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Analysis of the herbicide clopyralid in cultivated soils

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Abstract

Clopyralid is a broad-spectrum herbicide for thistle control in vegetable cultivation. Its application rate amounts to 0.2 kg/ha. However, contradictory results in soil leaching experiments require a reliable routine procedure for trace analysis of clopyralid in soil. Previously reported methods for clopyralid analysis in several matrices either proved to be insufficient for analysis in soil and leaching water or require the use of critical chemicals and are too complicated for routine analysis. The problem of poor extraction recovery of clopyralid from soil rich in humic acids was solved by phase transfer extraction of soil samples. Analysis of the extract was performed by GC–MS directly after the extractive phase transfer catalytic reaction. Tetrabutylammoniumhydroxide served as a phase transfer catalyst. For improving analytical reliability, 2.5-dichlorobenzoic acid was used as an internal standard. Detection limit (1 μg/kg=1 ppb) and quantification limit (10 μg/kg) of clopyralid in soil were found to be appropriate for determining the behaviour in herbicide treated soils. The leaching and disappearance of clopyralid in two differently tilled soils were determined in field trials.

Keywords: Soil; Environmental analysis; Derivatisation, GC; Sample preparation; Pesticides; Clopyralid

1. Introduction

Clopyralid (3,6-dichloropicolinic acid) is a systemic residual herbicide widely used for thistle control in cultivation of vegetables, sugar beets and rape seed [1–3]. Its main characteristics are low toxicity and good plant compatibility. The polar herbicide is water soluble so that leaching seems to be possible under field conditions. First experiments using standardised soil column leaching studies confirmed the expected mobility of clopyralid [4]. Lysimeter studies and field experiments showed a fast disappearance of clopyralid as a result of microbiological degradation. So the herbicide was characterised as immobile [5,6]. These contradictory results prompted further studies on the behaviour of clopyralid in soils taking into consideration different tillage intensities.

Previously described analytical methods contain at least one extraction and a following derivatisation step. Both steps seem to be necessary to obtain a reliable analysis of the herbicide in soil samples. Extraction is often carried out under strongly alkaline [6,7] or strongly acidic conditions [8] resulting in recoveries in the range of 80%. So far derivatisation requires the use of hazardous/corrosive chemicals such as diazomethane [9] pentafluorobenzylbromide [6,10–12] or 1-butanol and concentrated sulphuric acid [7].

In this paper a one-step method for extracting and derivatising clopyralid within sieved real soil samples and the analysis as its butyl ester is described. With this method the behaviour of clopyralid in differently tilled soils is studied.

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2. Experimental

2.1. Chemicals

With the exception of water, all solvents used were of a purity compatible with organic trace analysis (Merck, Darmstadt, Germany, residue analysis grade). Water was ion-exchanged and membrane filtered with a Milli-Q-Plus apparatus (Millipore, Bedford, MA, USA). Inorganic chemicals such as Na_2SO_4 and buffer were of analytical quality (Merck, pro analysi). Tetrabutylammoniumhydroxide (TBAOH) was delivered as solution of 20% in water, standards of 2,5-dichlorobenzoic acid (DBA) (Merck) and clopyralid (Riedel-de Haën, Seelze, Germany) were of 99% purity. Standards were stored in siliconised flasks to prevent adsorption of the carboxylic acids to glass surfaces.

2.2. Instrumentation

Measurements were performed on a GC–MS system (Hewlett-Packard, Palo Alto, CA, USA) consisting of a MS engine HP 5989A and a gas chromatograph HP 5890 Series II. The HP 5989A is a quadrupole type mass spectrometer with a mass resolution of $M/\Delta M = 1000$ over a mass range from 10 to 1000 u. Experimental parameters and data acquisition were controlled by a HP-UX Chem-Station type HP 59940A running under the software HP 59944B version B.04.03. The mass spectrometer was connected to the gas chromatograph via direct coupling.

The gas chromatograph was equipped with a HP-1 column (cross-linked methyl silicone gum) with a length of 25 m, an I.D. of 0.2 mm and a film thickness of 0.5 μm. A cold injection system (Gerstel, Muelheim, Germany, KAS 2, abbreviated CIS) allows cooling of the injection port by a Peltier element.

