

Determination of Bentazone, Dichlorprop, and MCPA in Different Soils by Sodium Hydroxide Extraction in Combination with Solid-Phase Preconcentration

Christian W. Thorstensen^{*,†} and Agnethe Christiansen[‡]

The Norwegian Crop Research Institute, Plant Protection, Høgskoleveien 7, N-1432 Ås, Norway,
and The Norwegian Crop Research Institute, Pesticide Laboratory, Osloveien 1, N-1430 Ås, Norway

A method for the extraction of bentazone, dichlorprop, and MCPA in three selected Norwegian soils of different textures is described. Initially three different extraction methods were tested on one soil type. All methods gave recoveries >80% for the pesticide mixture, but extraction with sodium hydroxide in combination with solid-phase preconcentration was used for further recovery tests with soils of different properties spiked at four herbicide concentration levels (0.001–10 µg/g of wet soil). The method was rapid and easy and required a minimum of organic solvents. The recoveries were in the range of 82–109, 80–123, and 45–91% for the soils containing 1.4 (Hole), 2.5 (Kroer), and 37.8% (Froland) organic carbon, respectively. Limits of quantification using GC-MS were 0.0003 µg/g of wet soil for bentazone and 0.0001 µg/g of wet soil for both dichlorprop and MCPA.

Keywords: Soil extraction; solid-phase extraction; phenoxy acids; bentazone

INTRODUCTION

Phenoxy acids have been extensively used for the control of weeds in agriculture since World War II. Although these herbicides are applied as salts or esters, they are hydrolyzed to their parent compounds and found in their acidic form in soil (1).

Phenoxy acids are often used in combination with bentazone or ioxynil. These pesticides have certain persistence in soil, which depends on the properties of the compounds, the soil characteristics, and the environmental conditions. The persistence in soil is important for obtaining good control of weeds during the growing season, although it may in turn produce contamination of ground and surface waters. From an agronomic viewpoint, any successfully applied herbicide should persist long enough to give acceptable weed control but not so long that it affects the growth of following crops. The determination of pesticide residual levels in soil is needed to better understand the behavior of these compounds in soil.

The extraction of phenoxy acids and bentazone from soil is mainly achieved using different organic solvents, such as acetone, diethyl ether, methylene chloride, and acetonitrile (2–8) or a mixture of solvents (9–12). Phenoxy acids have also been extracted from soil by esterification in situ to their methyl esters using an organic solubilizer (13, 14). Extraction by supercritical fluids has also been proposed (15, 16). However, supercritical fluid extraction is considered to be too expensive because of instrument cost and time used per sample.

The primary goal of this work was to develop a simple and rapid procedure for the determination of bentazone, dichlorprop, and MCPA in Norwegian reference soils.

This procedure will later be used to study the degradation of bentazone, dichlorprop, and MCPA in the same soils. The intention was, if possible, to preconcentrate the soil extracts according to a solid-phase extraction (SPE) procedure that has recently been developed for bentazone, 2,4-D, dichlorprop, MCPA, and mecoprop in water (18).

EXPERIMENTAL PROCEDURES

Chemicals. Methanol and acetonitrile far-UV were of HPLC grade, and dichloromethane was of pesticide grade; all were obtained from Labscan (Dublin, Ireland). Orthophosphoric acid (H₃PO₄) 85%, ammonia solution (NH₃) 25%, hydrochloric acid fuming (HCl) 37%, formic acid (HCOOH) 98–100%, dipotassium hydrogen phosphate trihydrate (K₂HPO₄·3H₂O), and anhydrous sodium sulfate (Na₂SO₄), of analytical grade, were purchased from Merck (Darmstadt, Germany). 2,3,4,5,6-Pentafluorobenzyl bromide and *n*-decane were from Fluka (Buchs, Switzerland). Potassium dihydrogen phosphate was obtained from Sigma (St. Louis, MO). Reference standards of bentazone, 2,4-D, dichlorprop, MCPA, and mecoprop were supplied by Dr. Ehrenstorfer (Augsburg, Germany). A formulation containing a mixture of bentazone, dichlorprop, and MCPA, with the trade name Triagran-P, was obtained from Collet Kjemis a/s (Asker, Norway). Dilutions of pesticides were made in 20 mM phosphate buffer, pH 3. All solutions were stored in the dark at 4 °C.

