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Multiresidue Herbicide Analysis in Soil: Subcritical Water Extraction with an On-Line Sorbent Trap

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We evaluated the feasibility of extracting selectively and rapidly herbicide residues in soils by hot water and collecting analytes with a Carbograph 4 solid-phase extraction (SPE) cartridge set on-line with the extraction cell. Phenoxy acid herbicides and those nonacidic and acidic herbicides which are often used in combination with phenoxy acids were selected for this study. Five different soil samples were fortified with target compounds at levels of 100 and 10 ng/g (30 ng/g of clopyralid and picloram) by following a procedure able to mimic weathered soils. Herbicides were extracted with water at 90 °C and collected on-line by the SPE cartridge. After the cartridge was disconnected from the extraction apparatus, analytes were recovered by stepwise elution to separate nonacidic herbicides from acidic ones. The two final extracts were analyzed by liquid chromatography/mass spectrometry with an electrospray ion source. At the lowest spike level considered, analyte recoveries ranged between 81 and 93%, except those for 2,4-DB and MCPB, which were 63%. For 16 herbicides out of 18, the ANOVA test showed recoveries were not dependent on the type of soil. The method detection limit was in the 1.7−10 ng/g range. For the analytes considered, method comparison showed this extraction method was overall more efficient than Soxhlet and sonication extraction techniques.

Extraction procedures adopted in many standardized analytical methodologies for determining pesticides in soils are both time and solvent consuming. As an example, U.S. EPA method 3540 for extracting semivolatile organic priority pollutants, including some classes of pesticides, from soils and solid wastes makes use of Soxhlet extraction with 300 mL of a solvent mixture for 16−24 h.[1] To shorten the analysis time and reduce the volumes of solvents which are toxic, flammable, and expensive, extraction techniques such as automated solvent extraction,[2] sonication extraction,[3,4] microwave-assisted solvent extraction (MASE),[5,6] supercritical fluid extraction (SFE),[7−9] and accelerated solvent extraction (ASE)[10,11] have been proposed as alternatives to the Soxhlet procedure. Among the new extraction techniques, ASE is the most recently introduced one. With this technique, a solid sample is packed into an extraction cartridge and analytes are extracted from the matrix with conventional low-boiling solvents or solvent mixtures at elevated temperatures (up to 200 °C) and pressures (up to 20 MPa) to maintain the solvent in the liquid state. Two comparative studies[12,13] showed ASE-extracted quantities of two neutral pesticides from soils equal to or larger than those found by other extraction techniques. ASE was also tested for the extraction of phenoxy acid herbicides from clay, loam, and sand.[10] However, the analytical performance was not quite satisfying, as recoveries were between 36 and 72%, with 11−55% RSD.

A limitation of the ASE technique, which is shared by the other extraction techniques mentioned above, is that selective extraction of organics on the basis of their polarities is difficult. Initially, we used ASE at 100 °C with either methanol or acetone as such or acidified with H_3PO_4 for extracting targeted pesticides from a soil sample with an unusually high organic content (9.5%). In every case, enormous amounts of waxlike substances, presumably cellulose, lignin, and waxes from plant cells, were co-extracted with the herbicides considered. The presence of these high molecular weight compounds in soil extracts made the rest of the analysis not practicable, unless resorting to tedious and time-consuming cleanup procedures. To a lesser extent, these naturally occurring species were also present in extracts of soil samples with lower organic contents.

Recently, Hawthorne et al.[14,15] reported that subcritical water efficiently extracted chlorophenols, alkylbenzenes, polycyclic aromatic hydrocarbons, and *n*-alkanes from solid matrixes. Class-

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(1) *Test Methods for Evaluating Solid Wastes*, 3rd ed.; USEPA SW-846; U.S. GPO: Washington, DC, July 1995; Method 3540, Update III.
(2) Lopez-Avila, V.; Bauer, K.; Milanes, J.; Beckert, W. F. *J. AOAC Int.* **1993**, *76*, 864−880.
(3) Chau, A. S. Y.; Babiak, L. J. *J.—Assoc. Off. Anal. Chem.* **1979**, *62*, 107−113.
(4) Eiceman, G. A.; Viau, A. C.; Karasek, F. W. *Anal. Chem.* **1980**, *52*, 1492−1496.
(5) Ganzler, K.; Salgo, A.; Valko, K. *J. Chromatogr.* **1986**, *371*, 299.
(6) Ganzler, K.; Salgo, A. *Z. Lebensm.-Unters.-Forsch.* **1987**, *184*, 274.
(7) Hawthorne, S. B. *Anal. Chem.* **1990**, *62*, 633A−642A.
(8) Chester, T. L.; Pinkston, J. D.; Raynie, D. E. *Anal. Chem.* **1992**, *64*, 153R−170R.
(9) Oostdyk, T. S.; Grob, R. L.; Snyder, J. L.; McNally, M. E. *Anal. Chem.* **1993**, *65*, 596−600.
(10) Ezzel, J. L.; Richter, B. E.; Felix, W. D.; Black, S. R.; Meikle, J. E. *LC-GC* **1995**, *13*, 390.
(11) Richter, B. E.; Jones, B. A.; Ezzell, J. L.; Porter, N. L.; Avdalovic, N.; Pohl, C. *Anal. Chem.* **1996**, *68*, 1033−1039.
(12) Frost, S. P.; Dean, J. R.; Evans, K. P.; Harradine, K.; Cary, C.; Comber, M. H. I. *Analyst* **1997**, *122*, 895−898.
(13) Conte, E.; Milani, R.; Morali, G.; Abballe, F. *J. Chromatogr.* **1997**, *765*, 121−125.
(14) Hawthorne, S. B.; Yang, Y.; Miller, D. J. *Anal. Chem.* **1994**, *66*, 2912−2920.
(15) Yang, Y.; Hawthorne, S. B.; Miller, D. J. *Environ. Sci. Technol.* **1997**, *31*, 430−437.