2.3. Extraction and clean-up

Soil samples (orthic luvisol) from a long-term tillage experimental field site (Wernborn, Taunus, Germany) were used for the analysis. The average composition was 26.5% clay, 55.9% silt, 17.6% sand, 1.3% organic matter and pH ($CaCl_2$) of 4.8. The test field (planted with rape seed) was divided into eight parts. Two different tillage systems plough and direct seed ("no-tillage") were applied to four randomised parts each. Six of the plots were treated with Lontrel 100 (containing clopyralid with 100 g/l as the monoethanolamine salt) at a rate of 200 g active ingredient/ha. This amounts to a calculated clopyralid concentration in the upper 5 cm of 270 μg/kg (=270 ppb). The remaining two plots were taken as control plots with cultivation but without clopyralid.

The soil samples were collected with drill tubes and stored at −20°C in the dark. The obtained sample cores were divided into three layers (0–5 cm, 5–10 cm, 10–30 cm) and passed through a 25 mesh sieve. In order to get calibration of the method, soil from the control plots were spiked with 1, 5, 10, 50, 100, 200, 500 μg clopyralid/kg soil. The spiked soil was stored two days at 4°C in the dark prior to extraction. Three replicates were injected three times for each calibration level.

A 10 g soil sample was shaken on a shaking table at 200/min with a solution consisting of 7 ml 0.5 *M* NaH_2PO_4, 1 ml 0.5 *M* Na_2HPO_4, 1.5 ml TBAOH (20% in water) and 5 ml dichloromethane containing 1 μg DBA (internal standard of 1 μg DBA/10 g soil=100 μg DBA/kg soil) for 2 h. The phase transfer catalyst supports the transfer of the extracted clopyralid from the aqueous into the organic phase and enhances the desorption of clopyralid from clay–humic complexes. Moreover, TBAOH acts as a derivatisation reagent that provides the butyl group in the formation reaction of the clopyralid butyl ester (Fig. 1). The sodium phosphate buffer (pH=8) provides optimal conditions for this reactive extraction. After centrifugation for 20 min (2500 *g*) the dichloromethane phase was separated, dried 20 min over 0.2 g Na_2SO_4 and filtered through an analytical filter into siliconised autosampler vials. Siliconisation of the vials improved the reliability of analytical results. Extreme care was taken to avoid any residual detergents on glass surfaces which are in contact with the derivatisation reagents.

2.4. Sample injection

All measurements were carried out using a 1 μl volume of the sample solution. Samples were manu-

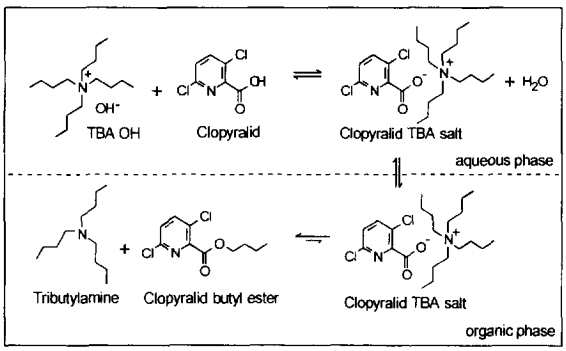

Fig. 1. Reaction scheme of clopyralid butyl ester formation during extractive derivatisation.

ally injected into the CIS using a 5 μl syringe (Hamilton). The microliter-syringe was stored in a refrigerator at 4°C in order to prevent the development of air bubbles when pulling up the sample. This precaution was found to be necessary because of the low boiling point of dichloromethane (40°C). The syringe was thoroughly rinsed with the sample-solution before each injection. Each gas chromatography (GC) run was followed by rinsing the CIS with dichloromethane, in order to minimise memory effects.

2.5. GC parameters

Helium was used as carrier gas with a column head pressure of 1 bar. The GC and CIS were operated with the temperature program (CIS: Initial temperature: 40°C, initial hold: 0 sec, heating rate 1: 5 C°/s to 50°C and 60 s hold, heating rate 2: 9 C°/s to 225°C, 60 s hold and subsequent cooling down to initial temperature), (GC: initial temperature: 100°C, initial hold: 2 min, heating rate 1: 60 C°/min to 160°C, 0 min hold, heating rate 2: 2 C°/min to 196°C, 0 min hold, heating rate 3: 20 C°/min to 270°C, 0 min hold and subsequent cooling down to the initial temperature).

