficient (0.980, $P = 0.02$).[7] Allophanic soils are derived from weathered basaltic-andesitic ashes and contain a high proportion of allophane, a poorly defined aluminum silicate, and appreciable amounts of Fe and Al amorphous oxides. Porosity is high and the bulk density is very low.

These facts and also the need for a better understanding of the environmental behaviour of this organic contaminant led us to evaluate the microwave-assisted solvent extraction (MASE) approach to develop an accurate, precise and fast method for MBT determination in soil samples.

Taking into account that the effect of soil composition on the extractability of pesticide residues is usually complex due to its heterogeneous nature, fortified soil samples having particularly different physico-chemical properties, and selected soil-derived matrices with diverse MBT adsorption capacity, all of them characterized by their K_f values, were used to verify the method applicability to a broad range of different soils. The spiking procedure was considered a crucial point, because it should reproduce as closely as possible the solute–soil adsorption taking place in the natural environment. Two principal fortification ways were employed to do recovery tests: in the first one soil samples were contaminated through a batch equilibrium method to secure a whole MBT adsorption process, the extent being controlled by the soil adsorption capacity; in the second one, through a more conventional criterion, by directly spiking samples at levels at which MBT could be detected in naturally contaminated soils.[5] Ageing effects in the adsorption process, where the compound can diffuse into inaccessible location within the soil matrix in view of its great stability, consequently with a considerable length of time available to interact, were also considered to be of particular concern.

Experimental

Reagents

Pure analytical Methabenzthiazuron (Pestanal®, chemical purity >99%) was purchased from Riedel de Häen (Seelze, Germany). Its solubility in water is 59 μg cm^{-3} at 20 °C. All stock solutions and further dilutions were prepared in acetonitrile or methanol for using as standards, and in water for spiking purposes. Acetonitrile and methanol, HPLC grade (J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA), were used for extraction. Water was provided by a NANO pure™ analytical deionisation system (Barnstead, Thermolyne Corporation, Dubuque, Iowa, USA).

Adsorbents

Three non-allophanic soils from the Aconcagua Valley (V Region of Chile), Pocuro (PCR), Quillota (QLT) and Los Hornos (LHS) and, four allophanic soils from the IX Region, Temuco (TEM), Galvarino (GLV) Cunco (CUN) and Gorbea (GRB) were used. The most relevant physical and chemical properties are given in Table 1. In order to increase the degree of complexity of the sample to be extracted, two of them (PCR and QLT) were amended with a commercial peat, its organic matter content (OM) being 50%; its pH and cation exchange capacity (CEC) were 2.8 and 1190 mmol kg^{-1}, respectively. Humic and fulvic acids and humin-mineral residues were extracted from GLV and GRB soils according to a method previously described for volcanic soils.[8] Ferrihydrite-coated allophane (Al–Si–Fe) was prepared by co-precipitating $AlCl_3$ and Na_4SiO_4 to obtain synthetic allophane, and by the subsequent coating with a $Fe(NO_3)_3 \times 9H_2O$ solution.[9] Particles <2 μm obtained by sedimentation of a Wyoming montmorillonite were saturated with Na by five successive treatments with NaCl.[10] The National Institute for Agricultural Research (INIA, Chile) supplied montmorillonite and illite. K_f values from the empirical Freundlich relationship describing adsorption behaviour were established for all of these materials. Concisely, 2.0 g of soil samples were treated with 10 mL of MBT solutions (5, 10, 15, 20 and 25 μg ml^{-1}) with 0.01 M $CaCl_2$ as background electrolyte; in the case of soil-derived matrices 100 mg were weighed. Suspensions were shaken at 30 ± 1 °C for 14 h and centrifuged. The equilibrium concentrations of supernatants were determined by HPLC-DAD.

Equipment

A Waters (Milford, MA, USA) HPLC chromatograph equipped with a Model 600 quaternary gradient pump, a Model 717 Plus autosampler and a Model 996 PDA detector was used. System control, data acquisition and process were made by the Millennium 2010 software. DAD instrument parameters were: wavelength, 200–300 nm; acquisition rate, 1 spectra s^{-1}; spectral resolution, 1.2 nm. The separation column was a Waters NovaPak C_{18} 60 Å, 3.9 × 300 mm, 4 μm particle size with a μBondapak™ C_{18} 120 Å, 3.9 × 20 mm, 10 μm particle size guard cartridge. The mobile phase used was H_2O:CH_3CN = 50:50 at a flow rate of 1.1 ml min^{-1} for MASE extracts from all soils and organic and mineral fractions under study. Detection wavelength was 225 nm. Column temperature was 35 °C. A 20 μl volume of the sample was injected.

