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# Liquid chromatographic method for the micro-quantitative determination of clodinafop in soil, wheat and *Phalaris minor*

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**Abstract**

A liquid chromatographic method was developed for the determination of clodinafop-propargyl herbicide. Clodinafop-propargyl was converted to clodinafop acid by alkaline hydrolysis as clodinafop-propargyl rapidly forms bioactive clodinafop acid in soil and plant environment. Recovery methods for both the acid and ester from different matrices were standardized. The sensitivity of the method for ester and acid was 5 and 2 ng, respectively, with limits of detection of 0.5 and 0.1 $\mu g\,ml^{-1}$. The method was standardized for the determination of clodinafop residues in soil and plant samples using HPLC. The recovery of clodinafop from soil and plant samples with ethyl acetate was significantly higher (78–83%) than those with dichloromethane, toluene and methanol (60–70%). The limit of determination of clodinafop in soil and plant samples ranged between 1 and 1.2 $ng\,g^{-1}$. In field soil, residues of clodinafop dissipated with a half-life of 3.44 days.


*Keywords:* Clodinafop; Recovery; Wheat; *Phalaris minor*; Residue; Soil

## 1. Introduction

Clodinafop-propargyl [prop-2-ynyl (*R*)-2-[(5-chloro-3-fluoro-2-pyridyloxy) phenoxy] propionate] (**1**; Fig. 1) is a recently introduced 'fop' group herbicide which effectively controls isoproturon resistant little seed canary grass biotypes (*Phalaris minor* Retz.) along with other broad leaved weeds of wheat (*Triticum aestivum*) [1–5]. This herbicide is used in combination with a safener, cloquintocet-mexyl, but has antagonistic effect with auxin type herbicides [6]. Previously, [$^{14}$C]-quantification procedure was followed by different workers for residue estimation of some other 'fop' members from different matrices [7]. Fluazifop was analysed by HPLC method for studying the persistence of this herbicide in soil [8]. There is, however, no information available on the methods for the detection of this herbicide. This paper presents a high-performance liquid chromatographic (HPLC) technique for the micro-quantitative determination of clodinafop-propargyl. The parent ester rapidly converts to clodinafop acid (Fig. 1) in the soil and plant environment, which is also responsible for the herbicidal activity. So, along with ester, analysis of clodinafop acid was also standardized.

The method is simple, sensitive and can be used conveniently for the detection of the herbicide at microgram levels. The technique is further extended and standardized for the determination of the residues of this herbicide in soil and crop (from plant material as well as harvested produce i.e. wheat grain and straw) material, so that it can be used for persistence and metabolism studies in the agro-ecosystem.

## 2. Experimental

### 2.1. Reagents and chemicals

Clodinafop-propargyl (97.8%, analytical) obtained from M/s Novartis India Limited was crystallized from *n*-hexane before use. Solvents like acetone, ethyl acetate, methanol, dichloromethane, toluene were analytical grade and distilled before use. Alumina, florisil and charcoal used for clean-up

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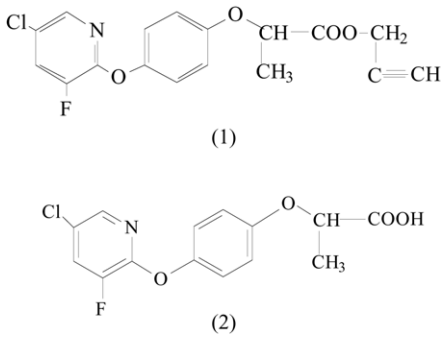


Fig. 1. Chemical structures of clodinafop-propargyl (**1**) and clodinafop acid (**2**).

were of AR grade. Anhydrous sodium sulphate was used as a drying agent for different samples. HPLC grade methanol and water used for HPLC were filtered through 0.02 mμ membrane before use.

### 2.2. Instruments

#### 2.2.1. Fourier transform-infrared spectroscopy (FT-IR)

The infrared spectra were recorded in a Nicolett Fourier transform-infrared spectrometer (model Impact 400) using chloroform and potassium bromide disc.