2.6. MS parameters

Qualitative characterisation of the clopyralid butyl ester and the DBA butyl ester was performed operating the ion source of the mass spectrometer in the positive chemical ionisation mode (PCI). Methane with a pressure of 0.8 Torr (1 Torr= 133.322 Pa) was used, resulting in an ion source pressure of $4.8 \cdot 10^{-6}$ Torr. For quantitative analysis the ion source of the mass spectrometer was operated in the electron-impact ionization (EI) mode with the following parameter: Electron energy=70 eV, emission current=300 μA, repeller voltage=7 V. The MS was operated in the selected-ion monitoring (SIM) mode. The dwell time for each ion was adjusted to 300 ms resulting in an overall cycle time slightly below 1 s. SIM measurements were carried out with a selection of three masses (147/173/174, mass accuracy: ±0.01 u) for clopyralid with 2,5-dichlorobenzoic acid as internal standard, because relative abundances of these ions were not sensitive to fluctuations in ionisation conditions and, therefore, yielded the best linearity in calibration curves. The simultaneous appearance of the three ions indicates the 2,5-dichlorobenzoic acid butylester used as internal standard whereas the ions with mass 147 and 174 show the clopyralidbutylester (Figs. 2 and 3). To ensure the reproducibility of the obtained mass spectra the MS parameter were tuned for stability at least once a day. A drift in the mass axis calibration, peak width, or ion abundance could be recognised by calibration with the test compound perfluorotributylamine (PFTBA) whose characteristic fragments appear over a mass range from 31 to 614 u. Small deviations from the optimum calibration were compensated by a manual tune of the MS parameter. The "AutoTune"-option of the mass spectrometer was

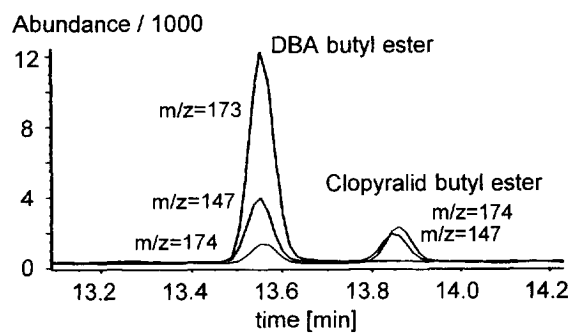

Fig. 2. Zoomed section of the ion chromatogram of a soil extract with an original clopyralid concentration of 100 μg/kg spiked with 200 μg/kg DBA as internal standard.

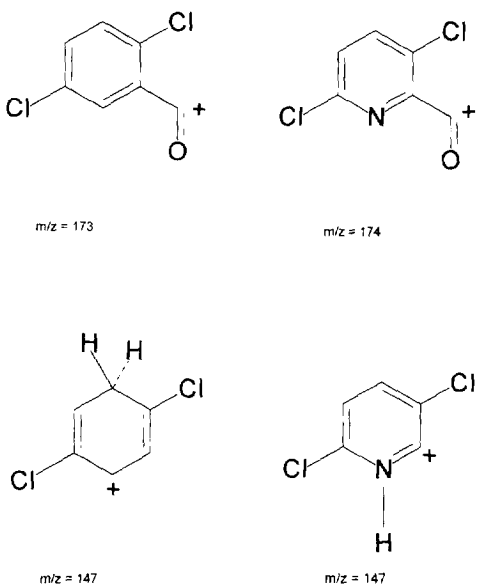

Fig. 3. Tentative structures of the ions employed for quantitative analysis.

used whenever the result of the manual tuning was unsatisfactory. MS temperatures were held constant during all measurements: source=250°C, quadrupole=100°C, GC–MS interface=250°C. Quantification calibration was performed with clopyralid and DBA standard solutions in phosphate buffer (1, 5, 10, 50, 100, 250, 500 μg/l each) derivatised and analysed according to the described procedure.

3. Results and discussion

In order to get calibration, the method of extractive derivatisation described above was applied to soil samples from the control plots spiked with 1, 5, 10, 50, 100, 200, 500 μg clopyralid/kg soil. Recovery rates at concentrations between 50 and 500 μg clopyralid/kg soil were determined (Table 1). Application of phase transfer catalysis enhances the desorption of clopyralid from the clay–humic acid complexes in soil and makes a simultaneous derivatisation possible without compromising reliability and recovery of clopyralid extraction significantly.

The combined extraction-derivatisation step produces the clopyralid butyl ester. This compound elutes from the column after (13.85±0.08)min. The derivative was characterised by MS in the SCAN mode using PCI (Fig. 4), because EI did not yield a molecular ion peak of sufficient intensity. The main by-product in this reaction is tributylamine eluting from the column with a retention time of 6 min. It does not interfere with the quantitative analysis of clopyralid (SIM mode). The validity of a calibration depends on the stability of the experimental parameters. Deviations in the efficiency of the apparatus will lead to errors in quantitative analysis. Therefore, an internal standard was used to compensate for day-to-day fluctuations in MS performance or in possible sampling errors. The 2,5-dichlorobenzoic acid butyl ester formed is eluted from the column after (13.55±0.07)min.