Equipment and Materials for Sample Preparation. An SPE vacuum manifold from Supelco was used for SPE. Disposable extraction cartridges of 6 mL packed with 500 mg of SDB from IST were used for preconcentration. The eluates from the cartridges were evaporated to dryness using a centrifugal evaporator from Savant Instruments (Farmingdale, NY).

Soil Samples. The main characteristics of the three soils selected for this study are given in Table 1. The soils differ in organic matter content, soil pH level, and particle size distribution. The soils adequately represent variation in parent material, profile development (soil properties), and land use. Soil samples were collected from the 0–30-cm layer of the soil profile and sieved to pass through a 2-mm sieve.

* Author to whom correspondence should be addressed (fax 47-64949226; e-mail christian.thorstensen@planteforsk.no).

[†] Plant Protection.

[‡] Pesticide Laboratory.

Table 1. Characteristics of Norwegian Reference Soils

description	location	sand (%)	silt (%)	clay (%)	pH	organic C (%)
fine sandy loam	Hole	49	46	5	6.3	1.4
loam	Kroer	36	45	19	5.5	2.5
highly decomposed organic matter	Froland				2.9	37.8

Table 2. Concentrations of Pesticides in Wet Reference Soils^a

concn level	pesticide concn ($\mu\text{g/g}$ of wet soil)		
	bentazone	dichlorprop	MCPA
1	11.2	8.0	9.8
2	1.12	0.8	0.98
3	0.0112	0.008	0.0098
4	0.00112	0.0008	0.00098

^a The difference in concentration within each level is caused by the use of a commercial formulation.

Soil Fortification. In the method screening, 10 g of wet soil from Kroer was spiked with bentazone, 2,4-D, dichlorprop, MCPA, and mecoprop to give a concentration of 5 $\mu\text{g/g}$ of wet soil. During extraction of reference soils, 25 g of wet soil was spiked with a mixture of bentazone, dichlorprop, and MCPA. Concentration levels are given in Table 2. The soil moisture contents after spiking were 22, 25, and 82% (calculated as percent of dry soil) in Hole, Kroer, and Froland soils, respectively, and correspond to 60% water holding capacity (WHC). Each spiked sample was shaken by hand and placed in the refrigerator at 4 °C for 15 h before extraction.

Extraction with Acetone/Water/Acetic Acid. A 10 g soil sample was extracted with 25 mL of acetone/water/acetic acid (80:19:1) for 24 h and centrifuged at 7600g for 10 min. The sample was reextracted with 10 mL of the same solvent mix for 1 h and centrifuged. The supernatants were combined, diluted with 500 mL of distilled water, and preconcentrated on a solid-phase cartridge as described below.

Extraction with Sodium Hydroxide and Partition into Dichloromethane. A 10 g soil sample was extracted twice with 25 mL of sodium hydroxide (0.01 M) for 1 h and centrifuged for 10 min at 7600g. The combined supernatants were acidified with 6 M HCl to pH 1 and partitioned into 50 mL dichloromethane (1 h). The partition was repeated with 25 mL of dichloromethane for 10 min. The combined extracts were dried over anhydrous Na_2SO_4 , evaporated to dryness, and redissolved in 1 mL of 20 mM phosphate buffer, pH 3.

Extraction with Sodium Hydroxide. A 10 g soil sample was extracted twice with 25 mL of 0.01 M NaOH for 1 h and then centrifuged at 7600g for 10 min. The combined supernatants were acidified with 6 M HCl to pH 2.5 ± 0.5 , centrifuged, and preconcentrated on a solid-phase cartridge. For extraction of reference soils, the amounts of soil and sodium hydroxide were increased to 25 g and 100 mL, respectively.