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selective extraction of these organics was simply achieved by adjusting the water temperature (50−300 °C). This finding was explained by considering that the polarity of water steadily decreases as the temperature is increased, thus making water more and more capable of competing with nonpolar organics for adsorption on soil particles or soil organic matter. Another consideration to be made is that higher temperatures also decrease sharply the viscosity of water,[16] thus allowing better penetration of matrix particles and enhancing extraction. Finally, water should be abler than conventional solvents to extract polar analytes when their sorption to soil is mainly controlled by specific interactions. Water has been used at ambient temperature to extract sulfonylurea herbicides[17,18] and phenoxy acid herbicides from soils.[19] MASE with water near its boiling point has been reported to extract selectively atrazine and its principal metabolites from agricultural soils.[20] Field et al.[21] used water at 50 °C and 200 bar for exhaustively extracting two acid metabolites of a herbicide in aged soils.

Selective extraction of polar and moderately polar organics from soil particles with heated water is as though natural processes occurring in the environment, where aged soils tend to be left with less polar compounds, are accelerated. In this sense, the hot-water extraction procedure could be called accelerated water extraction. From an operative point of view, subcritical water extraction is like ASE. In principle, when relatively polar target compounds in soils are analyzed, the former technique offers distinct advantages over the latter one in that selective extraction of the analytes is achieved with a nontoxic and inexpensive solvent.

Phenoxy acid herbicides have become widely used because of their relative cheapness and effectiveness in controlling the presence of unwanted broad-leaf weeds in crops. Several methods have been proposed for extracting phenoxy acid residues from soils.[10,22−27] A weakness of these methods is that they are specifically designed for the above class of herbicides and do not consider those pesticides which are often used in combination with phenoxy acids. This means that, for analyzing soil samples contaminated by phenoxy acids and associated herbicides, more than one extraction procedure should be followed.

The purpose of this work has been that of evaluating the feasibility of using hot water as extractant and liquid chromatography (LC)/mass spectrometry (MS) with an electrospray (ES) ion source for simultaneously analyzing phenoxy acid herbicides and those herbicides which are commonly used in combination with phenoxy acids to inhibit the growth of a larger number of weed species in soil. The extraction device used in this study was similar to that employed by Hawthorne et al.,[14] with the difference that analytes in the water stream leaving the extraction apparatus were successively re-extracted with an on-line Carbograph 4 solid-phase extraction (SPE) cartridge instead of being collected in a chloroform-containing vial.

EXPERIMENTAL SECTION

Reagents and Chemicals. Atrazine, simazine, cyanazine, diuron, isoproturon, linuron, clopyralid, picloram, dicamba, bentazone, 2,4-D, MCPA, bromoxynil, dichlorprop, mecoprop, ioxynil, 2,4-DB, MCPB, *sec*-butylazine, and monuron were purchased from Alltech, Sedriano, Italy. *sec*-Butylazine and monuron, the latter being a superseded herbicide, were used as internal standards (IS's) for analyzing neutral herbicides. *n*-Octylbenzenesulfonate (C_8-LAS) (Aldrich, Milwaukee, WI) was used as the IS for acidic herbicides. Individual standard solutions of the above compounds were prepared by dissolving 25 mg of each compound in 100 mL of methanol. Two composite working standard solutions of nonacidic and acidic herbicides were separately prepared by mixing the above solutions and diluting with methanol to obtain analyte concentrations of 30 and 3 μg/mL, except for clopyralid and picloram, whose concentrations in the most diluted solution were 9 μg/mL. For analysis of nonacidic herbicides, the internal standard working solution was prepared by diluting the methanol solutions of *sec*-butylazine and monuron with water/methanol (60:40, v/v) acidified with 30 μmol/L trifluoroacetic acid (TFA) to obtain final concentrations of 2 μg/mL. For analysis of acidic herbicides, the C_8-LAS solution was further diluted with water/acetonitrile (80:20, v/v) containing 0.3 mmol/L tetrabutylammonium fluoride ((TBA)F) to obtain a final concentration of 2 μg/mL. When unused, all solutions mentioned above were stored at 4 °C.

For LC, distilled water was further purified by passing it through the Milli-Q Plus apparatus (Millipore, Bedford, MA). Acetonitrile "Plus" and methanol "Plus" of gradient grade were obtained from Carlo Erba, Milano, Italy. To minimize formation of adduct ions (especially of sodiated ions) in the ES source, in addition to the protonated ones, inorganic salt impurities present in methanol were eliminated by distillation. TFA and (TBA)F were from Aldrich. All other solvents and chemicals were of analytical grade (Carlo Erba), and they were used as supplied.