#### 2.2.2. Nuclear magnetic resonance spectroscopy (NMR)

$^1$H NMR spectra were recorded on Varian EM 360L (60 MHz) instrument. Deutero-chloroform ($CDCl_3$) was used as a solvent with tetramethyl silane (TMS) as internal standard. The chemical shifts are expressed in $\delta$-value (mg g$^{-1}$) and coupling constants (*J*-values) in Hz.

#### 2.2.3. High performance liquid chromatography (HPLC)

A reverse phase high-performance liquid chromatographic technique was used for quantitative analysis. A Hewlett–Packard HPLC instrument (series 1100) equipped with degasser, quarternary pump, photodiode-array detector connected with rheodyne injection system and a computer (model Vectra) was used for analysis. The stationary phase consisted of Lichrospher on RP-8 packed stainless steel column (250 mm × 4 mm i.d). Chromatogram was recorded in a Window'95 NT based HP Chemstation programme. Methanol:water (4:1) was used as mobile phase with a flow rate of 1 ml min$^{-1}$. All the samples were filtered through 0.02 mμ membrane (Millipore) using a filtration syringe system.

### 2.3. Alkaline hydrolysis of clodinafop-propargyl: formation of clodinafop acid (2)

Clodinafop-propargyl (500 mg) was taken in a round bottom flask and 20 ml of methanolic KOH (1%) was added to it. The contents were stirred on a magnetic stirrer at room temperature. The progress of the reaction was monitored by checking TLC periodically. After 3 h, the reaction mixture was diluted with distilled water, pH was adjusted to neutral by addition of dilute (1N) HCl. The contents were transferred to a separatory funnel and extracted thrice with ethyl acetate (3 × 50 ml). The ethyl acetate layer was dried (anhydrous $Na_2SO_4$) and the solvent evaporated on a rotary vacuum evaporator. The solid thus obtained was chromatographed on a column of silica gel and eluted with *n*-hexane, *n*-hexane:benzene (9:1), *n*-hexane:benzene (1:1), acetone:benzene (5:95) in succession. TLC was checked after concentrating each fraction of elutes. The acetone:benzene (5:95) fraction on concentration gave a white solid, which was recrystallised from benzene to give the compound **2** (310 mg; yield 70%). Compound **2** (Fig. 1) melted at 212 °C. The relative retention factor on TLC in benzene and acetone:benzene (1:4) were 0.257 and 0.261, respectively, (Table 1). The compound gave a single sharp peak in HPLC at $\lambda_{max}$ 240 nm.

$$\text{IR } \nu^{\text{KBr}}_{\text{max}}: \ 3175 \text{ (O—H str.)}; 1729 \text{ (>C=O str.)}$$

$^1$H NMR ($CDCl_3$) $\delta$: 1.64 (d, $J$ = 7 Hz, 3H, $CH_3$); 4.55–4.95 (q, $J$ = 7 Hz, 1H, (O C<u>H</u>($CH_3$)COOH) 6.82 and 7.18 (each d, $J$ = 10.5 Hz, 4H, 2′,3′,5′,6′-Ar-H); 7.32–7.65 (dd, $J$ = 10.5 Hz and 2.5 Hz, 1H, H-4); 7.85 (d, $J$ = 2.5 Hz, 1H, H-6); 8.50 (bs, exchangeable with $D_2O$, 1H, –COOH).

### 2.4. Preparation of standards

Clodinafop-propargyl (**1**) (10 mg) was taken in a 10 ml volumetric flask, dissolved in methanol and the volume was made upto the mark to obtain a stock solution containing 1000 μg ml$^{-1}$. From this stock solution, 20, 10, 5, 2.5, 1.0, 0.5, 0.1 μg ml$^{-1}$ solutions of clodinafop-propargyl were prepared by serial dilution. In a similar manner, standards of clodinafop acid (**2**) were also prepared of 20, 10, 5, 2.5, 1.0, 0.5, 0.1 μg ml$^{-1}$ concentration in methanol.

