Bull. Environ. Contam. Toxicol. (2003) 71:414–421
© 2003 Springer-Verlag New York Inc.
DOI: 10.1007/s00128-003-0180-4

Bulletin of Environmental Contamination and Toxicology

Improved Analytical Procedure for Determination of Clopyralid in Soil Using Gas Chromatography

R. Ahmad,[1] A. Rahman,[1] P. T. Holland,[2] D. E. McNaughton[2]

[1] AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
[2] HortResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand

Received: 23 January 2003/Accepted: 20 April 2003

Herbicides are an important component of modern agricultural production systems and are widely used to restrict the losses from weeds. The herbicide clopyralid (3,6-dichloropyridine-2-carboxylic acid) was introduced in the mid 1970's as a broad-spectrum herbicide for selective control of weeds in crops such as sugar beet, fodder beet, maize, brassicas, cereals, strawberries as well as pastures (Haagsma, 1975; Naish, 1975; Rutherford and Lobb, 1979). In New Zealand, it is currently registered for the control of certain broadleaf weeds in a range of crops, turf and amenity areas, pastures, forestry, as well as for conservation tillage (Hathway, 1989; O'Connor, 2002).

Published methods to determine trace level residues of clopyralid have been based on high performance liquid chromatography with UV detection (HPLC-UV) (Pik et al., 1977; Lauren et al., 1988; Cox et al., 1999) and gas chromatography with electron capture detection (GC-ECD) (Pik and Hodgson, 1976; Cotterill, 1978; Galoux et al., 1985; Tan et al., 1996). However, the reported methods proved to be inadequate for analysis in soil. The available HPLC-UV methods suffer from a general lack of sensitivity (Schütz et al., 1994). The GC-ECD method involves alkaline extraction and methylation of clopyralid. The percentage recovery obtained following these GC-ECD methods is dependent on soil type (Pik and Hodgson, 1976) and unacceptably high background responses and instabilities of baselines have been observed (Cotterill, 1978; Schütz et al., 1996). Another concern is that these methods use diazomethane for the derivatization reaction. The drawbacks for the use of diazomethane are that it is extremely toxic, carcinogenic, and can explode unaccountably both as a gas and as a liquid. Moreover, the methyl ester of 3,6-dichloropyridine-2-carboxylic acid is volatile and losses may occur during extract concentration. Cotterill (1978) reported a GC-ECD method using the 1-butyl ester of clopyralid, which involved the liquid-liquid partitioning using chloroform. We observed, using the Cotterill method, that the high organic matter contents of New Zealand soils gave very high background responses and the GC autosampler syringe needle tended to block due to humic matter extracted from soil matrices (coextractants). The lack of a final clean-up step makes this method unreliable. Therefore, there was a need to fill the gaps in available GC-ECD methods for determination of clopyralid in soils.

Correspondence to: R. Ahmad

This paper describes an improved method for determination of clopyralid in soil which involves extraction with methanolic alkali, derivatization with 1-butanol, partitioning into dichloromethane, a simple and fast clean-up technique using activated Silica gel, and separation and detection by GC-ECD. The technique has proved suitable for analyses of clopyralid in both cultivated and pasture soils with high organic matter contents.

MATERIALS AND METHODS

All solvents used were of purity compatible with pesticide residue analysis (Nanograde, Mallinckrodt Baker, Inc, Paris, Kentucky, USA). Water was distilled and membrane filtered with a Milli-Q-Plus apparatus (Millipore, Bedford, MA, USA). Inorganic chemicals such as $Ca(OH)_2$ were of analytical quality (BDH AnalaR®). Sodium sulfate obtained from Mallinckrodt was heated at 600°C for 6 h. Silica gel, (Davisil™ 60-100 mesh, 60°A, 99+ %) was obtained from Aldrich Chemical Company, USA. Butan-1-ol was 99.5% (BDH AnalaR®). Clopyralid (CAS number 1702-17-6) was obtained from Dr Ehrenstorfer GmbH, Germany and was of 98.5 % purity.

A primary standard solution (200 μg mL^{-1}) was prepared by dissolving clopyralid (5.08 mg) in ethyl acetate and the volume made up to 25 mL. All gas chromatographic standards were prepared by derivatizing this stock solution. A 5-mL aliquot of the stock was transferred into a 15 mL glass tube, evaporated with oxygen-free nitrogen and low heat (<30°C) to just dryness and derivatized as described below. The stock derivatized standard was made up to 50 mL with ethyl acetate. Portions of the butyl ester of clopyralid standard solution (equivalent to 20 μg mL^{-1}) were diluted with ethyl acetate to prepare GC calibration standards (0.02-1.0 μg mL^{-1}).