Sand (crystobalite, 40−200 mesh) was obtained from Fluka AG, Buchs, Switzerland. Sand was extensively cleaned by washing it with methanol. Humic acid sodium salts were purchased from Aldrich. A water solution containing 3 g/L humic acids was prepared as reported elsewhere.[28] SPE cartridges filled with 0.5 g of Carbograph 4,[29] which is a new example of graphitized carbon black (GCB) with a surface area of 210 m^2/g, were supplied by LARA, Rome. A Teflon piston with a Luer tip and a conically indented base (LARA) was forced to enter the cartridge until it reached the upper frit.[29] After this device was fitted into a sidearm filtering flask, it was washed with 10 mL of the eluent phase for eluting acidic herbicides (see below), followed by 5 mL of methanol and 10 mL of distilled water. Liquids were forced to pass through the cartridge by the aid of vacuum from a water

(16) Yang, Y.; Belghazi, M.; Lagadec, A.; Miller, D. J.; Hawthorne, S. B. *J. Chromatogr.* **1998**, *810*, 149−159.
(17) Galletti, G. C.; Bonetti, A.; Dinelli, G. *J. Chromatogr.* **1995**, *692*, 27−37.
(18) Li, L. Y. T.; Campbell, D. A.; Bennet, P. K.; Henion, J. *Anal. Chem.* **1996**, *68*, 3397−3404.
(19) Kim, I. S.; Sasinos, F. I.; Stephens, R. D.; Wang, J.; Brown, M. A. *Anal. Chem.* **1991**, *63*, 819−823.
(20) Steinheimer, T. R. *J. Agric. Food Chem.* **1993**, *41*, 588−595.
(21) Field, J. A.; Monohan, K.; Reed, R. *Anal. Chem.* **1996**, *70*, 1956−1962.
(22) Cotterill, E. G. *Analyst* **1982**, *107*, 76−81.
(23) Lee, H. B.; Chau, S. Y. A. *J.−Assoc. Off. Anal. Chem.* **1983**, *66*, 1023−1028.
(24) Tsukioka, T.; Murakami, T. *J. Chromatogr.* **1989**, *675*, 351−359.
(25) Rochette, E. A.; Harsh, J. B.; Hill, H. H., Jr. *Talanta* **1993**, *40*, 147−155.
(26) Sánchez-Brunete, C.; García-Valcárcel, A. I.; Tadeo, J. L. *J. Chromatogr.* **1994**, *675*, 213−218.
(27) Reighard, T. S.; Olesik, S. V. *Anal. Chem.* **1997**, *69*, 566−574.
(28) Senseman, S. A.; Lavy, T. L.; Mattice, J. D.; Gbur, E. E. *Environ. Sci. Technol.* **1995**, *29*, 2467−2453.
(29) Di Corcia, A.; Bellioni, A.; Madbouly, M. D.; Marchese, S. *J. Chromatogr.* **1996**, *733*, 383−393.

Table 1. Physical and Chemical Characteristics of the Soils Used in This Study

	%				
soil	sand	silt	clay	organic matter	pH[a]
Cadriano	45	24	31	1.4	6.8
Camporeale	55	27	18	5.2	6.2
Mantova	19	57	24	2.0	7.7
Paliano	23	44	33	2.3	6.1
Trivero	54	39	7	9.5	4.6

[a] 1:1 soil:water (w/v).

pump. The SPE device was then attached to the outlet of the soil extraction apparatus by a PEEK connector.

Soil Samples. Five herbicide-free soil samples originating from various locations of Italy were considered in this study. Table 1 gives some details of their compositions. Soils were heated at 110 °C until they were free flowing and sieved to a particle size of less than 2 mm. Individual and pooled soil samples were amended with the herbicides at two concentration levels, namely 10 ng/g (30 ng/g for clopyralid and picloram) and 100 ng/g. Each sample was prepared by putting 60 g of soil in an amber glass bottle and wetting it with acetone until the solvent completely covered the soil particles Then, known volumes of the two composite standard solutions were added to the soaked soil. The bulk of the solvent was slowly evaporated at room temperature by thoroughly mixing the suspension with a spatula. When the soil particles became a slushlike mass, the bottle was sealed and stored at ambient temperature in the dark for 4 months. This procedure is similar to that previously reported to mimic native samples.[30] Thereafter, acetone was completely removed by putting the bottle in a ventilated oven at 50 °C. With some types of soils, the treatment with acetone provoked deaggregation of the particulate matter with formation of very fine particles which obstructed the passage of water into the extraction cell. These soils were then resieved by excluding particles <40 μm.

Extraction Apparatus. A homemade apparatus was used for extracting the analytes from soil samples (Figure 1). It consisted of an LC pump to supply water through $^1/_{16}$-in.-o.d. ($^1/_{30}$-in.-i.d.) stainless steel tubing (including a 2-m preheating coil) to the extraction cell. Both the preheating coil and the extraction cell were placed inside a GC oven. A 25-cm × 4.6-mm-i.d. stainless steel LC column was used as the extraction cell. Outside the GC oven, a Carbograph 4 cartridge was set on-line with the extraction cell for trapping analytes extracted from the soil. No cooling loop was set after the extraction cell, as analyte collection was not affected by the temperature at which water passed through the SPE cartridge. To avoid plugging of the extraction device by fine soil particles, the lower frit of the extraction cell was renewed after each extraction.