With each of the derivatised samples three GC–

Table 1
Comparison of methods for clopyralid analysis in soil

	Conventional alkaline[a]	Conventional acidic[b]	Ultrasound enhanced[c]	Phase–transfer
Concentration range	50–500 μg/kg	100–500 μg/kg	50–500 μg/kg	50–500 μg/kg
Different concentrations	4	3	4	4
Replicates	3	3	4	3
Recovery (mean) in %	70	80	78	75
Recovery (S.D.) in %	10	8	5	5

[a] Conventional alkaline extraction [6,7,9,13]: 20 g soil shaken (200/min) with 100 ml 0.1 *M* NaOH for 2 h.
[b] Conventional acidic extraction [8]: 20 g soil shaken (200/min) with 100 ml 0.1 *M* HCl in acetone for 2 h.
[c] Ultrasound enhanced extraction [11]: 10 g soil shaken (200/min) with 35 ml 0.1 *M* NaOH for 20 min, ultrasonication for 10 min, repeated once.

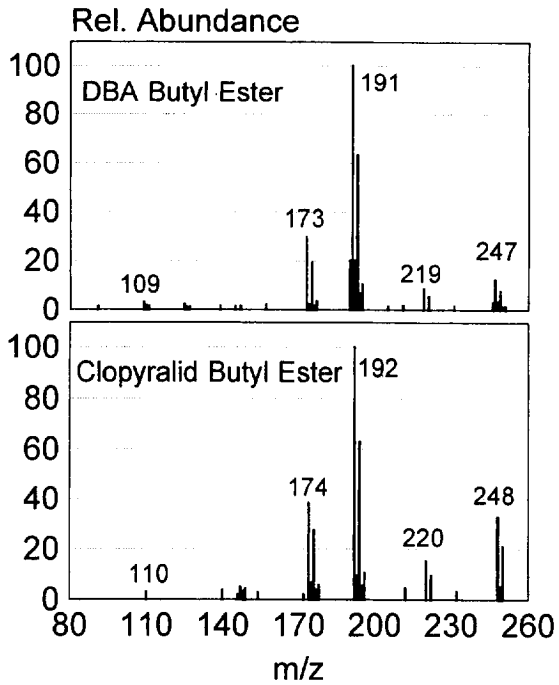

Fig. 4. Positive chemical ionisation mass spectra of clopyralid butyl ester and 2,5-dichlorobenzoic acid butyl ester.

MS measurements were performed. The regression line fitted to the data resulting from spiked soils at concentrations between 10 and 500 μg/kg with three replicates each, written in the form c(clopyralid)= $a\times$peak area(clopyralid)−constant:

Clopyralid in soil [μg/kg]

= ((436.3 μg/kg)/Peak area(DBA − standard)) × Peak area(clopyralid)

− (2 221 000/Peak area(DBA − standard)) μg/kg

has a R^2 value of 0.995 in accordance with the good linearity of the calibration.

The peak area of the internal standard DBA influences slope and constant value. Therefore, this equation is valid when the area of the DBA peak (internal standard, 100 μg/l) amounts to 70 000±7000 and the peak area quotient (Peak area DBA(100 μg/kg)/peak area clopyralid(100 μg/kg)) is 3.33±0.3. This can be checked by measurement of 100 μg/kg standard samples of clopyralid and DBA. If this check yields parameters outside the specification, the equation will have to be modified respectively.

Lastly, four different methods for clopyralid analysis in soil were compared for quantification limit, linear range, sensitivity, reliability and capability for automation (Table 2). Three of these methods, namely HPLC–UV [13,14], GC–electron-capture detection (ECD) [7,15] and GC–MS [6,11] were taken from the literature and reproduced. Limit of detection was taken as analyte concentration producing three times higher peaks than background noise, limit of quantification was taken as analyte concentration producing peak areas with standard deviations less than 10% of the absolute value. The R^2 values of the calibration curves fitted to three replicates of spiked soil samples containing 5 different concentrations of clopyralid within the designated concentration range were used as estimate of reliability ($R^2>0.99$: high, $0.99>R^2>0.90$: intermediate, $R^2<0.90$: low). Our results show that the GC–MS methods are superior in sensitivity and reliability when DBA internal standard is used. Whereas published HPLC–UV methods [13,14,16,17] suffer from a general lack of sensitivity, the GC–ECD methods [6,7,15,18] fall short in reliability because of instabilities of baselines due to numerous unidentified by-products formed in the derivatisation step. Another published GC–MS method employing clopyralid methyl ester [9] has not been considered because of the toxic, carcinogenic and explosive properties of diazomethane needed for the derivatisation reaction. Our previous GC–MS method based on the derivatisation of clopyralid by pentafluorobenzyl (PFB) ester formation [11] is more sensitive than the extractive derivatisation described here. However, the extractive derivatisation is easy to perform and capable of automation, whereas the previous method based on PFB ester formation contained an ultrasound catalysed derivatisation step which needs some experimental skill to guarantee reliable results and is difficult to automate. Moreover, handling of pentafluorobenzylbromide which is corrosive and irritant, is not necessary any more.