Extractions were performed with a Milestone (Sorisole, Bergamo, Italy) MLS-1200 Mega microwave digestion system configured with a MDR-600/10 carousel. Maximum pressure for this configuration is 30 bar (430 psi) and 10 vessels can be simultaneously used.

Table 1 Soils physico-chemical characteristics

Soil	OM[a] (%)	pH	CEC[b]/ cmol kg^{-1}
PCR	1.4	7.2	18.0
LHS	2.5	6.7	22.5
QLT	3.1	7.4	22.8
GLV	3.9	5.7	28.5
GRB	14.7	4.9	62.0
CUN	13.9	5.8	79.2
TEM	11.4	6.3	55.5

[a] OM: organic matter. [b] CEC: cation exchange capacity.

Soil spiking methods

Three spiking methods were used to establish recoveries, including the studies to assess the influence of ageing in the soil matrix:

(1) Four soils, plus two of them amended with peat, were fortified at five high levels of concentration according with the following procedure: 8 g of air-dried soil samples were equilibrated with 40 ml of aqueous standard solutions (2, 4, 6, 8 and 10 μg ml^{-1} for QLT and PCR and 5, 10 15, 20 and 25 μg ml^{-1} for the other soils) into 60 ml sealed tubes by shaking mechanically at 30 °C ± 0.5 °C for 14 h. After equilibration, the suspensions were centrifuged at 4000 rpm for 20 min and MBT was determined in the supernatant. The residual moisture content was determined in all samples by drying at 105 °C for 24 h. The amount of sorbed herbicide was considered to be the difference between the initially present and that in the equilibrium solution. After drying overnight at 30 °C, samples were homogenized by grinding and stored at 4 °C.

(2) Freshly spiked soil samples were obtained by weighing 1.00 g of soil into the extraction vessels followed by the addition of 1 ml of aqueous spiking solution (0.45, 0.90, 1.35 and 1.80 μg ml^{-1}); the samples were equilibrated by shaking for 1 h and standing overnight before extraction. The same procedure was used to get freshly spiked organic fractions and mineral adsorbents. The amounts of adsorbents weighed were: 0.075 g of humic and fulvic acids, montmorillonite-Na, illite and Al–Si–Fe, and 0.4 g of peat and humin-mineral residues. All tests were done in duplicate.

(3) Three sets of sample soils fortified at 2.0, 0.5 and 0.15 μg g^{-1} levels were used to evaluate the effect of sample weight, type of soil, and the interaction between these factors on the extraction, and the effect of ageing at low analyte concentration. The first one was obtained by fortifying 50 g of PCR, QLT, TEM and CUN soils by the addition of 25 ml of an aqueous spiking solution at a proper concentration to reach the 2.0 μg g^{-1} level. As above, suspensions were equilibrated by shaking for 1 h, standing overnight at 4 °C, subsequently they were dried at 30 °C, homogenized by grinding and stored at 4 °C until the analysis. In this type of sample, the residual moisture content was also determined. A similar procedure was used to get the 0.5 and 0.15 μg g^{-1} levels for PCR and TEM soils. Ageing periods considered for the analysis of these samples were: 7, 60, 105 and 150 days.

General MASE procedure

A 1.00 g spiked soil sample, weighed on aluminium paper, was quantitatively transferred to the extraction vessel. Subsequently, 1 ml of water and 10 ml of acetonitrile or methanol were added to each sample. Unless otherwise stated, all samples were extracted in duplicate and 10 simultaneous extractions were always performed. Blank tests for all the soils and adsorbent materials used in the present work were carried out to

evaluate the co-extracted components, which could interfere in the HPLC-DAD determination. The starting parameter settings in the microwave system were: 2 min at 350 W, 3 min at 500 W and, finally, a 2 min vent step at 0 W. Once the digestion program was completed, the rotor was cooled down inside the cooling system for 15 min before opening the vessels. The effect of water content, extracting solvents, and instrumental parameters such as time and power for each heating step was preliminary studied by using a contaminated sample of the QLT soil. The heating program was modified by adding 2 min to or by subtracting 1 min from the first two steps or by adding 150 W to the second step or by subtracting 100 W from the first two steps. After the extraction, vessel contents were centrifuged at 4000 rpm for 15 min and filtered through a 0.22 μm membrane (GV, Durapore) into a vial.