SPE Procedure. The extraction cartridge was rinsed by passing 5 mL of methanol through the cartridge followed by 10 mL of water adjusted to pH 2.5 ± 0.5 . Sample loading was performed at a flow rate of 30 mL/min under vacuum. The sorbent was never allowed to dry during the rinsing and sample loading procedures. After extraction, the cartridges were dried with a gentle stream of nitrogen. Elution was performed by gravity using 2 mL of methanol with 5% NH_3 , but the vacuum was turned on at the end of elution. The eluate was evaporated to dryness. At concentration levels 1 and 2 (see Table 2) the residue was redissolved in 1 mL of 20 mM phosphate buffer, pH 3, and analyzed by HPLC-UV. At concentration levels 3 and 4 the residue was dissolved in 4 mL of phosphate buffer, pH 8. The pesticides were derivatized with pentafluorobenzyl bromide (19) and analyzed by GC-MS.

HPLC Analysis. The liquid chromatograph was composed of a Spectra-Physics model SP8000 pump, a SP4270 integrator (San Jose, CA), a Gilson autosampler (Middleton, WI), and a Milton Roy UV detector (Staffordshire, U.K.) set at 200 nm.

A C_8 analytical column of stainless steel, 25 cm \times 4.6 mm i.d., packed with Spherisorb 5 μm particles from Phase Separations Ltd. (Deeside, U.K.) was used. The mobile phase was 20 mM phosphate buffer, pH 3/acetonitrile (65:35 v/v) at a flow rate of 1 mL/min, and the injection volume was 50 μL . Quantification was performed by external calibration by measuring the peak areas.

GC-MS Analysis. All GC-MS measurements were performed with a Hewlett-Packard 5971A MSD combined with a Hewlett-Packard 5890A GC (Palo Alto, CA) fitted with a CP-SIL 5CB MS capillary column, 50 m \times 0.25 mm i.d. \times 0.40 μm film thickness, from Chrompack (Middelburg, The Netherlands). The carrier gas was helium (Ultra Plus 6.0, Hydro gas, Oslo, Norway) set to a head column pressure of 18 psi at 80 °C (linear velocity = 29.9 cm/s). The injector was operated in the splitless mode (60 s), and the injection volume was 2 μL . Injector and detector temperatures were 240 and 260 °C, respectively. The oven temperature was held at 80 °C for 1 min, then raised at 20 °C/min to 160 °C followed by 5 °C/min to 280 °C, and finally held for 5 min. The MSD was operated in the selected ion monitoring mode (SIM, 70 eV). Two characteristic ions were selected for each compound: bentazone, m/z 378 and 420; dichlorprop, m/z 414 and 416; and MCPA, m/z 380 and 382. Quantification was performed by external calibration by measuring the peak areas.

RESULTS AND DISCUSSION

The soils were selected to represent the common Norwegian soil, and these will later serve as reference soils for ecotoxicological tests of pesticides in the Norwegian environment. Therefore, a great effort was made to select the most common soils in Norway. Two of the agricultural soils, a fine sandy loam from Hole and a loam from Kroer, were selected on the basis of information from the Norwegian Institute of Land Inventory's Digital Soil Database, which currently covers ~ 2500 km² of agricultural land. A forest soil of highly decomposed organic matter from Froland was selected as the third soil due to its high organic content.

Our primary goal was to develop a method that could be used to study the degradation of acidic herbicides in Norwegian reference soils. An underlying aim was that the method could be used for routine analysis in Norway. The soil from Kroer is routinely used for various studies of pesticide behavior in soil, and it was therefore decided to use this soil type for the initial extraction method screening. The first method tested was a modification of a procedure proposed by Herber and Stan (20). A time-saving modification was accomplished by directly diluting the soil extract with water prior to preconcentration, instead of evaporation of the soil extract followed by dilution and preconcentration. This modification also involves fewer steps that might cause contamination and loss of analytes. Both the second and third tested methods involved extraction with sodium hydroxide followed by either acidification and partition into dichloromethane or solid-phase preconcentration as described for analysis of phenoxy acids and bentazone in water samples (18). A problem with the second method was the formation of emulsions during the partition step, with much time needed to break up the emulsions. With the third method a soil extract of 100 mL could rapidly be preconcentrated on SDB solid-phase cartridges using a flow of 30 mL/min. Earlier studies have shown that silica-based sorbents are not suitable for cleanup of water extracts of soil (18) due to plugging.