Extraction Procedure. Three grams of soil was mixed with 2 g of sand, and the mixture was poured into the extraction cell, taking care to tap the tube to avoid loose packing of the soil particles. Any void space remaining after packing the soil was filled with sand. The soil-containing tube was then put into the oven

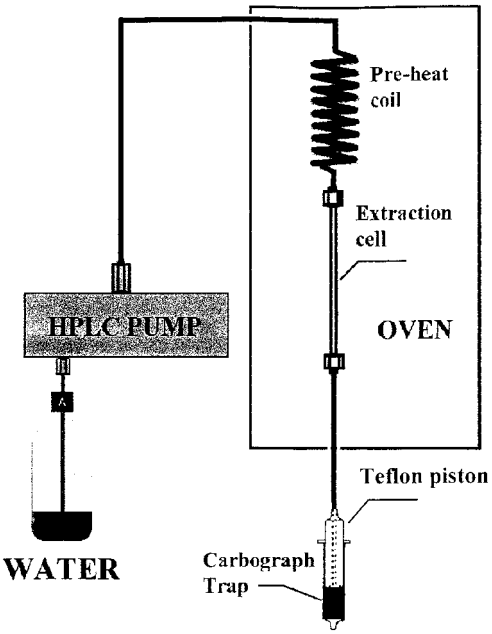

Figure 1. Schematic view of the laboratory-made extraction device used in this work.

and heated at 90 °C for 5 min. Twenty five milliliters of water was used to extract the analytes. The first 2.5 mL of water was passed through the cell at a flow rate of 0.4 mL/min. The flow rate was increased to 1 mL/min in 1 min. The Carbograph 4 cartridge was then disconnected and fitted into a sidearm filtering flask. Inorganic salts co-extracted from the soil were removed from the cartridge by passing 100 mL of distilled water at a flow rate of ca. 80 mL/min. The presence of even small amounts of salts was observed to affect base−neutral/acid class fractionation of the analytes (see below). Water remaining in the Carbograph 4 cartridge was partially removed by drawing room air through the cartridge for 1 min. The water content was further decreased by slowly passing 0.5 mL of methanol. Again, the trap was air-dried for 1 min. By exploiting a singular feature of the Carbograph 4 material,[31] we isolated nonacidic herbicides from acidic ones by differential elution, following in part a procedure reported elsewhere.[32] Briefly, the cartridge device was turned upside down and nonacidic herbicides were back-eluted by passing through the sorbent bed 1.5 mL of methanol followed by 8 mL of CH_2Cl_2/CH_3OH (95:5, v/v). Subsequently, acidic pesticides still adsorbed on the Carbograph 4 surface were eluted by using 10 mL of CH_2Cl_2/CH_3OH (80:20, v/v) acidified with 50 mmol/L formic acid. These two fractions were separately collected in 1.4-cm-i.d. glass vials with a conical bottom, and solvents were removed at 40 °C in a water bath under a gentle flow of nitrogen. Residues were reconstituted with 200 μL of the solutions containing the respective internal standards (see above). In both cases, 30-μL portions of the final extracts were then injected into the LC column.

LC-ES/MS Analysis. The analytes were chromatographed on an Alltima 25-cm × 4.6-mm-i.d. column filled with 5-μm C-18 reversed phase packing (Alltech). Initially, a Varian (Walnut

(30) Sparks, D. L. *Kinetics of Soil Chemical Processes*; Academic Press: San Diego, CA, 1989.

(31) Andreolini, F.; Borra, C.; Caccamo, F.; Di Corcia, A.; Samperi, R. *Anal. Chem.* **1987**, *59*, 1720−1725.

(32) Crescenzi, C.; Di Corcia, A.; Passariello, G.; Samperi, R.; Turnes Carou, M. I. *J. Chromatogr.* **1996**, *733*, 41−55.

Table 2. Time-Scheduled Multiple-Ion SIM Conditions for Detecting the Herbicides Considered

compound	channel mass, *m*/*z* (rel abund)	cone voltage, V	retention window, min
nonacidic analytes			
cyanazine	136 (20), 214 (100), 241 (70)	60	10−17
monuron (IS)[a]	72 (100), 199 (30)	60	17−21
simazine	104 (40), 124 (60), 202 (100)	60	
atrazine	104 (20), 174 (100), 216 (40)	60	21−26.5
isoproturon	72 (100), 165 (30), 207 (40)	60	
diuron	72 (100), 233 (30), 235 (15)	50	26.5−29
sec-butylazine (IS)	174 (100), 230 (30)	60	29−30.7
linuron	161 (30), 182 (80), 249 (90)	50	30.7−33
acidic analytes			
clopyralid	190 (100), 192 (60)	35	10−14
picloram	195 (100), 239 (70), 241 (60)	35	
dicamba	175 (40), 219 (100), 221 (60)	30	14−15.5
bentazone	132 (60), 197 (60), 239 (100)	50	15.5−18.5
MCPA	141 (50), 199 (100), 201 (40)	35	
2,4-D	161 (100), 163 (60), 219 (40)	35	
mecoprop	141 (50), 213 (100), 215 (40)	35	
dichlorprop	161 (100), 163 (60), 233 (40)	35	
bromoxynil	274 (50), 276 (100), 278 (50)	35	
ioxynil	127 (20), 370 (100)	35	18.5−22
2,4-DB	161 (100), 163 (60), 247 (20)	35	
MCPB	141 (100), 143 (80), 227 (80)	35	
C8-LAS (IS)	170 (80), 269 (100)	45	22−25

[a] IS = internal standard.