Surface soils (0 - 10 cm) from two locations were used for the fortification and recovery studies. Table 1 gives some details of their physico-chemical characteristics. Each soil was passed through a 3-mm sieve prior to fortification. An aqueous solution containing 1 μg clopyralid mL^{-1} was prepared from the ethyl acetate stock solution after evaporating the solvent. Aqueous solutions of the herbicide were used to spike the soil to achieve concentrations of 0.05, 0.3, 0.5 and 0.7 μg g^{-1} dry soil, mixed thoroughly, and then allowed to stand at 4°C for 24 h before extraction.

Table 1. Properties of the soils used.

Soil	pH	Clay	Silt	Sand	Water holding capacity	Organic C content
	(1:2 H_2O)	(g kg $soil^{-1}$)				
Dunmore	5.4	300	620	80	922	120
Horotiu	6.9	230	580	190	955	55

Ten grams soil (dry mass) was weighed into a 250 mL polypropylene bottle, mixed with ca 1 g of $Ca(OH)_2$ powder, and distilled deionized water (40 g total water content) was added. The bottle was capped and left to stand at ambient temperature overnight (~16 h). The mixture was shaken on an end to end shaker with 100 mL methanol for 1 h. The bottle then stood on the bench for 1h.

An aliquot (20 mL) of the supernatant was pipetted into a 100 mL separating funnel, acidified to a pH less than 2 using 5M sulphuric acid and extracted twice with 20 mL portions of dichloromethane:ethanol (19:1, v/v). The organic layers were combined and evaporated to dryness by rotary evaporation at <50°C. The rotary flask was rinsed with 2×2 mL dichloromethane:ethanol and the solutions quantitatively transferred into a 15 mL Kimax tube and evaporated to dryness under oxygen-free nitrogen (30°C).

Esterification of the extracted material was carried out following the method of McKone and Hance (1972). The extract in the Kimax tube was dissolved in 0.5 mL butan-1-ol followed by 3 drops of conc. H_2SO_4. Tubes were capped firmly and heated at 100°C for 30 min in a heating block. The contents were cooled and 10 mL of H_2O was added. A 4-mL volume of cyclohexane was added, shaken well and the tube centrifuged @ 1800 rpm for 5 min to separate the upper cyclohexane layer containing the clopyralid ester.

The butylated ester fraction was further cleaned up using Silica gel (Davisil). A column was prepared using a disposable glass pasteur pipette (length approx. 150 mm) containing a small amount of glass wool in the bottom and 0.4 ± 0.02 g activated Davisil (150°C for 48 h) topped with 0.5 ± 0.05 g anhydrous Na_2SO_4 (ashed at 600°C). The column bed was made uniform by tapping the column gently after each material was added. The column was conditioned with 4 mL cyclohexane which was discarded. The cyclohexane extract was transferred directly to the column with a pasteur pipette and the eluent discarded. Partitioning of the derivatization reaction mixture was repeated with a second 4-mL aliquot of cyclohexane and the cyclohexane layer was transferred to the column, the eluent again being discarded. The retained herbicide ester was eluted from the column with 4 mL of cyclohexane:ethyl acetate (9:1, v/v). The eluate was concentrated with a gentle stream of oxygen-free nitrogen, made to final volume 2 mL with cyclohexane:ethyl acetate (9:1), and transferred to a GC autosampler vial for analysis.

Clopyralid butyl ester was measured using a Varian 3500/3600 gas chromatograph fitted with a model 8200 autosampler and electron capture detector. The GC conditions employed were as follows:
Column: 25m×0.2mm id, Hewlett Packard Ultra2 (5% phenyl methyl siloxane), film thickness 0.33 μm; carrier gas: Helium; temperature, injector 220°C, detector 320°C and the column was temperature programmed from 80°C (1 min hold time) @ 40°C min^{-1} to 150°C (1 min hold time) to 170°C @ 3°C min^{-1} (0 min hold time) and @ 40°C min^{-1} to 265°C (15.22 min hold time); split ratio 30:1; pressure 41.0 psig; flow 1.6 mL min^{-1}. Injection of 1 μL volume was made using a Varian 8200 autosampler and the split-splitless injector was operated in the split mode.

The detector signal was processed using Millennium[32] Chromatography Manager (version 4.0).

