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USE OF ACETONITRILE FOR THE EXTRACTION OF HERBICIDE RESIDUES FROM SOILS

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SUMMARY

Using a 10% aqueous acetonitrile solution for extraction and an identical solvent clean-up procedure, soil-based residues of the herbicides alachlor, benzoylprop-ethyl, flufenprop-isopropyl, flufenprop-methyl, dichlorfop-methyl, nitrofen, and profluralin were recovered reproducibly from three prairie soils fortified at 0.5 and 0.1 ppm levels. The acidic herbicides benazolin, 2,4-D, and 2,4,5-T, together with the acids derived from benzoylprop-ethyl, dichlorfop-methyl, flufenprop-isopropyl, and flufenprop-methyl were reproducibly recovered from the three prairie soils fortified at 0.5 and 0.1 ppm levels using 30% aqueous acetonitrile containing 1% acetic acid after identical clean-up stages. All compounds were analysed by gas chromatographic means utilising an electron-capture detector. The two procedures described were developed for the routine extraction and analysis of neutral and acidic herbicide residues from field soil persistence studies.

INTRODUCTION

For toxicological reasons the detection of herbicide residues in crops, fruits, vegetables, and other foods at the 0.05 ppm level, or even lower, is desirable. However, for soil persistence studies such low detection limits are generally unnecessary, as the measurement of herbicide residues is required chiefly to establish breakdown rates and to determine whether or not phytotoxic amounts are carried over from one growing season to the next. Carry-over could cause crop damage when further applications are made. Most herbicides are applied at rates of at least 1 kg/ha and with incorporation assumed throughout the top 5 cm of soil; this is equivalent to residues of approximately 2 ppm. Hence, a residue level of 0.1 ppm represents only 5% of such an initial treatment. With the exception of picloram and certain triazine herbicides, residues of this magnitude would generally not be expected to result in subsequent crop damage.

Detection of soil residues at the 0.1 ppm level poses less of a problem to the analyst than would residues present at the 0.05 ppm level, and lower, as a less rigorous clean-up for the removal of substances which may interfere with the final gas chromatographic (GC) analysis is necessary. For persistence studies, especially where a

large number of herbicides are being tested at more than one location and over a number of years a routine analytical procedure is desirable that is quick, reliable, versatile in that it can be applied to the extraction of many different herbicide residues, and allows detection at the 0.1 ppm level with minimum clean-up.

The two procedures to be described here, using aqueous acetonitrile as soil extractant, fulfil the above requirements and were developed for the routine extraction with GC estimation of individual neutral and acidic herbicides commonly applied to Canadian prairie soils.

MATERIALS AND METHODS

Soils

The composition and physical characteristics of the soils used in these studies are shown in Table I.

TABLE I

CHARACTERISTICS OF SOILS

Soil	*% clay*	*% silt*	*% sand*	*% organic carbon*	*pH in water (1:1)*
Jameson sandy loam	6	9	85	3.2	7.5
Regina heavy clay	69	26	5	4.2	7.7
Melfort silty clay	30	38	32	11.7	5.2

Herbicides

The following technical-grade herbicides were included in these investigations: alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide]; benazolin (4-chloro-2-oxobenzothiazolin-3-ylacetic acid); benzoylprop-ethyl [ethyl(±)-2-(N-benzoyl-3,4-dichloroanilino)propionate]; flufenprop-isopropyl [isopropyl-N-benzoyl-N-(3-chloro-4-fluorophenyl)-2-aminopropionate]; flufenprop-methyl [methyl-N-benzoyl-N-(3-chloro-4-fluorophenyl)-2-aminopropionate]; dichlorfop-methyl {methyl-2-[4-(2,4-dichlorophenoxy)phenoxy]propionate}; nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether); profluralin [N-(cyclopropylmethyl)-*α*,*α*,*α*-trifluoro-2,6-dinitro-N-propyl-*p*-toluidine]; 2,4-D (2,4-dichlorophenoxyacetic acid); and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). The free acids derived from benzoylprop-ethyl and dichlorfop-methyl and the two flufenprop esters, *viz.* 2-(N-benzoyl-N-3,4-dichloranilino)-propionic acid, 2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid, and N-benzoyl-N-(3-chloro-4-fluorophenyl)-2-aminopropionic acid were also included as they are formed hydrolytically in soils from the parent ester herbicides. Individual stock solutions were prepared containing 50 μg of each herbicide per millilitre of methanol, except for the dichlorfop acid, which was dissolved in ethanol.