Table 3. Analyte Recoveries and Their Neutral/Acid Class Fractionations with the Carbograph 4 SPE Cartridge by Extraction from 25 mL of Water Amended with 0.5 g/L Humic Acids (Spike Level 100 ng/g)

	recovery,[a] %	
compound	8 mL of CH_2Cl_2/CH_3OH (95:5)	8 mL of CH_2Cl_2/CH_3OH (80:20) + 50 mmol/L HCCOH
nonacidic analytes		
cyanazine	97	
simazine	101	
atrazine	98	
isoproturon	98	
diuron	98	
linuron	100	
acidic analytes		
clopyralid		93
picloram		91
dicamba		94
bentazone		100
MCPA		96
2,4-D		97
mecoprop		96
dichlorprop		96
bromoxynil		98
ioxynil		96
2,4-DB	9	86
MCPB	10	88

[a] Mean values from triplicate measurements.

Table 4. Mean Percentage Recoveries of Target Herbicides by Extraction from a Pooled Soil Sample with Water at Increasing Temperatures (Spike Level 100 ng/g)

	recovery,[a] %		
compound	50 °C	90 °C	120 °C
nonacidic herbicides			
cyanazine	79	87	90
simazine	81	89	90
atrazine	73	89	87
isoproturon	69	88	63
diuron	70	86	59
linuron	63	84	67
acidic herbicides			
clopyralid	85	93	93
picloram	85	91	93
dicamba	85	90	89
bentazone	77	85	83
MCPA	74	87	88
2,4-D	70	85	86
mecoprop	67	84	82
dichlorprop	58	86	84
bromoxynil	72	84	88
ioxynil	74	84	84
2,4-DB	47	63	80
MCPB	49	62	82

[a] Mean values from triplicate measurements.

Creek, CA) model 9010 liquid chromatograph coupled to a Micromass (Manchester, U.K.) Platform ES/MS system was used to analyze final extracts of soils. With this instrumentation, experimental conditions were substantially equal to those previously reported for analyzing nonacidic[33] and acidic herbicides.[34] Final measurements were performed by a Thermoquest (Manchester, UK) model P4000 liquid chromatograph equipped with a membrane degasser, which was coupled to a recently introduced Navigator *aQa* ES/MS device. The advantage of using this device over the former one was that, despite the presence of 0.3 mmol/L (TBA)F (see below) in the LC mobile phase, it produced a sufficiently stable ion abundance over several working days without the need for cleaning the sample cone.

For fractionation of nonacidic analytes, phase A was methanol and phase B was water. Both solvents contained TFA, 30 μmol/L. The initial composition of the mobile phase was 40% A, which was increased linearly to 80% in 40 min. For fractionation of acidic analytes, both water and acetonitrile contained 0.3 mmol/L (TBA)F. In this case, the initial percentage of the organic modifier was 15%, which was increased first to 55% after 20 min and then to 75% after an additional 5 min. In both cases, the flow rate of the mobile phase was 1 mL/min, and 0.3 mL/min of the column effluent was diverted to the ES source. Nonacidic herbicides were analyzed in the positive-ion mode by applying a voltage of 4.0 kV to the capillary, while acidic herbicides were analyzed in the negative-ion mode by applying a voltage of 3.0 kV to the capillary. In both cases, the probe temperature was set at 250 °C. Structure-significant fragment ions were obtained by the collision-induced decomposition (CID) process, after suitably adjusting the sample cone voltage. In Table 2, conditions followed to detect target compounds in the time-scheduled multiple ion selected ion monitoring mode are reported.

The mass spectrometry data handling system used was the Mass Lab software from Thermoquest.

Quantitation. Recovery of each analyte was assessed by selecting the ion current profiles for both parent and fragment

(33) Crescenzi, C.; Di Corcia, A.; Guerriero, E.; Samperi, R. *Environ. Sci. Technol.* **1997**, *31*, 479−488.

(34) Crescenzi, C.; Di Corcia, A.; Marchese, S.; Samperi, R. *Anal. Chem.* **1995**, *67*, 1968−1975.

Table 5. Accuracies and Precisions of the Method on Analyzing Five Types of Soils Amended with 10 ng/g (30 ng/g of Clopyralid and Picloram) of Selected Herbicides

	mean concn found[a] and standard deviation for soil sample, ng/g										
	Cadriano		Camporeale		Mantova		Paliano		Trivero		
compound	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$F_{4,15}$[b]
cyanazine	8.5	0.8	9.2	0.5	9.1	0.7	8.9	0.6	8.2	0.6	1.7
simazine	8.6	0.8	9.7	0.8	9.5	0.5	8.7	0.8	8.3	0.6	2.9
atrazine	8.2	0.9	8.6	0.7	9.5	0.6	8.3	0.7	7.9	0.8	2.7
isoproturon	8.3	0.6	8.9	0.7	8.8	0.7	8.6	0.6	8.0	0.6	1.3
diuron	8.4	0.8	8.0	0.7	9.2	0.5	8.2	0.6	7.9	0.9	2.1
linuron	8.4	0.7	8.7	0.9	9.7	1.1	8.7	0.7	7.8	0.9	2.5
clopyralid	26.1	1.8	25.0	3.6	30.6	4.1	29.6	3.9	25.8	3.0	2.2
picloram	28.6	3.0	26.0	3.1	28.1	2.9	31.4	3.6	25.4	3.3	2.2
dicamba	9.4	0.9	8.6	0.8	9.6	0.9	9.5	0.8	8.1	0.6	2.7
bentazone	8.7	0.5	7.9	0.8	8.5	0.5	8.4	0.4	7.6	0.6	1.7
MCPA	8.4	0.4	7.8	0.6	8.6	0.4	8.9	0.7	7.8	0.7	2.9
2,4-D	8.8	0.6	8.0	0.9	9.3	0.7	8.9	0.5	8.0	0.8	2.4
mecoprop	8.3	0.6	8.1	0.5	9.0	1.0	8.5	0.6	7.6	0.8	2.1
dichlorprop	8.7	0.6	7.7	0.6	8.6	0.9	8.5	0.6	7.6	0.8	2.2
bromoxynil	7.8	0.6	7.6	0.9	9.0	0.6	8.3	0.6	7.4	0.7	2.2
ioxynil	8.4	0.6	7.7	0.7	8.7	0.7	8.0	0.8	7.5	0.8	1.9
2,4-DB	6.8	0.6	5.9	0.5	6.7	0.8	6.6	0.5	5.4	0.7	3.7
MCPB	7.0	0.6	5.9	0.5	6.7	0.7	6.6	0.6	5.5	0.8	3.7