Alkaline extraction is seldom accomplished, and to our knowledge a combination of alkaline extraction with

Table 3. Percent Recovery of Phenoxy Acids and Bentazone Using Three Different Sample Preparation Methods^a

extractant	bentazone	2,4-D	dichlorprop	MCPA	mecoprop	average
acetone/water/acetic acid (80:19:1) ^b	91.0 ± 1.0	90.0 ± 1.0	89.0 ± 1.0	89.0 ± 1.0	90.0 ± 1.0	89.7 ± 1.3
sodium hydroxide ^c	84.8 ± 2.5	80.4 ± 2.3	81.7 ± 2.4	80.9 ± 2.2	79.1 ± 2.4	81.6 ± 3.0
sodium hydroxide ^b	88.3 ± 1.2	85.9 ± 1.3	85.3 ± 1.0	85.7 ± 1.3	84.6 ± 0.8	86.0 ± 1.6

^a Concentration: 5 µg of pesticide/g of wet soil, *n* = 4. Quantification was performed with HPLC-UV. ^b Extraction with acetone/water/acetic acid and sodium hydroxide was followed by solid-phase preconcentration. ^c Followed by partition into dichloromethane.

Table 4. Average Percent Recovery of Pesticides from Three Different Soils at Four Different Concentration Levels^a

herbicide	concn level	soil		
		Hole	Kroer	Froland
bentazone	1	90.3 ± 2.8 ^b	109.9 ± 5.2	87.2 ± 7.3
	2	91.8 ± 2.9	89.2 ± 2.1	68.1 ± 4.9
	3	101.3 ± 10.5	106.7 ± 6.9	74.5 ± 8.6
	4	96.7 ± 4.8	127.5 ± 24.8	91.8 ± 31.1
average	1-4	94.1 ± 6.7	108.3 ± 18.9	79.6 ± 16.2
dichlorprop	1	85.0 ± 4.7	101.1 ± 3.6	63.8 ± 5.5
	2	90.1 ± 3.7	86.4 ± 2.4	57.1 ± 5.2
	3	86.7 ± 11.9	92.0 ± 9.8	54.4 ± 5.5
	4	96.2 ± 17.4	133.0 ± 19.4	89.0 ± 30.8
average	1-4	88.6 ± 6.8	103.2 ± 21.8	64.5 ± 18.1
MCPA	1	83.7 ± 5.3	99.8 ± 3.8	61.4 ± 4.8
	2	85.3 ± 4.0	80.2 ± 2.7	50.3 ± 5.6
	3	82.2 ± 9.3	84.9 ± 7.5	45.2 ± 4.5
	4	109.5 ± 20.9	123.2 ± 17.6	69.8 ± 22.4
average	1-4	88.9 ± 5.9	96.8 ± 20.1	58.8 ± 13.4

^a Quantification was performed with HPLC-UV (concentration levels 1 and 2) and GC-MS (concentration levels 3 and 4).

^b Average of four replicates ± standard deviation.

solid-phase preconcentration for determination of phenoxy acids has never been applied. HPLC-UV was used for quantification during method screening due to its rapidity compared to GC-MS of derivatized pesticides.

Initial recovery experiments using the three different sample preparation methods were performed by spiking soil from Kroer with the pesticide mixture to a concentration of 5 µg of pesticide/g of wet soil. The results are reported in Table 3. Average recoveries of the pesticide mixture were in the range of 82–90% for all tested methods, which showed that they were all satisfactory. However, extraction followed by sodium hydroxide with SPE was chosen for further work because it was easy to perform and required a minimum of organic solvents compared to the other methods.