### 2.5. HPLC analysis

Aliquot (20 μl) of each solution containing 20, 10, 5, 2.5, 1.0, 0.5, 0.1 μg ml$^{-1}$ clodinafop-propargyl was injected into

Table 1
Retention factor (TLC), retention time and sensitivity of clodinafop ester and acid in HPLC

| Compound | Retention factor (TLC) | | Retention time (HPLC) (min) | HPLC sensitivity (ng) |
|---|---|---|---|---|
| | Benzene | Acetone:benzene (1:4) | | |
| Clodinafop ester (**1**) | 0.469 | 0.484 | 3.91 | 5 |
| Clodinafop acid (**2**) | 0.257 | 0.261 | 1.31 | 2 |

HPLC at wavelength of 240 nm which was detected for absorption maxima using photodiode array. Each run was repeated thrice and the detector response was measured in terms of peak areas. Calibration curve was prepared by plotting concentrations of clodinafop-propargyl in μg on *x*-axis against average peak area on *y*-axis.

In a similar procedure, standards of clodinafop acid were also analysed and HPLC response was measured through peak area at a wavelength of 240 nm.

### 2.6. Extraction of clodinafop (ester and acid) from different matrices

#### 2.6.1. Extraction from soil

Extraction of clodinafop ester and acid were standardized separately. Clodinafop ester was standardized by two different methods. First, ester quantification and second, by acid quantification method. The IARI soil was a sandy loam with a composition of 17.5% clay, 18.7% silt, 63.8% sand and 0.26% organic carbon and had a pH of 8.2.

*2.6.1.1. Recovery of clodinafop-propargyl (ester).*

*Ester quantification method.* Sieved and air-dried control soil (50 g) was taken in each set of four Erlenmeyer flasks. Soil samples in 3 flasks were fortified at the required level (different fortification levels used were 0.5, 1.0 and 5.0 $\mu g\,g^{-1}$) with the standard solution of clodinafop-propargyl and mixed thoroughly. One of the flasks was not fortified and kept as control. Solvent (methanol) of equal amount was added to the control flask. The samples were brought to the field capacity by the addition of the distilled water. After 1 h, 3–4 drops of ammonia were added to each flask and stirred with glass rod. The flasks were kept undisturbed until the smell of ammonia disappeared. 100 ml of solvent (different solvents tried were methanol, dichloromethane, toluene, ethyl acetate) was added to each of the flasks and shaken on a horizontal shaker for 30 min. The contents of the flasks were allowed to settle and the supernatant phase was filtered through Buchner funnel using water pump. The extraction was done twice more with the same solvent (50 ml in each time) and filtered in the same way. The combined filtrate was then concentrated by evaporating the solvent on a rotary vacuum evaporator at 35–40 °C to dryness.

*Acid quantification method.* After extracting the ester with ethyl acetate as in the previous case, the solvent was evaporated to dryness on a rotary evaporator. The residue was dissolved in 50 ml of 0.1N KOH (aqueous) and the content of the flasks were heated at 60 °C on a water bath for half an hour. After cooling, the mixture was neutralized (pH 7) by addition of dilute (1N) HCl. The neutralized mixture was diluted with water (100 ml) and transferred to a 250 ml separatory funnel. The aqueous solution was partitioned with ethyl acetate (3 × 50 ml). The organic layer was dried (anhydrous $Na_2SO_4$) and the solvent evaporated to dryness on rotary evaporator.

*2.6.1.2. Recovery of clodinafop acid.* The control soil samples were fortified by clodinafop acid (as prepared by Section 2.3) at 0.5, 1.00 and 5.00 $\mu g\,g^{-1}$ and as mentioned in the procedure 2.6.1.1, soils were extracted by ethyl acetate, dichloromethane and toluene separately. After filtration, filtrate was concentrated by evaporating the solvent on a rotary vacuum evaporator to dryness.

#### 2.6.2. Extraction from wheat straw and grain

The chopped control straw (20 g) was taken in each of four beakers. The straw of the three beakers was fortified separately at the required level (different fortification levels used were 0.5 and 1.0 $\mu g\,g^{-1}$) with the standard solution of clodinafop ester and acid separately and mixed thoroughly. One of the beakers was not fortified and kept as control. After one hour, the contents of the beaker were transferred to a filter paper thimble and extracted with 250 ml of solvent (solvents tried were acetone, ethyl acetate separately) using Soxhlet apparatus for 4 h. The contents of the round bottom flask were concentrated to dryness on a rotary vacuum evaporator.

For the recovery experiment from the grain sample, powdered control grain (50 g) was taken, fortified (at 0.5 and 1.0 $\mu g\,g^{-1}$ level) and extracted using Soxhlet apparatus as in the procedure mentioned for the straw sample. Ethyl acetate and acetone (250 ml each separately) were tried as the extracting solvent for the grain samples. Control grain was also processed in the same manner.