[a] Number of replicates: 4. [b] $F_{4,15}$ critical value: 3.1.

ions (see Table 2), measuring the peak area relative to that of the IS (note that monuron was chosen as the reference IS for cyanazine and simazine, while *sec*-butylazine was the reference IS for the other four nonacidic herbicides), and comparing this result with that obtained for a standard solution. These solutions were prepared by dissolving known and appropriate volumes of the two working standard solutions in the solvent systems used respectively for eluting nonacidic and acidic analytes from the Carbograph 4 cartridge and then following the final part of the procedure reported above.

RESULTS AND DISCUSSION

Setup of the Extraction Device. The subcritical water extraction device designed by Hawthorne et al.[14,15] involved collection of the extracted analytes into a glass vial containing chloroform. For the organics with higher water solubility, 82−86% removal from water was obtained only after re-extracting water with an additional volume of chloroform. In this study, analyte collection was accomplished by a Carbograph 4 SPE cartridge set on-line with the extraction device. This sorbent was shown to be very effective in extracting highly polar pesticides from water[33,35] and provides neutral/acid class fractionation by differential elution.[31,32,36] Since different instrumental conditions are needed for analyzing nonacidic and acidic herbicides, this fractionation step does not lengthen the analysis time. On the contrary, one inherent positive feature of analyte class fractionation is that also co-extracted species naturally occurring in the soil at relatively large concentration levels are distributed in two fractions. This condition decreases the probability of coelution of the above species with the small amounts of the analytes from the LC column. When this occurs, saturation effects of the ES/MS detector can provoke large and unpredictable ion signal weakening of the analytes.[37] Neutral/acid class fractionation with a GCB sorbent relies upon the presence on its surface of relatively few particular active sites able to exchange anions. The concentration of these anion exchange sites was estimated to be about 16 μequiv/g of Carbograph 4.[32] Saturation effects of these specific adsorption sites due to co-extracted humic acids abundantly present in soils can cause acidic analytes to be partly adsorbed on the nonspecific adsorption sites of the Carbograph surface. As a consequence, this fraction of the acidic analytes is not quantified because it is prematurely eluted by the eluent phase designed to re-extract nonacidic analytes. Preliminary experiments showed that hot water is able to extract abundant amounts of humic acids from soils, as evidenced by the yellow-brown coloring of the water stream leaving the extraction cell. Thus, a preliminary study for evaluating the practicability of using a Carbograph 4 SPE cartridge set on-line with the extraction cell for trapping the analytes and successively fractionating them by differential elution was conducted. For this purpose, 25 mL of water containing 0.5 g/L humic acids was spiked with 100 ng of each analyte and this solution was passed through the SPE cartridge. Thereafter, the final part of the analytical procedure (see the Experimental Section) was followed. To detect the eventual presence of acidic analytes in the neutral final extract, this was reanalyzed, after setting the ES/MS system in the negative-ion acquisition mode. Experiments were performed in triplicate, and results are reported in Table 3. It can be seen that target compounds were completely recovered from water containing abundant amounts of humic acids and that base−neutral/acid class fractionation was accomplished with only some carryover of the two most weakly acidic herbicides, namely MCPB and 2,4-DB, in the neutral fraction.

(35) Sabik, H.; Jeannot, R. *J. Chromatogr.* **1998**, *818*, 197−207.
(36) Bucheli, T. D.; Grüebler, F. C.; Müller, S. R.; Schwarzenbach, R. P. *Anal. Chem.* **1997**, *69*, 1569−1576.
(37) Di Corcia, A.; Crescenzi, C.; Laganà, A.; Sebastiani, E. *J. Agric. Food Chem.* **1996**, *44*, 1930−1938.