Recovery experiments on three different reference soils were performed over a wide range of concentrations, which are shown in Table 2 (0.001–10 µg/g of wet soil, approximate concentrations). Because the method will later be used to study the degradation of a mixture of bentazone, dichlorprop, and MCPA in reference soils, only these three pesticides were tested for recovery. The results are given in Table 4. Mean percent recovery for all levels of bentazone ranged from 80% in the organic-rich soil from Froland to 108% for the fine sandy loam from Kroer. The mean levels of recovery for dichlorprop ranged from 65% in the soil from Froland to 103% in the soil from Kroer and for MCPA from 59% in the soil from Froland to 97% in the soil from Kroer. For each set of percent recovery data, the Froland soil ranked the lowest. Sorption of these acidic compounds to soil is known to increase with increasing organic content and decreasing pH (21), and this may explain the lower recovery in the forest soil from Froland compared to the agricultural soils. The phenoxy acids may be incorporated into the three-dimensional structure of the humic

compounds or be subjected to radicals from the humic compounds forming complexes during the extraction with sodium hydroxide in the presence of air.

Average recovery for all pesticides was >88% in both the Hole and Kroer soils. Irrespective of the higher organic content in the soil from Kroer, there was a tendency toward a higher recovery of all pesticides from the Kroer soil than from the Hole soil. The reason for a higher recovery in the Kroer soil could be that other soil constituents, such as clay, may also interfere with the sorption of pesticides and thereby influence their recovery. Several authors have noted that negatively charged pesticides could be expelled by negatively charged clay (22, 23). The soil from Kroer has a higher clay content than the Hole soil, and this could explain the higher recovery from the Kroer soil compared to the Hole soil. The organic matter contributes with negative pH-dependent charges. Because the acidic herbicides are in their anionic form in both agricultural soils, the higher organic matter content in the soil from Kroer may actually decrease the binding to the soil matrix and thereby increase recovery. It may therefore be assumed that both clay and organic content have a positive effect on the recovery of anionic pesticides.

Limits of quantification using GC-MS were calculated as the sample analyte concentration that gives a signal-to-noise ratio ≥ 10 (24). The determinations were performed by injection of soil extracts at the lowest concentration level (Table 2), and the limits of quantification were 0.0003 µg/g of wet soil for bentazone and 0.0001 µg/g of wet soil for both dichlorprop and MCPA.

The novelty of this work is a combination of alkaline extraction with SPE. Small amounts of organic solvents are used. Samples with a high content of organic matter can be handled without any plugging of the SDB cartridges, in contrast to the C₈/C₁₈ silica sorbents. The developed method has been used in degradation studies of phenoxy acids and bentazone in soil and was satisfactory for this purpose for all pesticides and soil types. The method is satisfactory for the routine analysis of bentazone, dichlorprop, and MCPA in Norwegian agricultural soils with recoveries in the range of 89–103% for the pesticide mixture.

ABBREVIATIONS USED

organic C, organic carbon; SPE, solid-phase extraction; SDB, styrene–divinylbenzene.

LITERATURE CITED

- (1) Tadeo, J. L.; Sanches-Brunete, C.; García-Valcarcel, A.; Martínez, I. L.; Pérez, R. A. Determination of cereal herbicide residues in environmental samples by gas chromatography. *J. Chromatogr. A* **1996**, *754*, 347–365.
- (2) Danis, T. G.; Albanis, T. A. Analysis of phenolic residues in soil as free and acetylated derivatives by capillary gas chromatography equipped with FID, ECD and MSD. *Toxicol. Environ. Chem.* **1996**, *53*, 9–18.
- (3) Khan, S. U. Electron capture gas–liquid chromatographic method for the simultaneous analysis of 2,4-D, dicamba, and mecoprop residues. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 1027–1031.