Results and discussion

Chromatographic performance

Quality parameters for the chromatographic determination of MBT were previously established. Analytical sensitivity, detection limit and quantification limit were calculated from a calibration curve at four concentrations levels,[11] 0.030, 0.060, 0.090 and 0.120 μg ml^{-1}, the corresponding values being 1.70×10^{-3}, 4.72×10^{-3} and 1.57×10^{-2} μg ml^{-1}. The chromatographic response was found to be linear with an r^2 value of 0.9998. A calibration curve at 1.30, 2.75, 5.50, 8.25, 11 and 13.75 μg ml^{-1} was used to evaluate recoveries from soils contaminated at high concentration levels with an r^2 value of 0.9999. Spectrum matching and purity testing allowed to determine the stability of MBT during the extraction from soil and soil-derived matrices, the probable degradation taking place during ageing time of residues, and the interferences from co-extracted compounds.

Preliminary optimisation of MASE

One of the most important parameters in the preliminary optimisation of MASE procedure with the QLT soil was the presence of water. The addition of 0, 0.5 and 1.0 ml to 1 g of the spiked soil was assayed. Recoveries varying from 36% for acetonitrile to 71% for methanol were obtained without the addition of water ($n = 2$); with 0.5 ml they reached 71 and 98% with a RSD of 6.5 and 3%, respectively ($n = 4$), and with 1 ml, these values were 87% and 99% with a RSD of 2.0% and 5% ($n = 4$). The effect of moisture content of samples on the recoveries of several apolar pollutants such as organochlorinated pesticides or PAHs by MASE extraction has already been discussed.[12,13] It was concluded that it is not possible to perform a good extraction in both completely dry and very wet samples when hexane or dichloromethane are used as the extraction solvents. The effect on recovery when different percentages of water (0–70%) were added to dried soil has been also studied for substituted urea herbicides such as linuron, diuron or monuron with dichloromethane–methanol (90:10).[14] The recoveries increased in the range 0–10%, and decreased significantly when the amount of water was >10% for all the evaluated compounds. The effect of this parameter probably depends on the extraction solvent used. The miscibility with the organic solvent and the effect of water hindering the transfer of the analytes from the matrix to the solvent, as well as the low dielectric constant and the low polarity for solvents such as hexane reaching a low temperature, have been proposed to explain low recoveries when the amount of water becomes too important. Taking into account the strong MBT–soil interactions, water may have a swelling effect on soil components such as clay minerals and organic matter, making the analyte more available to the extraction solvent. Lower recoveries were obtained in all cases when power or time length of each step were reduced and an increased power or time length led to similar recoveries for both solvents, so the definitive settings for microwave heating for further experiments were: 2 min at 350 W, 3 min at 500 W and, finally, a 2 min vent step at 0 W.

Soil spiking methods

Batch equilibrium method. Fortified samples to validate extraction techniques are often obtained by weighing soils followed by the addition of a low volume of spiking solution, frequently in methanol. Samples are allowed to air dry overnight or during several days with occasional stirring.[14–20] With this method it is assumed that the contaminants are uniformly distributed in the sample; however, in spite of the "equilibration time", analytes will persist partially as a deposit on the adsorbent surface, without undergoing a whole adsorption process, the extent being controlled by the soil adsorption capacity, so the efficiency to extract residues could be overestimated and, consequently, contamination in a field sample could be seriously underestimated. For this reason, a different way of fortification was assayed to establish the efficiency of the MASE procedure to extract the residues actually adsorbed: samples were contaminated through the batch equilibrium method at a broad range of concentrations (4–155 μg g^{-1}). Results for recoveries of MBT with methanol and acetonitrile are shown in Table 2. Equations correlate the amount of recovered analyte with the amount sorbed by the soil. The extent of working ranges was dependent on soil adsorption behaviour, so the highest levels of fortification were obtained for soils with the highest K_f values. When the slope and intercept values are 1 and zero, respectively, systematic errors will not be present at the concentration range under study.[21,22] Recoveries for both solvents were highly correlated with the corresponding sorbed amount for each soil with r values ≥ 0.995 ($P < 0.01$). The hypothesis test concerning the intercept and the slope of the fitted linear regression model allowed rejection of the hypothesis that the slope equals 1 at the 99% confidence level only for the extraction of MBT with both solvents from TEM soil and with acetonitrile from QLT soil.