#### 2.6.3. Extraction from plant samples (wheat and P. minor)

For plant samples also the technique was standardized for clodinafop ester and acid separately.

*2.6.3.1. Recovery of clodinafop ester from plant samples.* Washed wheat plant sample was cut into small pieces. The chopped plant sample (10 g) was taken in beakers. After that, contents of the three beakers were fortified at the1.0 and 5.00 $\mu g\,g^{-1}$ level with the standard solution of clodinafop ester and mixed thoroughly. One of the beakers was not fortified and kept as control. After 1 h, the plant material was crushed in a pastel and mortar with acetone (20 ml). The contents were decanted and filtered through filter paper. The extraction was repeated twice with 10 ml acetone each time. Combined acetone extract was evaporated and the residue was kept for further cleanup. Similar procedure of extraction was also adopted for *P. minor* plant samples.

*2.6.3.2. Recovery of clodinafop acid from plant samples.* Different matrices (wheat and *P. minor*) were fortified at 1.0 and 5.00 $\mu g\,g^{-1}$ level by the standard solution of clodinafop acid and extracted as mentioned in the procedure for clodinafop ester.

### 2.7. Clean-up

The soil, wheat grain and straw sample extracts were dissolved in methanol and filtered for HPLC analysis and no further cleanup was required. But, for the plant samples of wheat and *P. minor*, three different methods of cleanup were used.

#### 2.7.1. Using charcoal

The residue was dissolved in *n*-hexane:acetone (9:1) and a pinch of activated charcoal (0.025 g) was added to the extract. The mixture was shaken for 2 min and filtered through a Whatmann no. 1 filter paper to get the clear solution.

#### 2.7.2. Using neutral alumina

In a glass column neutral alumina (4 g) was packed sandwiched between anhydrous $Na_2SO_4$ (2 g) on both the sides. The concentrated plant extract (in acetone) was added at the top after pre washing of column with *n*-hexane. It was then eluted with *n*-hexane, *n*-hexane:benzene (1:1) and *n*-hexane:acetone (9:1). For ester quantification *n*-hexane:benzene (1:1) fraction was collected. For acid quantification, *n*-hexane:acetone (9:1) fraction was collected.

#### 2.7.3. Using florisil

In a glass column, florisil (4 g) was packed sandwiched between anhydrous sodium sulphate (2 g) on both sides. The concentrated plant extract (in acetone) was added at the top. It was then eluted with *n*-hexane, *n*-hexane:benzene (1:1) and *n*-hexane: acetone (9:1). The *n*-hexane: acetone (9:1) fraction was collected.

After the extraction and cleanup, (for each case) the ultimate fraction was concentrated to dryness on a rotary evaporator (at 35–40 °C) and the residue was dissolved in 5 ml methanol and transferred to a test tube for HPLC analysis. A 20-μl volume of this concentrated extract was injected for HPLC analysis after filtering through sample filtration membrane. This was preceded by the injection of standard solutions of both clodinafop ester and acid of known concentration under standardized conditions of HPLC. The retention time ($R_t$) and peak area of the standard solution and the samples were recorded. The quantity of the clodinafop ester or the clodinafop acid present in the extract was calculated using following equation:

$$Y = \frac{(\alpha \times C \times V)}{\beta \times W}$$

where $Y$ is the concentration of herbicide residue in sample ($\mu g\, g^{-1}$); $\alpha$ the peak area of sample aliquot; $\beta$ the peak area of standard solution; $C$ the concentration of the standard solution ($\mu g\, g^{-1}$); $V$ the volume of the sample extract (ml); $W$ the weight of the sample (g).

### 2.8. Collection of field sample and processing

A piece of barren land was sprayed with Topik formulation (Clodinafop-propargyl 15WP) at 60 g a.i. $ha^{-1}$. Soil samples were drawn randomly from 0 to 15 cm depth using a tube auger from 6 to 7 spots in triplicate. Around 500 g soils were collected from each plot. The samples were drawn on 0 (4 h), 1, 3, 5, 10, 15, 20 days after treatment (DAT) from the treated and control plots. Samples were mixed thoroughly, air-dried, grounded and passed through 2 mm sieve. Representative sample (50 g) was taken by quartering for the final analysis.