The successful measurements of real soil samples from field trials in Wernborn prove the suitability of

Table 2
Comparison of methods for clopyralid analysis in soil

	HPLC–UV[a]	GC–ECD[b]	GC–MS[c]	GC–MS[d]	GC–MS
Limit of detection	400 μg/kg	20 μg/kg	2 μg/kg	1 μg/kg	1 μg/kg
Limit of quantification	1000 μg/kg	50 μg/kg	20 μg/kg	10 μg/kg	10 μg/kg
Linear range	1–100 mg/kg	50–250 μg/kg	20–1000 μg/kg	10–1000 μg/kg	10–500 μg/kg
Reliability	High	Low	Intermediate	High	High
Time/sample	4h	4.5h	4.5h	4.5h	3h
(no parallel work)	Auto[e] ++	Auto +	Auto +	Auto −	Auto ++
Working steps	6	11	11	11	5

[a] HPLC–UV [13,14]: RP8, 250×4 mm, 1% acetic acid–methanol (70:30), 1 ml/min, UV diode array detector (280 nm).
[b] GC–ECD [7,15]: OV-17, 25 m×0.32 mm, 1 μm, on column injection, 140°C, 2 min, 20°C/min, 160°C, 2°C/min, 210°C, 20°C/min, 270°C.
[c] GC–MS [6]: HP-1, 25 m×0.20 mm, 0.5 μm, cold injection system, 100°C, 2 min, 60°C/min, 160°C, 2°C/min, 210°C, 20°C/min, 270°C.
[d] GC–MS [11]: HP-1, 25 m×0.20 mm, 0.5 μm, cold injection system, 100°C, 2 min, 60°C/min, 160°C, 2°C/min, 210°C, 20°C/min, 270°C.
[e] Capability for automation, estimates based on number of unit operations/extraordinary operations.

the method (Fig. 5). Clopyralid leaching from ploughed soil is more pronounced than from no-tilled soil, presumably because of the higher organic compound content in the soil top layer of no-tilled soil. In laboratory trials those soils showed higher immobilisation capability for clopyralid than soils with lower content of organic compounds [19]. Moreover, the half-dissipation times of total clopyralid were increasing with decreasing cultivation intensity. This is consistent with findings in a comparative study of dissipation behaviour of clopyralid in soils from set aside fields and ploughed fields [11]. More detailed interpretation of the dissipation data requires further information regarding soil structure, microbiological activity in soil and climate conditions. The detailed discussion of field data will be published elsewhere.

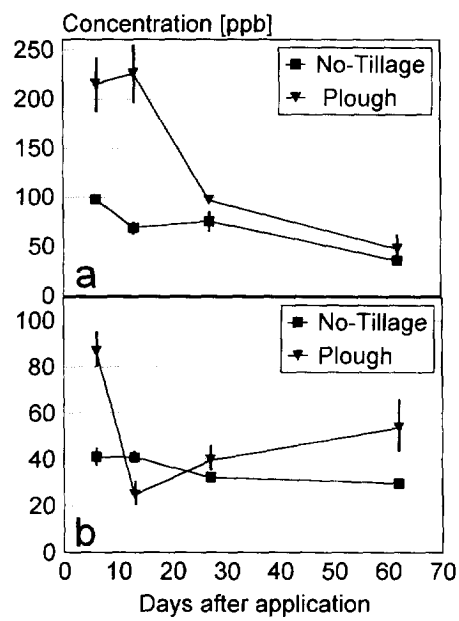

Fig. 5. Translocation of clopyralid under different tillage conditions (plough and no-tillage). (a) Soil layer 0–5 cm, (b) soil layer 5–10 cm.

4. Conclusions

The GC–MS method presented here is suitable for analysis of clopyralid in soil and leaching water with detection limits of about 1 μg/kg (=1 ppb, ≈2.7 nanomolar concentration) and quantification limits of about 10 μg/kg. Application of phase transfer catalytic extractive derivatisation provides an easy and quick method performing routine analysis of clopyralid in soil.

Soil samples from field trials were analysed. It could be shown that leaching of clopyralid from ploughed soil is more pronounced than from no-tilled soil.

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