Table 2 Recovery data sets obtained for the extraction of MBT from different soil types. $Y = a + bX$, where Y is the amount of recovered analyte and X, the amount sorbed by the soil. (a = intercept, b = slope and r = relation coefficient)

Soil	K_f/cm^3 g^{-1}	Concentration range in fortified soils/μg g^{-1}	Methanol			Acetonitrile		
			b	a	r	b	a	r
PCR	5.3	4.2–11.3	1.008	−0.107	0.998	1.003	−0.575	0.995
LHS	11.5	8.7–59.5	1.032	0.515	0.999	0.991	1.470	0.999
QLT	16.1	5.8–18.5	0.965	−0.047	0.998	0.900	−0.110	0.997
TEM	82.1	41–155	0.918	−3.246	0.999	0.874	−1.010	0.998
PCR + Peat	39.7	25–93.5	0.974	2.366	0.999	0.998	1.662	0.999
QLT + Peat	124	25–97.3	0.995	−1.571	0.999	0.975	0.219	0.996

Acetonitrile provided recoveries from 83 to 94% for QLT soil and from 83 to 89% for TEM soil at the different fortification levels under study. The corresponding values for methanol were 93 to 100% and 84 to 93%, respectively.

To have a basis for comparison, six samples belonging to the different fortified soils (75–156 μg g^{-1}) were also treated using Soxhlet extraction.[6] Data from Soxhlet extraction were always 5–20% lower than those obtained with MASE, the lowest differences were for the TEM soil, where the two methods presented low recoveries, values being 116 and 122 μg g^{-1}, respectively, for a sample fortified at 137 μg g^{-1}.

The TEM soil has to be considered as a complex matrix due to its high organic matter content and consequently, with a strong ability to adsorb MBT. From the K_f values (Table 2) it is inferred that the MBT adsorption capacity follows the order TEM > QLT > LHS > PCR, likewise, an enhancement in the adsorption capacity is produced in peat-amended soils. In spite of the heterogeneity of physico-chemical properties of the specific soils under study, the extraction efficiency was nearly independent of the soil matrix, with recoveries always higher than 90%, the results allowed the selection of methanol as the extraction solvent for the following recovery tests.

Freshly fortified soils and soil-derived matrices. Results from freshly contaminated soils and the selected organic and mineral fractions (through the procedure 2) with concentration levels ranging from 0.45 to 1.80 μg of MBT are shown in Table 3. As can be observed, good recoveries with values higher than 90% for all natural soils (except for CUN) were obtained. The performance of the MASE procedure was highly correlated with the adsorption capability of each soil-derived matrix under study, represented by the corresponding K_f value and/or by the organic matter content. The lowest values were for illite (67–79%), between the mineral surfaces and for the humic acid extracted from the GRB soil (67–72%), between the organic fractions both presenting a very high K_f, 678 and 345 cm^3 g^{-1}, respectively, the humic acid having the highest organic matter content. In these types of matrices nearly all the herbicide was adsorbed, therefore it represents the most complex situation within this way of fortification, where the matrix is wet and a fraction of the herbicide will remain in the solution. This fact can also explain the total recovery obtained in this case for TEM soil, its structure remaining wet therefore making easier accessibility of methanol to the adsorption sites. Lower recoveries for CUN soils are probably dependent on some different structural components, such as amorphous mineral oxides or silicates.

Chromatograms from extracts of several soil-derived matrices freshly spiked and the corresponding blanks overlaid are shown in Fig. 1. When blank samples of montmorillonite-Na, illite, Al–Si–Fe, humin-mineral residue and soils were analysed no peak was observed interfering in the HPLC-DAD determination of MBT. As can be seen in Fig. 1, a large signal from humic and fulvic acids is produced (HA and FA), however, no peak was detected at the MBT retention time according to the chromatographic processing method used and, purity tests performed in the spiked samples gave always a peak spectrally homogeneous for MBT. On the other hand, in spite of the high organic matter content in several soil samples (TEM, GRB and CUN), the use of methanol as the extraction solvent provided a good selectivity for the UV determination of MBT. Chromatograms obtained for GLV and GRB soils spiked at 0.45 μg g^{-1} level and the corresponding blanks are shown in Fig. 2.