RESULTS AND DISCUSSION

The improved method consists of 4 main steps: extraction, derivatization, clean-up and GC-ECD analysis. A methanol-water mixture was found to be suitable for extraction of clopyralid from the soils. We observed that if 20 g or more soil was used in the extraction, an emulsion formed during the dichloromethane partition step that prevented clopyralid partitioning to the organic phase. Therefore, 10 g soil was used for sample extraction. We also compared the efficiency of dichloromethane with chloroform in the partitioning step and found that both solvents had a similar recovery (average 92% and 96%, respectively). The proposed silica clean-up step is fast, effective and economical. The final eluate used for GC analysis was a clear solution.

Figure 1 illustrates chromatograms of Dunmore soil control samples before and after column clean-up and clearly demonstrates the efficiency of the column clean-up procedure. In chromatogram A, prior to clean-up, the region of clopyralid is masked by a large interfering peak. Chromatogram B was obtained with the same extract but after column clean-up as described. The background was greatly reduced after clean-up, and there were no peaks in the appropriate region that could cause interference. Therefore, the limit of detection was improved significantly.

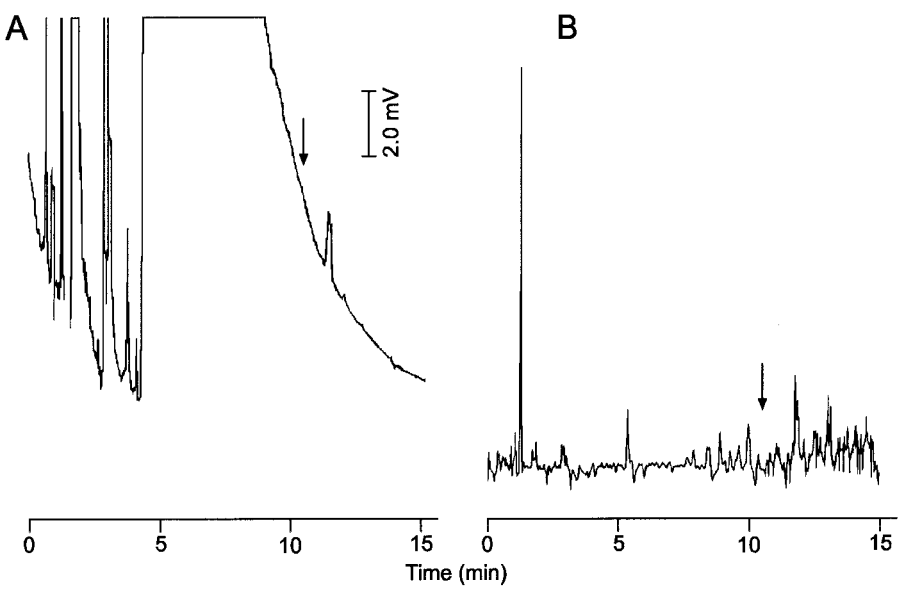

Figure 1. Typical GC chromatograms of Dunmore soil extracts. A, untreated sample without prior clean-up; B, same sample after clean-up. The retention time of the butyl ester of clopyralid is marked by arrows. Chromatographic conditions are as described in procedure.

Using the GC conditions listed above, clopyralid was well separated from any interferences. Its retention time, under the conditions described above, was 10.40 ± 0.02 minutes. A comparison of the retention times and the peak areas for the standards (0.1 - 1.0 μg mL^{-1}) at different times (1, 3, 7 and 14 days) showed no significant difference (data not presented). Calibration curves for the clopyralid showed good linearity within the concentration range studied (0.02 - 1.0 μg mL^{-1}) with R^2 of 0.99.

Representative chromatograms of fortified Dunmore and Horotiu soils (fortified level, 0.05 μg clopyralid g^{-1} dry soil) are shown in Figure 2. The chromatograms are free from matrix interferences in the regions of clopyralid elution.