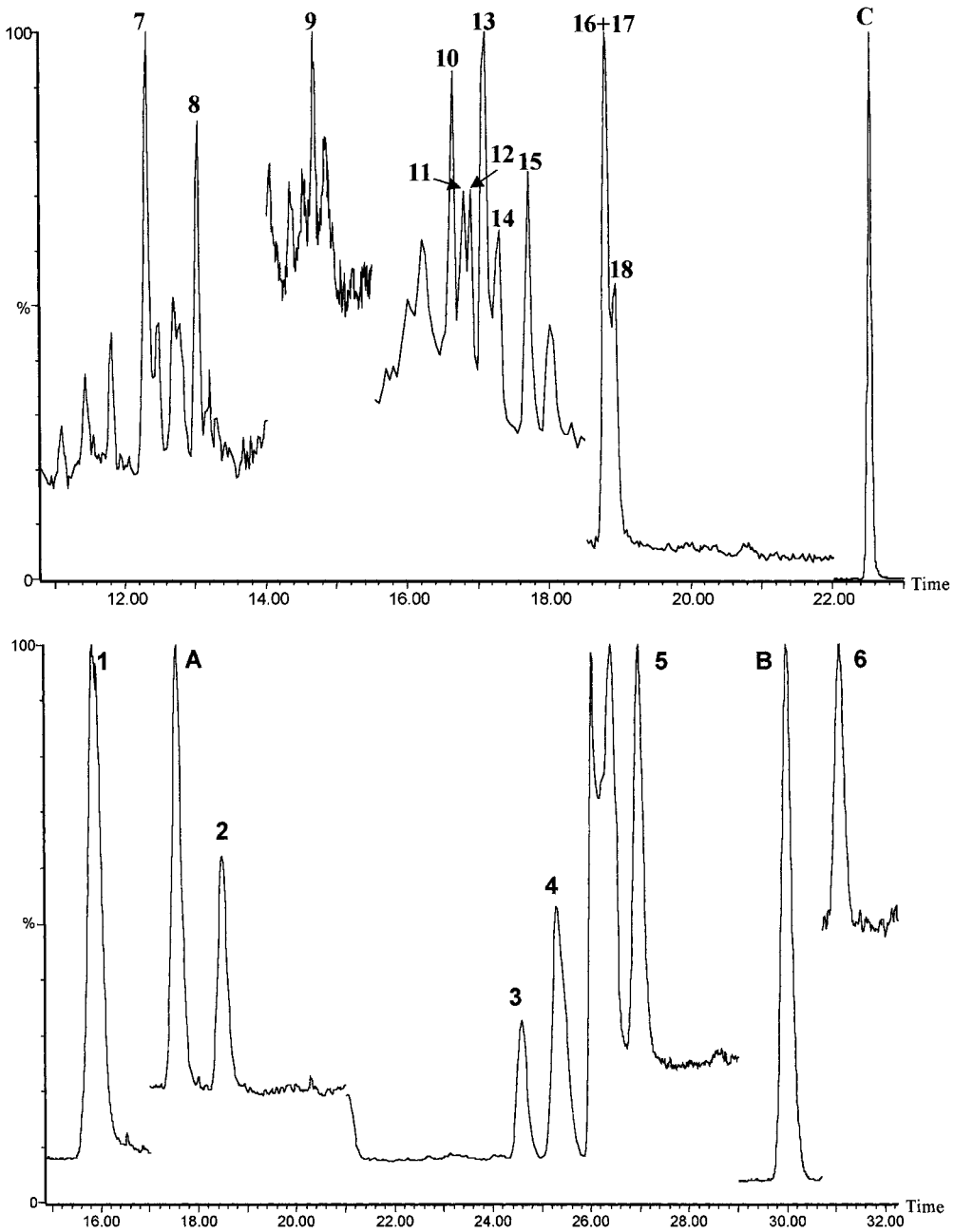

Figure 2. LC/MS chromatograms resulting from injecting (bottom) the nonacidic herbicide-containing extract and (top) that containing the acidic herbicides. These extracts were obtained by analyzing a laboratory-weathered pooled soil sample amended with herbicides at the level of 10 ng/g (30 ng/g of clopyralid and picloram). Peak assignation: 1, cyanazine; A, monuron (IS); 2, simazine; 3, atrazine; 4, isoproturon; 5, diuron; B, *sec*-butylazine (IS); 6, linuron; 7, clopyralid; 8, picloram; 9, dicamba; 10, bentazone; 11, MCPA; 12, 2,4-D; 13, mecoprop; 14, dichlorprop; 15, bromoxynil; 16, ioxynil; 17, 2,4-DB; 18, MCPB; C, C_8-LAS (IS).

The extraction efficiency of water steadily increases as its temperature is increased.[14,15] On the other hand, subcritical water extraction performed at excessively high temperatures could give soil extracts containing abundant amounts of unwanted low-polar species. More important, those analytes prone to hydrolytic attack could be rapidly degraded by hot water. Therefore, recovery studies were initially conducted to find the best temperature at which efficient extraction of all of the analytes was obtained. Three-gram aliquots of an in vitro weathered pooled soil sample spiked with the analytes at the 100 ng/g level were extracted at increasing temperatures from 60 to 120 °C. These experiments were done in triplicate, and mean recoveries are reported in Table 4. A general enhancement of the extraction yield for all the analytes was achieved by raising the extraction temperature from 50 to 90 °C. When the extraction temperature was raised to 120 °C, more abundant fractions of the least polar acidic herbicides, i.e. 2,4-DB and MCPB, were extracted from the soil. Under this condition, however, severe losses of the three phenylurea herbicides, namely isoproturon, diuron and linuron, were experienced. It was reported that phenylureas are quantitatively converted to their corresponding anilines by catalytic hydrolysis on silica at 165 °C for 20 min.[38]

(38) De Kok, A.; Vos, Y. J.; Van Garderen, C.; De Jong, T.; Van Opstal, M.; Frei, R. W.; Geerdink, R. B.; Brinkman, U. A. Th. *J. Chromatogr.* **1984**, *288*, 71−89.