Directly spiked soils at low concentration levels. Samples fortified through procedure 3 were employed to evaluate the precision, the effect of sample weight, type of soil and the interaction between these factors on the extraction, and finally, the effect of ageing at low analyte concentration.

To evaluate the precision three recently contaminated samples from PCR, QLT and TEM soil containing the herbicide at ≅ 2 μg g^{-1} (the highest level at which MBT could be detected in naturally contaminated soils,[5]) were extracted within the same run the procedure being repeated twice to evaluate the run-to-run variation in the method. The three variance components were estimated considering each factor after the first nested in the one above: between run precision (s_b^2), between type of sample precision (s_s^2) and within run precision s_w^2, being the total variance of measurements expressed as the sum of $s_b^2 + s_s^2 + s_w^2$. The total variance was 0.0992 and 1.40% was estimated to be due to variation within run, 98.52% to variation between type of samples and 0% to the variation run-to-run. Intra-assay variation for each type of sample soil was between 0.40–3.89% (RSD).

The effect of sample weight, type of soil, and the interaction amongst these factors on the extraction was established through a multilevel factorial design consisting of 27 experiments. The study was performed using PCR, QLT and CUN soil spiked at 2 μg g^{-1} and sample weights were 1, 2 and 3 g. Runs were carried out in 3 blocks and the order of the experiments was fully randomised. The *P* values for each factor under study and

Table 3 Recovery of MBT from freshly spiked soil samples, organic fractions and mineral adsorbents

			Recovery (%) (n = 2)			
Soils and adsorbents	K_f/cm^3 g^{-1}	OM (%)	0.45 μg	0.90 μg	1.35 μg	1.80 μg
PCR	5.3	1.4	104	104	102	100
LHS	11.5	2.5	98	100	99	100
QLT	16.1	3.1	101	102	101	99
GLV	17.0	4.0	90	98	94	94
GRB	30.1	14.7	90	90	90	95
CUN	80.6	13.8	80	83	83	81
TEM	82.1	11.4	89	95	97	98
TEM + peat	124	14.8	82	85	91	91
Peat	383	50.0	85	76	75	75
HA-GLV	184	53.0	80.5	86	86	—
HA-GRB	345	86.0	72	68	68	67
FA-GLV	25.1	33.4	103	97	92	99
FA-GRB	39.0	57.6	—	84	81	—
Hum-mineral GLV	20.0	1.7	97	98	98	97
Hum-mineral GRB	96	5.8	90	93	94	91
Montmorillonite-Na	45.5	0	103	93	98	96
Al–Si–Fe	6.3	0	—	101	103	—
Illite	678	0	73	73	67	79

for the interaction term weight × type of soil were less than 0.05 (Table 4), consequently these factors have a significant effect on recovery at the 95.0% confidence level. To sum up, PCR and QLT soil allowed recoveries ≥90% with 1 and 2 g of sample, decreasing to ≅ 80% with 3 g. For CUN soil, markedly different recoveries were obtained, the lowest level also being for the highest sample weight. These results indicate that significant differences may be found on the analyte recovery, related to the type of soil when different amounts of sample are employed, illustrating the need to optimise conditions specially for soils with a high organic matter content.

The effect of ageing was studied on samples spiked at two low levels in PCR and TEM soils (0.15 and 0.50 μg g^{-1}, Table 5). Analyte residence time was not a very significant factor on the extractability, specially taking into account the high organic matter content of TEM soil. With 5 months of residence in this soil, the extractability decreased to 90%, with a RSD of 5.5%. In this ageing period the extraction was done with a 5 ml volume of solvent. The same volume was used for the extraction of samples spiked at 0.15 μg g^{-1}. In this case, an ageing period of 3.5 months was studied with similar recoveries to those