Soil fortification

To 20-g samples of the sieved air-dried soils in screwcapped glass bottles of 70-ml capacity were added either 200 or 40 μl of a particular solution to give a herbicide concentration of 0.5 or 0.1 ppm. After thorough mixing to ensure even distri-

bution of the chemicals throughout the soil, the bottles containing the fortified soils were capped, equilibrated for 24 h at room temperature, and then frozen at −5° for four weeks before extraction and analysis. There were four replicates for each chemical concentration in each of the three soil types.

Extraction procedure for alachlor, benzoylprop-ethyl, dichlorfop-methyl, flufenprop-isopropyl, flufenprop-methyl, nitrofen, and profluralin

Each fortified soil sample was placed in a 100-ml capacity beaker, covered with 50 ml of acetonitrile containing 10% by volume of distilled water, and extracted for 2 min using a Sonic Dismembrator (Artek Systems, Farmingdale, N.Y., U.S.A.) at maximum power. After settling, 25 ml of the acetonitrile extract, corresponding to 10 g of the treated soil, was decanted into a measuring cylinder and added to 100 ml of 5% aqueous sodium carbonate solution in a 250-ml capacity separatory funnel and extracted with a 25-ml portion of *n*-hexane. The aqueous phase was discarded and the organic layer dried over sodium bicarbonate when 3- or 5-μl aliquots were examined by GC.

Extraction procedure for benazolin, benzoylprop acid, dichlorfop acid, flufenprop acid, 2,4-D, and 2,4,5-T

Each soil sample (20 g) was placed in a 100-ml beaker together with 50 ml of a solution containing acetonitrile–water–acetic acid in the proportions 70:29:1 and extracted for 2 min using the Sonic Dismembrator at maximum power. Following centrifugation at 5000 rpm for 5 min, 25 ml of the clear extract, equivalent to 10 g of soil, was decanted into a measuring cylinder and added to 100 ml of 5% aqueous sodium carbonate solution in a 250-ml separatory funnel, and extracted with a 25-ml portion of *n*-hexane which was then discarded. The aqueous phase was acidified by the addition of 15 ml concentrated hydrochloric acid and any acidic substances extracted using one 50-ml and two 25-ml portions of diethyl ether. The ether extracts were pooled and shaken with two 25-ml portions of 5% aqueous sodium bicarbonate to recover any acidic residues. Following acidification of the combined bicarbonate extracts with 7 ml concentrated hydrochloric acid, the acidified solution was shaken with one 50-ml and two 25-ml portions of diethyl ether. The pooled ether extracts were evaporated to dryness at 30° using a rotary evaporator. Traces of water were removed from the flask by adding equal portions of methanol and benzene, followed by evaporation under reduced pressure when the water was removed by azeotropic distillation. The residue was quantitatively transferred, using diethyl ether, to a 100-ml glass tube and the ether evaporated to approximately 10 ml by immersing the tube in a water-bath set at 50°. The ether extracts, containing any acid residues, were methylated using 2 ml of a solution of diazomethane in *n*-hexane[1]. After evaporation of excess reagent and diethyl ether the volume was adjusted to 25 ml with *n*-hexane when 3- or 5-μl aliquots were analysed gas chromatographically.

Gas chromatographic analysis

A Hewlett-Packard Model 5713A gas chromatograph was used equipped with means for on-column injection and a radioactive nickel electron-capture detector operated at 300°. The column was of glass (1.5 m × 6.0 mm O.D.) and the carrier gas was argon containing 5% of methane at a flow-rate of 40 ml/min. The various

column packings and column temperatures used for the analyses of the various compounds are shown in Table II, together with the retention times.