- (4) Ngan, F.; Ikesaki, T. J. Determination of 9 acidic herbicides in water and soil by gas-chromatography using an electron-capture detector. *J. Chromatogr. A* **1991**, *537*, 385–395.
- (5) Sánchez-Brunete, C.; García-Valcárcel, A. I.; Tadeo, J. L. Determination of residues of phenoxy acid herbicides in soil and cereals by gas chromatography ion-trap detection. *J. Chromatogr. A* **1994**, *675*, 213–218.
- (6) Gennaro, M. C.; Giacosa, D.; Baglietto, C. Simultaneous separation of phenylurea-, triazine- and phenoxy-acid herbicides by reversed phase ion-interaction HPLC. Application to soil analysis. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *6*, 911–924.
- (7) Smith, A. E. The hydrolysis of herbicidal phenoxyalkanoic esters to phenoxyalkanoic acids in Saskatchewan soils. *Weed Res.* **1976**, *16*, 19–22.
- (8) Tsukioka, T.; Murakami, T. J. Capillary gas-chromatographic mass-spectrometric determination of acid herbicides in soils and sediments. *J. Chromatogr. A* **1989**, *469*, 351–359.
- (9) Lee, H-B.; Chau, A. S. Y. Analysis of pesticide residues by chemical derivatization. VI. Analysis of ten acid herbicides in sediment. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1023–1028.
- (10) Bruns, G. W.; Nelson, S.; Erickson, D. G. Determination of MCPA, bromoxynil, 2,4-D, trifluralin, triallate, picloram, and diclofop-methyl in soil by GC-MS using selected ion monitoring. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 550–553.
- (11) Ahmed, A. W.; Mallet, V. N.; Bertrand, M. J. Determination of acidic herbicides and related compounds in water and soil by capillary gas chromatography using a nitrogen-phosphorus detector. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 365–367.
- (12) Purkayastha, R. Simultaneous determination of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and 2-methoxy-3,6-dichlorobenzoic acid in soil and water by gas chromatography with electron capture detector. *J. Agric. Food Chem.* **1974**, *22*, 453–459.
- (13) Brumley, W. C.; Brownrigg, C. M. Electrophoretic behavior of aromatic-containing organic acids and the determination of selected compounds in water and soil by capillary electrophoresis. *J. Chromatogr.* **1993**, *646*, 377–389.
- (14) Li, C.; Markovec, L. M.; Magee, R. J.; James, B. D. A convenient method using methanolbenzyltrialkylammonium reagents for simultaneous extraction and methylation of 2,4-dichlorophenoxyacetic acid in soil, with subsequent analysis via gas-chromatography. *J. Agric. Food Chem.* **1991**, *39*, 1110–1112.
- (15) Chiang, L.; Magee, R. J.; James, B. D. Chromatographic determination of chlorinated phenoxyacetic acids by simultaneous extraction methylation and application to soil analysis. *Anal. Chim. Acta* **1991**, *255*, 187–196.
- (16) Rochette, E. A.; Harsh, J. B.; Hill, H. H. Soil component interactions with 2,4-dichlorophenoxyacetic acid under supercritical fluid conditions. *Environ. Sci. Technol.* **1996**, *30*, 1220–1226.
- (17) Lopez-Avila, V.; Dodhiwala, N. S. Developments in the supercritical-fluid extraction of chlorophenoxy acid herbicides from soil samples. *J. Agric. Food Chem.* **1993**, *41*, 2038–2044.
- (18) Thorstensen, C. W.; Lode, O.; Christiansen, A. L. Development of a solid-phase extraction method for phenoxy acids and bentazone in water and comparison to a liquid-liquid extraction method. *J. Agric. Food Chem.* **2000**, *48*, 5829–5833.
- (19) Holen, B.; Christiansen, A. *Handbook of Analytical Methods for Pesticide Residues*; The Norwegian Crop Research Institute: Ås, Norway, 1994.
- (20) Herber, T.; Stan, H.-J. Determination of trace levels of dichlorprop, mecoprop, clofibric acid, and naphthylacetic acid in soil by gas chromatography mass spectrometry with selected-ion monitoring. *J. AOAC Int.* **1996**, *79*, 1428–1433.
- (21) Voos, G.; Groffman, P. M.; Pfeil, M. Laboratory analysis of 2,4-D and dicamba residues in soil. *J. Agric. Food Chem.* **1994**, *42*, 2502–2507.
- (22) Frissel, M. J.; Bolt, G. H. Interaction between certain ionizable organic compounds (herbicides) and clay minerals. *Soil Sci.* **1962**, *94*, 284–291.
- (23) Weber, J. B. Mechanisms of adsorption of s-triazines by clay colloids and factors affecting plant availability. In *Residue Reviews*; Gunther, F. A., Gunther, J. G., Eds.; Springer Verlag: New York, 1970; pp 93–130.
- (24) Dyson, N. *Chromatographic Integration Methods*; RSC Chromatography Monographs; The Royal Society of Chemistry: Cambridge, U.K., 1990.

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