## 3. Results and discussion

IR spectrum of the compound **2** (hydrolyzed product of **1**) showed absorption at 3175 and 1279 $cm^{-1}$, a typical stretching for –OH and C=O respectively, indicating the presence of a free –COOH group. More over disappearance of absorption for C≡CH confirmed the hydrolysis of propargyl ester to carboxylic acid.

Proton NMR spectrum of compound **2** showed a doublet ($\delta$ 1.64) and a quartet ($\delta$ 4.55–4.95) with coupling constant $J = 7$ Hz typical of –OCH–$CH_3$ group. Six aromatic protons resonated at $\delta$ 6.82–7.85 as in parent ester, but, signals for alkynic proton (≡C<u>H</u>) and –OC<u>$H_2$</u>– group were absent. This clearly showed the absence of propargyl group and confirming the hydrolysis of ester to acid. In addition, a downfield peak at $\delta$ 8.50 as a broad singlet, which was exchangeable with $D_2O$, confirmed the presence of –COOH group. On the basis of the above spectral features, the compound **2** was characterized as (*R*)-2-[(5-chloro-3-fluoro-2-pyridyloxy) phenoxy] propionic acid {clodinafop acid} (**2**; Fig. 1).

Clodinafop-propargyl and clodinafop showed a sharp single peak in HPLC (Fig. 2), though the title molecule contains a stereogenic center. The standard curve obtained by HPLC analysis was linear from 0.1 to 20 $\mu g\, ml^{-1}$. The regression equations best fitted for HPLC standard curves for clodinafop-propargyl and clodinafop were $Y = 223.68X$ and $Y = 982.5X$, respectively. The limit of detection of clodinafop ester and acid was 0.5 and 0.1 $\mu g\, ml^{-1}$ with sensitivity of 5 and 2 ng, respectively (Table 1).

After optimizing the HPLC conditions for both clodinafop ester and acid, the method was standardized for the determination of clodinafop residues in soil. Recovery of clodinafop ester (by ester quantification method for the quantification of the ester) using methanol, dichloromethane and toluene ranged from 60 to 70%. Extraction by ethyl acetate gave 81.3, 77.2 and 83% recovery at 0.5, 1.00 and 5.00 $\mu g\, g^{-1}$ fortification level. So ethyl acetate was selected as the best solvent for the quantitative recovery of clodinafop ester from soil. The method showed no interfering peak from soil matrix (Fig. 2). Ester quantification method is useful for determining the residues of ester and to quantify the amount of unhydrol-

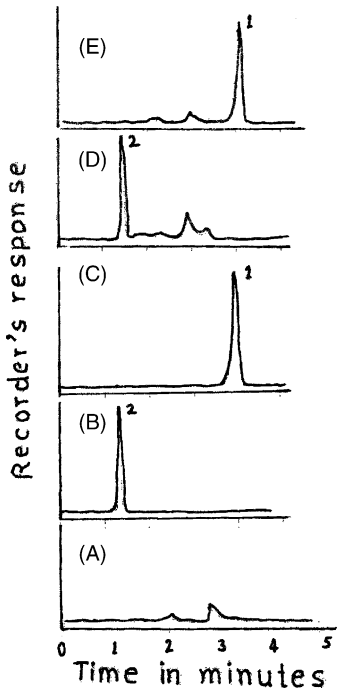


Fig. 2. HPLC chromatogram of: (A) control soil; (B) standard clodinafop acid (**2**); (C) standard clodinafop-propargyl (**1**); (D) soil fortified with clodinafop acid (**2**); (E) soil fortified with clodinafop-propargyl (**1**).

ysed ester present in soil especially for zero day soil samples as after that ester generally converts to acid.

In acid quantification method, after fortification with clodinafop-propargyl (**1**) and extraction with suitable solvents, the residue was hydrolyzed by alkali to clodinafop acid and extracted with ethyl acetate. Ethyl acetate gave 83.2, 78.9 and 81.0% recovery at 0.5, 1.00 and 5.00 $\mu g\,g^{-1}$ level of fortification. (Table 2) In soil, clodinafop acid is the major metabolite and also herbicidal in action. Hence, this method is useful for the estimation of total herbicidally active compound whether in the form of acid or ester. A similar method has been used for the total residues of fluazifop herbicide for real world samples [8]. GC has also been used for analysis of fluazifop residues in soybean grains, oil and cake, after derivatization of the extract by diazomethane [9].