Table 6. Mean Percentage Recoveries of Herbicides from Soil by the Proposed Method Compared with Those Obtained by Two Other Extraction Methods (Spike Level 10 ng/g (30 ng/g of Clopyralid and Pichloram))

	recovery,[a] %		
compound	this method	Soxhlet extraction	sonication extraction
nonacidic herbicides			
cyanazine	85	61	
simazine	92	87	
atrazine	86	88	
isoproturon	88	63	
diuron	83	50	
linuron	85	77	
acidic herbicides			
clopyralid	88		42
picloram	90		61
dicamba	82		83
bentazone	89		82
MCPA	83		84
2,4-D	88		86
mecoprop	84		84
dichlorprop	80		83
bromoxynil	79		80
ioxynil	83		82
2,4-DB	64		79
MCPB	66		79

[a] Mean values from duplicate measurements.

Conceivably, this reaction partly occurred on extracting the above three compounds at temperatures higher than 90 °C. To avoid hydrolytic decomposition of the three phenylureas, further recovery studies were conducted by setting the temperature of the extraction cell at 90 °C.

The addition of 20% acetone or salts to water at 90 °C did not significantly improve recovery of the analytes. This suggested that incomplete recovery of some of the analytes could not be traced to some inefficiency of water as the extracting medium. Probably, those fractions of unextracted herbicides were irreversibly bound to soil or soil organic matter by covalent binding. Strong interactions between particular compounds and soil organic matter slowly becoming irreversible chemisorption is a process that may occur on weathered environmental solid matrixes.[39]

Matrix Effect. It is now well established that the fraction of certain analytes in soils extractable by a particular technique depends on the type of solid matrix. We assessed the extent to which the type of matrix could affect extraction by hot water. For this study, five different soil samples (see Table 1) that were amended with the herbicides at the 10 ng/g level (30 ng/g of both clopyralid and picloram) and in vitro aged were analyzed. Each soil was analyzed four times, and results are reported in Table 5. To check that mean concentrations of each herbicide measured in the five different types of soils did not differ significantly, means were compared among them by using the one-way ANOVA (analysis of variance) test at the $P = 0.05$ significance level. Except those for MCPB and 2,4-DB, the calculated $F_{4,15}$ values were lower than the critical values, showing that the extraction method was not influenced by the type of soil. Statistical analysis using the least significant difference method suggested that the relatively lower concentrations of MCPB and 2,4-DB measured in Camporeale and Trivero soil samples differed significantly from those in the other three types of soils. The reason for this was unclear to us. The organic matter coating the soil particles might be responsible for these underestimations, considering that the two soils mentioned above have the largest organic content, among the soils considered.

Precision and Method Detection Limits. The precision and the method detection limits (MDL's) were estimated by averaging data reported in Table 5. The precision of the method was between 6.7% (MCPA) and 12% (clopyralid). The MDL is here defined as 3 times the standard deviation calculated at the lowest spike level considered. MDL's ranged between 1.7 ng/g (bentazone) and 10 ng/g (clopyralid).

Figure 2 shows typical LC/MS chromatograms obtained by analyzing a pooled soil sample spiked with the target compounds at the level of 10 ng/g (30 ng/g of both clopyralid and picloram).

Method Comparison. We compared results obtained by our method with those obtained by two previously reported methods which were devised to extract respectively nonacidic, polar, and medium polar herbicides[40] and acidic herbicides[41] from soils. Briefly, the first method involved Soxhlet extraction with methanol for 12 h, while the second one extracted analytes for 2 h with an acetonitrile/water/acetic acid (80:20:2, v/v/v) mixture in an ultrasonic bath. For these experiments, aliquots of a pooled soil sample spiked with the analytes at 10 ng/g (30 ng/g of both clopyralid and picloram) levels were used. Measurements were made in duplicate, and results are presented in Table 6. Compared to Soxhlet extraction, subcritical water extraction was shown to be more efficient in extracting phenylurea herbicides. Compared to sonication extraction, this method appeared to be more effective in removing polar acidic herbicides from the soil but less efficient in extracting the least polar acidic herbicides, namely 2,4-DB and MCPB. This gap could be filled by increasing the extraction cell temperature to 120 °C (see Table 3). Under this condition, however, one should consider that phenylurea herbicides, if present in soil, could be partially decomposed.

In conclusion, water, an "environmentally-friendly" solvent, heated at a moderately high temperature appears to be an efficient medium for extracting polar and medium polar compounds from solid matrixes. Hot water extraction coupled with a Carbograph 4 cartridge and followed by LC/MS can offer sufficiently rapid and accurate determination of herbicide residues without the need for any cleanup step. Besides being tedious and time-consuming, purification procedures designed for few analytes having similar chemical characteristics cannot be applied when affording simultaneous analysis of several target compounds having a broad spectrum of chemical characteristics.

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(39) Thorn, K. A.; Pettigrew, P. J.; Goldenberg, W. S.; Weber, E. J. *Environ. Sci. Technol.* **1996**, *30*, 2764−2773.

(40) Abián, J.; Durand, G.; Barceló, D. *J. Agric. Food Chem.* **1993**, *41*, 1264−1273.

(41) Smith, A. E.; Aubin, A. *J. Agric. Food Chem.* **1991**, *39*, 2019−2021.