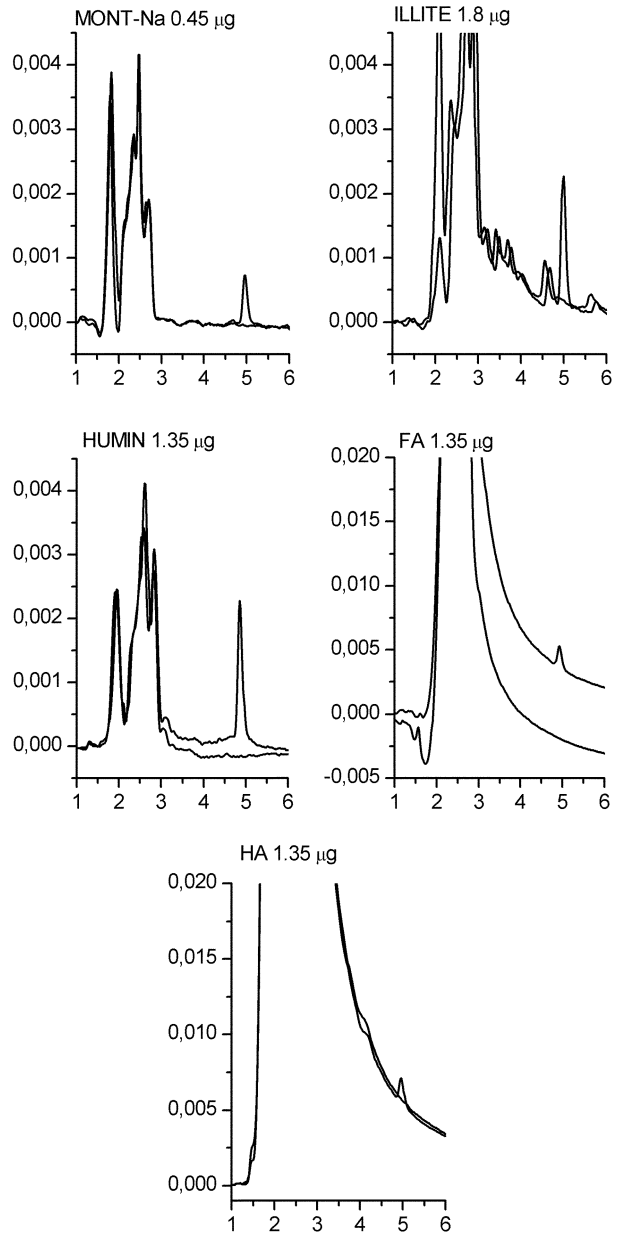

Fig. 1 HPLC-DAD chromatograms using MASE from some soil natural organic and inorganic constituents spiked with MBT (blanks overlaid).

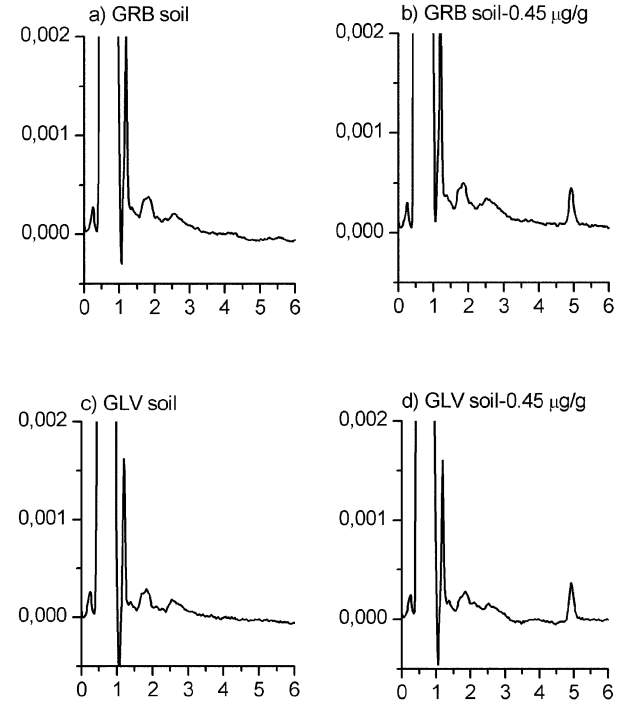

Fig. 2 HPLC-DAD chromatograms of extracts obtained by MASE from the allophanic GRB and GLV soils. (a) and (c) extracts from the corresponding blank samples, (b) and (d) extracts from GRB and GLV soils spiked at 0.45 μg g^{-1} level.