Chromatographic standards of the esters and other non-acidic herbicides were prepared by adding 200 or 40 μl of each herbicide solution to 50 ml of *n*-hexane to give solutions containing 0.2 or 0.04 ng of the chemical per μl. In the case of the acidic compounds, 200 or 40 μl portions of the stock solutions, in 5 ml diethyl ether, were methylated as described and taken up in 50 ml of *n*-hexane to give methylated standards equivalent to 0.2 or 0.04 ng of the respective acids per μl. The concentrations of the herbicides present in the samples were calculated by comparing the sample peak heights with those of the appropriate standards.

TABLE II

COLUMN PACKINGS AND TEMPERATURES WITH RETENTION TIMES

Column packings: (A) 2% DC-200 + 3% QF-1 on Gas-Chrom Q, 60–80 mesh. (B) 3% Dexsil-300 + 3% OV-210 on Chromosorb W-HP, 80–100 mesh. (C) 10% OV-1 on Chromosorb G-HP, 80–100 mesh.

Herbicide	*A*		*B*		*C*	
	Column temperature (°C)	*Retention time (min)*	*Column temperature (°C)*	*Retention time (min)*	*Column temperature (°C)*	*Retention time (min)*
Alachlor	180	3.10	190	5.00	240	3.00
Benzoylprop-ethyl	210	5.60	220	9.50	240	12.00
Dichlorfop-methyl	210	4.00	220	7.00	240	10.50
Flufenprop-isopropyl	210	3.75	220	5.75	240	7.80
Flufenprop-methyl	210	3.00	220	4.75	240	6.30
Nitrofen	210	2.80	220	5.80	240	7.00
Profluralin	180	2.50	190	3.10	230	2.50
Benazolin methyl ester	180	4.70	220	2.75	230	5.00
Benzoylprop methyl ester	210	5.00	220	8.20	240	10.50
2,4-D methyl ester	180	1.20	190	1.70	230	1.60
2,4,5-T methyl ester	180	1.80	190	2.80	230	2.60

RESULTS AND DISCUSSION

Following fortification, the soil samples were frozen for four weeks prior to extraction and analysis, as it is the custom in this laboratory to air-dry field soils containing herbicide residues to constant weight at room temperature and then to store at −5° while awaiting assay.

The 10% aqueous acetonitrile was selected as extraction solvent for the non-acidic chemicals as this has been shown to be suitable for the recovery of several different herbicides from soils[2,3]. The 30% aqueous acetonitrile containing 1% acetic acid has been recommended for the soil extraction of the acid soil-hydrolysis product derived from benzoylprop-ethyl[4]. As initial studies using this solvent system resulted in very satisfactory recoveries of benzoylprop acid from Saskatchewan soils, its effects on the extraction of other acidic residues was tried.

Recoveries of the thirteen chemicals from treated soils (Table III) were in general excellent and reproducible. Although recoveries of dichlorfop-methyl, di-

TABLE III
RECOVERY OF HERBICIDES FROM FORTIFIED SOILS

Herbicide	*Amount added (ppm)*	*Recovery (%)* *		
		Regina heavy clay	*Melfort silty clay*	*Jameson sandy loam*
Alachlor	0.5	99 ± 2	98 ± 3	92 ± 1
	0.1	95 ± 2	94 ± 3	95 ± 3
Benzoylprop-ethyl	0.5	95 ± 3	99 ± 1	97 ± 2
	0.1	93 ± 9	95 ± 4	96 ± 3
Flufenprop-isopropyl	0.5	89 ± 3	99 ± 1	90 ± 3
	0.1	95 ± 6	99 ± 1	81 ± 14
Flufenprop-methyl	0.5	91 ± 2	92 ± 4	94 ± 6
	0.1	92 ± 9	93 ± 5	92 ± 6
Dichlorfop-methyl	0.5	90 ± 2	86 ± 3	84 ± 3
	0.1	87 ± 8	88 ± 4	82 ± 4
Nitrofen	0.5	97 ± 2	98 ± 3	96 ± 2
	0.1	98 ± 3	97 ± 3	94 ± 2
Profluralin	0.5	100 ± 1	100 ± 2	96 ± 2
	0.1	98 ± 2	93 ± 3	90 ± 2
Benazolin	0.5	80 ± 4	68 ± 6	84 ± 12
	0.1	89 ± 7	79 ± 8	92 ± 8
Benzoylprop acid	0.5	94 ± 3	90 ± 4	97 ± 3
	0.1	96 ± 5	94 ± 7	95 ± 6
Dichlorfop acid	0.5	65 ± 2	64 ± 1	64 ± 3
	0.1	54 ± 3	59 ± 4	53 ± 5
Flufenprop acid	0.5	94 ± 4	93 ± 4	96 ± 4
	0.1	93 ± 4	98 ± 5	97 ± 6
2,4-D	0.5	99 ± 3	94 ± 4	99 ± 2
	0.1	98 ± 2	88 ± 3	94 ± 4
2,4,5-T	0.5	100 ± 2	87 ± 3	99 ± 1
	0.1	87 ± 4	79 ± 3	100 ± 2