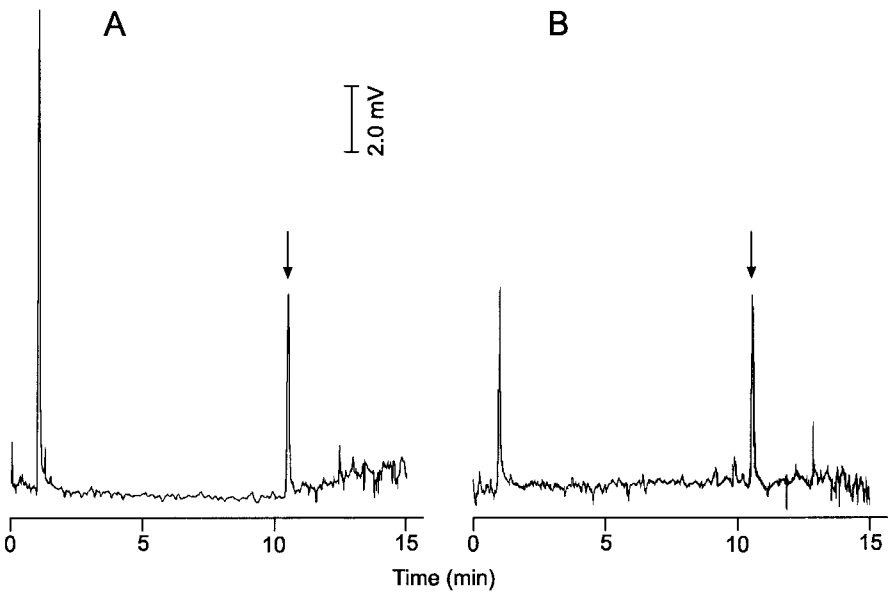

Figure 2. Typical GC chromatograms. A, Dunmore soil; B, Horotiu soil after fortification with clopyralid at 0.05 μg g^{-1}. The butyl ester peaks of clopyralid are marked by arrows. Chromatographic conditions are as described in procedure.

Without the Davisil clean-up, the final extract contained extracted natural soil constituents that provided significant interference in the baseline and clogged the autosampler syringe needle after a few injections. Therefore, a clean-up step in the procedure was developed to remove these interfering substances. Similar interference problems from coextractants from soil with high organic contents (10%) were encountered by Tan et al. (1996) in the GC-ECD measurement of clopyralid following extraction with $Ca(OH)_2$ and water. With the Davisil clean-up described above, the removal of this interference reduced noise level and provided significantly improved detection limits. It was also noted that overall the reproducibility improved when the clean-up was used and recoveries were higher using activated rather than unactivated Davisil.

The tests showed high recoveries (>84%) of clopyralid in both soils using 0.5 mL of butanol for the derivatization. With a lower volume (<0.5 mL) of butanol the recovery dropped significantly, most likely due to incomplete derivatization. Higher volumes (>0.5 mL) of butanol resulted in losses of clopyralid esters from the Davisil column during the application/rinse steps.

The recovery data for the soil samples spiked with clopyralid are presented in Table 2. Recoveries varied from 84 - 112 % over the spiking range of 0.05 - 0.7 μg g^{-1} dry soil with excellent reproducibility, even at the lowest spike level.

Table 2. Recovery of clopyralid from fortified soils (mean % ± SD).

Fortification (μg g^{-1} soil)	% recovery	
	Dunmore	Horotiu
0.05	98±12 (n=3)	N.T
0.3	96±8 (n=8)	96±4 (n=3)
0.5	90±9 (n=3)	N.T
0.7	91±1 (n=3)	89±4 (n=12)

N.T = not tested

The limit of detection for clopyralid was established to be 0.003 μg g^{-1} dry soil. This quantification limit reflects the fortification level at which an analyte peak was consistently generated at a level approximately 3 times the average background noise on the chromatogram at a retention time of the target compound. Different GC-ECD methods from the literature for clopyralid analysis in soils were compared for detection limit (Table 3).

Table 3. A comparison of detection limits for clopyralid in soil reported in published GC-ECD methods.

Detection limit (μg g^{-1})	Reference
0.06	Smith and Aubin (1989)
0.05	Galoux et al. (1985)
0.01	Tan et al. (1996)
0.005 - 0.01	Cotteril (1978); Pik & Hodgson (1976)
0.003	This study

Our laboratory studies of clopyralid confirmed the good reproducibility and accuracy of the method. Persistence data of clopyralid in Horotiu soil (0 - 10 cm) spiked at 0.7 μg g^{-1} soil incubated at different temperatures (water content at 60% of water holding capacity) are summarized in Table 4. The data showed good agreement between the results from the two replicates at each sampling interval. The reliable measurements of clopyralid in soil samples from our field studies of

clopyralid degradation under various management regimes have also proved the suitability of this method (Ahmad et al., 2003).

Table 4. Persistence (μg g^{-1} soil) of clopyralid in Horotiu soil at 60% of water holding capacity following incubation at 10°C, 20°C, and 30°C.