Table 4 Effect of sample weight and type of soil on recovery from samples fortified at 2 μg g^{-1} level (n = 3)

	Mean recovery and RSD (%)		
Soil	1 g	2 g	3 g
PCR	92.7 ± 2.0	91.1 ± 1.4	83.3 ± 2.5
QLT	94.7 ± 0.1	91.0 ± 0.7	79.1 ± 4.2
CUN	81.5 ± 3.2	70.2 ± 1.9	59.3 ± 2.8
Overall mean	89.7	84.1	73.9
Analysis of variance			
Source of variation	F test significance		
Sample weight	$P < 0.001$		
Soil	$P < 0.001$		
Block	$P < 0.001$		
Sample weight × soil	$P < 0.001$		

Table 5 Effect of ageing on recovery at low concentration levels

	Mean recovery and RSD (%)			
Soil	7 days 0.5 μg g^{-1} (n = 3) 10 ml	60 days 0.5 μg g^{-1} (n = 3) 10 ml	150 days 0.5 μg g^{-1} (n = 5) 5 ml	105 days 0.15 μg g^{-1} (n = 5) 5 ml
PCR 1.4% OM	98.7 (0.6)	96 (1.5)	93 (3.2)	97 (4.4)
TEM 11.4% OM	97.3 (4.2)	101 (7.0)	90 (5.5)	88 (4.5)

previously obtained. Mean recoveries of aged residues of several phenylurea herbicides ranging from 41 to 113% (RSD 1–35%) have been obtained for samples spiked at 0.10 μg g^{-1} level and stored over a period of 40 days.[14] Similar experiences with aged residues have been done for several acidic pesticides with samples stored for 120 days.[19] The recovery of most analytes were significantly lower (23–72%) in peat samples (with an OM content of 10.4–12.9%) than those obtained in sand or clay samples (40–100%) (with an OM content of 0.3–3.9%). For several sulfonylureas recoveries achieved for aged residues (60 days at 4 °C) at 0.10–0.02 μg g^{-1} level ranged 69–91%.[18] However, 2,4-D or compounds such as monuron, monolinuron or isoproturon are frequently quickly biodegraded in soils and this process can be favoured in spite of the slower biotic degradation achieved at low temperatures. On the other hand, compounds such as sulfonylureas are degraded *via* chemical hydrolysis and microbial processes in soils. So, some contribution to the lower recoveries can be expected from these facts. Results obtained by MASE allow to establish that difficulty presented with conventional methods for the extraction of aged residues of MBT is completely overcome in spite of its adsorption behaviour in soils and great stability allowing bound residues.

MBT degradation products imputable to the extraction procedure were never observed. On the other hand, a good selectivity was provided for the UV determination of MBT including the more complex matrices from allophanic soils with no interferences that could affect quantification of MBT. In soil-derived matrices the only exceptions were humic and fulvic acids, where serious interferences could be expected for a good quantification of recoveries in spite of the spectroscopic results obtained.

The significant reduction in sample preparation time, along with the relatively ease of handling are attributes of choice of MASE to carry out environmental concern studies of this herbicide in soil. In this type of laboratory experiments no pre-concentration techniques are currently required. The limit of detection (LOD) under the selected liquid chromatographic conditions was 0.047 μg g^{-1}. The quantification limit allows determining MBT at a level of approximately 0.15 μg g^{-1} Improvement of the sensitivity can be obtained with a better ratio between sample weight and solvent volume. This approach has been applied to detect sulfonylureas herbicides to a level of at least 5 μg kg^{-1} by reversed-phase liquid chromatography and UV detection at 226 nm.[18] A simple concentration step of extracts by evaporation to dryness under a stream of nitrogen or vacuum and re-dissolution to a lower volume can also be used. Losses are unlikely to occur because MBT has a low vapour pressure.[1] Both procedures were evaluated in this study and all analyses were carried out in triplicate or quintuplicate. In the first one, the extraction volume was changed to 5 ml and samples used were PCR and TEM soils spiked at 0.15 and 0.5 μg g^{-1} levels. Recoveries reached were higher than 90% in all samples. In the second one, the same volume was used and after the extraction, a 4 ml volume from the organic solvent was taken and evaporated to dryness under vacuum. Residue was dissolved by adding 1 ml of methanol. Samples used were PCR and TEM soils spiked at 0.15 μg g^{-1}. Mean recoveries reached 93 and 84%, respectively. Several chromatograms from these tests are shown in Figs. 3 and 4. Under both extraction conditions, MBT can be determined without any interference in PCR soil in spite of the simultaneous matrix concentration, including the 0.15 μg g^{-1} level. With the second approach, the co-extracted humic substances owing to the high organic matter content of TEM soil are larger than those obtained with the first one at the same low level. However, purity testing gave a peak spectrally homogeneous in that condition and no spectroscopic evidence for co-elution of some soil constituent at the corresponding retention time was found in the chromatogram of the blank sample.