* Mean and standard deviation from four determinations.

chlorfop acid, and benazolin were lower than those for the other chemicals, the amounts recovered were quite reproducible. This lower recovery of dichlorfop-methyl and dichlorfop acid may perhaps be due to some unknown reactivity of the molecule, as it has been observed[5] that dichlorfop acid in methanol undergoes complete esterification to the methyl ester at room temperature in fourteen days. For this reason the acid in these studies was dissolved in ethanol, as ethylation of the dichlorfop acid does not appear to occur at laboratory temperatures, or is very slow.

For the analyses of the neutral herbicides obtained from the soils fortified at the 0.5-ppm level the choice of GC column was not critical, as interfering substances co-extracted from the soils were present in such comparatively small quantities that adequate quantitative measurements could be made. At the higher attenuations required for detection of the lower herbicide concentrations extracted from the soils fortified at the 0.1-ppm level signals from soil co-extracts did interfere with the detection of some chemicals on certain columns and the choice of column thus became important. The 10% OV-1 column proved to be the most useful for the analysis of alachlor, benzoylprop-ethyl, dichlorfop-methyl, nitrofen, and profluralin residues,

while flufenprop-isopropyl and flufenprop-methyl could be analysed most satisfactorily using the mixed DC-200 and QF-1 column.

The more aqueous and acidic solvent used for the extraction of the acid residues also resulted in the presence of more interfering substances in the final *n*-hexane solution. However, at the higher fortification level any of the three GC columns could be used to obtain adequate measurements. For analyses of the extracts obtained from soils treated at the 0.1-ppm level, the 10% OV-1 column proved the most suitable for estimation of dichlorfop, 2,4-D, and 2,4,5-T methyl esters, while the mixed DC-200 and QF-1 column was found to be excellent for the analysis of extracts containing flufenprop, benzoylprop, or benazolin methyl esters.

Although the solvent mixture used for the recovery of the acidic compounds would also extract the non-acidic and ester herbicides, use of the 10% aqueous acetonitrile resulted in much cleaner extracts, and the gas chromatograms indicated the presence of less interfering substances. Thus, it is better to use this solvent to extract benzoylprop-ethyl or the two flufenprop esters or dichlorfop-methyl from treated soils and then use further soil samples and 30% aqueous acetonitrile to separately extract the acid hydrolysis products, rather than use the latter solvent to extract both ester and acid derivatives for subsequent separation and analysis as reported for benzoylprop-ethyl and acid[4]. Experiments showed that any esters extracted using the 30% aqueous acetonitrile were subsequently removed from the alkaline sodium carbonate–acetonitrile mixture by shaking with *n*-hexane. As these hexane extracts were discarded, possible contamination of dichlorfop and flufenprop acids (which are analysed as their methyl esters) by any of the original unhydrolysed parent herbicide would not occur.

The two extraction procedures described would thus appear to be satisfactory for the detection of soil-based residues of the thirteen neutral and acidic chemicals tried, down to at least the 0.1-ppm level. The procedures are quick and reproducible and versatile with an added benefit that the extraction is reasonably inexpensive, especially for the non-acidic compounds, where only 50 ml acetonitrile and 25 ml of *n*-hexane are required per analysis.

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