Day after application	10°C	20°C	30°C
0	0.59 (0.05)	0.63 (0.02)	0.65 (0.06)
3	0.56 (0.04)	0.52 (0.06)	0.39 (0.05)
7	0.52 (0.03)	0.38 (0.02)	0.16 (0.01)
14	0.48 (0.01)	0.30 (0.03)	0.03 (0.03)
21	0.45 (0.03)	0.14 (0.02)	0.02 (0.00)
28	0.44 (0.04)	0.06 (0.03)	0.01 (0.00)
42	0.36 (0.04)	0.01 (0.00)	ND
56	0.28 (0.01)	ND	
84	0.16 (0.07)		

ND= not detectable; Numbers in parentheses show standard deviations based on two replicate measurements.

We have demonstrated from this work that the method outlined here is more sensitive and gives lower limits of detection than the previous methods developed for clopyralid using GC and ECD detection. The introduction of a less toxic partitioning solvent, elimination of losses due to volatilisation of the methyl ester (by replacing diazomethane with 1-butanol in the esterification step) and a simple clean-up technique were advantageous to the analysis of soils containing high organic matter contents.

Acknowledgments. Our appreciation is extended to Mr Alastair Boyd for his technical support in GC analyses.

REFERENCES

Ahmad R, James TK, Rahman A, Holland PT (2003) Dissipation of the herbicide clopyralid in an allophanic soil: laboratory and field studies. J Environ Sci Health-Part B (submitted)

Cotterill EG (1978) Determination of 3,6-dichloropicolinic acid residues in soil by gas chromatography of 1-butyl ester. Bull Environ Contam Toxicol 19: 471-474

Cox L, Calderón MJ, Hermosín MC, Cornejo J (1999) Leaching of clopyralid and metamitron under conventional and reduced tillage systems. J Environ Qual 28: 605-610

Galoux MP, Bernes AC, Damme J.-C. Van (1985) Gas chromatographic determination of 3,6 dichloropicolinic acid residues in soils and its application to the residue dissipation in soil. J Agric Food Chem 33: 965-968

Haagsma T (1975) Dowco 290 herbicide - a coming new selective herbicide. Down to Earth 30: 1-2

Hathway DE (1989) Molecular Mechanisms of Herbicide Selectivity. Oxford University Press Oxford, UK

Lauren DR, Taylor HJ, Rahman A (1988) Analysis of the herbicide dicamba, clopyralid and bromacil in asparagus by high-performance liquid chromatography. J Chromatog 439: 470-475

McKone CE and Hance RJ (1972) Determination of residues of 2,4,5-trichlorophenoxyacetic acid in soil by gas chromatography of the n-butyl ester. J Chromatog 69: 204-206

Naish RW (1975) Dowco 290 - a new growth regulator herbicide. Proc 28th NZ Weed and Pest Control Conf Hastings, August 5-7, pp 177-180

O'Connor B (2002) Versatill. Novachem Manual 2002/2003. A New Zealand Guide to Agrichemicals for Plant Protection pp 418-419. Novachem Services Limited, New Zealand

Pik AJ, Hodgson GW (1976) Gas liquid chromatographic determination of 3,6-dichloropicolinic acid in soils. J Assoc Off Anal Chem 59: 264-268

Pik AJ, Peake E, Strosher MT, Hodgson GW (1977) Fate of 3,6-dichloropicolinic acid in soils. J Agric Food Chem 25: 1054-1061

Rutherford GR, Lobb PG (1979) The use of 3,6-dichloropicolinic acid mixtures for broadleaf weed control in cereal crops. Proc 32nd NZ Weed and Pest Control Conf Dunedin, August 7-9, pp 139-143

Schütz S, Weißbecker B, Hummel HE (1996) Gas chromatography-mass spectrometry analysis of the herbicide clopyralid in differentially cultivated soils. Environ Toxicol Chem 15: 244-252

Schütz S, Weißbecker B, Wittig C, Hummel HE (1994) Ultrasound enhanced derivatisation and GC-MS analysis of clopyralid. Med Fac Landbouww Univ Gent 59/3b: 1415-1422

Smith AE, Aubin AJ (1989) Persistence studies with the herbicide clopyralid in prairie soils at different temperatures. Bull Environ Contam Toxicol 42: 670-675

Tan LK, Humphries D, Yeung P, Florence LZ (1996) Determination of clopyralid, picloram, and silvex at low concentration in soils by calcium hydroxide-water extraction and gas chromatography measurement. J Agric Food Chem 44: 1135-1143