Conclusion

This study has been conducted taking into account the effects of environmental behaviour on the extractability by MASE of a very stable herbicide in natural soils. The three different ways for spiking samples to do recovery tests reproduce the equilibrium in the solute–soil adsorption taking place in the natural environment. This strategy will permit the achievement of results without any overestimation of recoveries that could

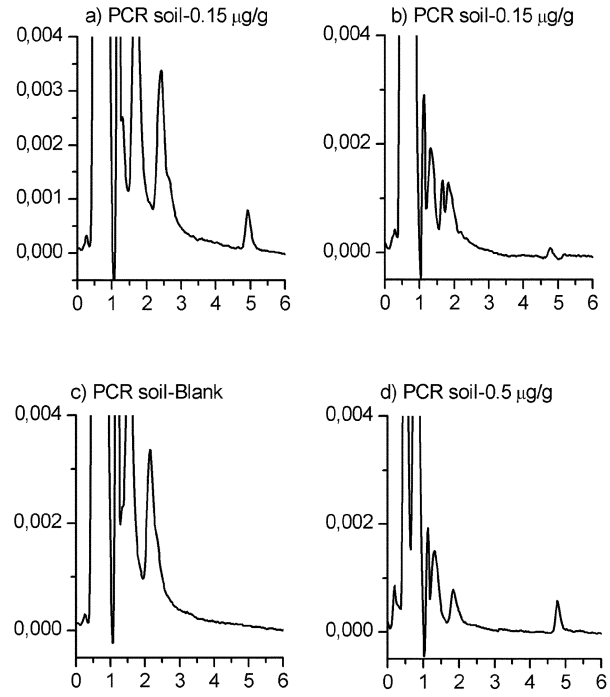

Fig. 3 Chromatograms from PCR soil extracts: (a) and (c) with 5 ml of methanol including a concentration step from 4 ml to 1 ml. (b) and (d) extractions with 5 ml without the concentration step.

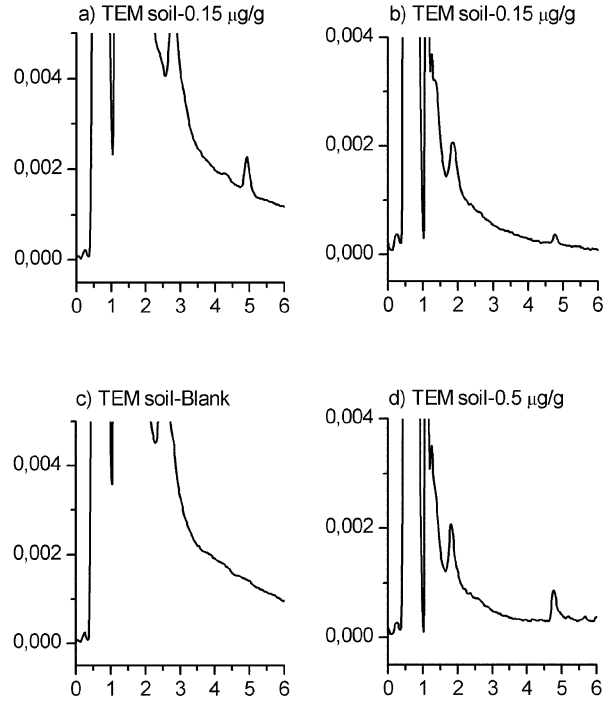

Fig. 4 Chromatograms from TEM soil extracts: (a) and (c) with 5 ml of methanol including a concentration step from 4 ml to 1 ml. (b) and (d) extractions with 5 ml without the concentration step.

underestimate contamination in field conditions. Quantitative recoveries have been obtained including soils with high organic matter content, the most complex matrices. The correlation between adsorption capacity and extractability of MBT from some mineral and organic soil-derived matrices have allowed to explain the contribution of these components in the low recoveries found with conventional methods. The ageing effect was not a significant factor in reaching an appropriate accuracy and repeatability including the lowest spiking level under study.

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