Extraction of clodinafop acid fortified soil by different solvents showed that, the extracting power of ethyl acetate is better than toluene. Where toluene gave 65–70% recovery, ethyl acetate gave more than 80% recovery. The limit of determination of clodinafop from soil ranged between 1 and 1.2 $ng\,g^{-1}$.

For the quantitative recovery of clodinafop ester and clodinafop acid from wheat straw and grain samples, the performance of acetone and ethyl acetate was found to be more or less similar (around 80%, Table 3), but ethyl acetate was selected, as the amount of co extractives were less in case of ethyl acetate (Fig. 3). The limit of determination of clodinafop ester and acid from straw and grain samples was 0.25 and 0.1 $\mu g\,g^{-1}$ respectively with sensitivity of 5 and 2 ng level. The percent recovery of clodinafop ester was 79.3, 81.7 and 82.6, 82.9 for straw and grain sample at the fortification level of 0.5 and 1.00 $\mu g\,g^{-1}$, respectively.

The recovery of clodinafop ester and acid was also standardized in wheat and *P. minor* plant samples (Table 4). This standardization was done keeping in view that in crops the

Table 2
Recovery of clodinafop-propargyl (ester) and clodinafop acid from soil using different solvents

| Compound (method) | Extracting solvent | Amount added ($\mu g\,g^{-1}$) | Amount recovered ($\mu g\,g^{-1}$) mean ± s.d. | Percent recovery |
|---|---|---|---|---|
| Ester (ester quantification) | Methanol | 0.50 | 0.315 ± 0.013 | 63.0 |
| | | 1.00 | 0.712 ± 0.022 | 71.2 |
| | Toluene | 0.50 | 0.627 ± 0.021 | 62.7 |
| | | 1.00 | 3.360 ± 0.018 | 67.1 |
| | Dichloromethane | 0.50 | 0.327 ± 0.011 | 64.4 |
| | | 1.00 | 0.592 ± 0.013 | 59.2 |
| | Ethyl acetate | 0.50 | 0.405 ± 0.004 | 81.3 |
| | | 1.00 | 0.772 ± 0.021 | 77.2 |
| | | 5.00 | 4.150 ± 0.007 | 83.0 |
| Ester (acid quantification) | Methanol | 0.50 | 0.307 ± 0.019 | 61.4 |
| | | 1.00 | 0.643 ± 0.015 | 64.3 |
| | | 5.00 | 3.256 ± 0.005 | 65.1 |
| | Toluene | 1.00 | 0.683 ± 0.002 | 62.4 |
| | Dichloromethane | 0.50 | 0.312 ± 0.013 | 68.3 |
| | Ethyl acetate | 0.50 | 0.415 ± 0.021 | 82.2 |
| | | 1.00 | 0.789 ± 0.013 | 78.9 |
| | | 5.00 | 4.050 ± 0.007 | 81.0 |
| Clodinafop acid | Toluene | 0.50 | 0.326 ± 0.12 | 65.2 |
| | | 1.00 | 0.697 ± 0.23 | 69.7 |
| | Ethyl acetate | 0.50 | 0.419 ± 0.05 | 83.8 |
| | | 1.00 | 0.813 ± 0.26 | 81.3 |
| | | 5.00 | 4.110 ± 0.13 | 82.2 |

Average of three replicates.

Table 3
Recovery of clodinafop-propargyl (ester) and clodinafop acid from wheat straw and grain using different solvents

| Compound (method) | Substrate | Extracting solvent | Amount added ($\mu g\,g^{-1}$) | Amount recovered ($\mu g\,g^{-1}$) mean ± s.d. | Percent recovery |
|---|---|---|---|---|---|
| Ester (ester quantification) | Straw | Acetone | 0.50 | 0.401 ± 0.013 | 80.3 |
| | | | 1.00 | 0.791 ± 0.018 | 79.1 |
| | | Ethyl acetate | 0.50 | 0.396 ± 0.020 | 79.3 |
| | | | 1.00 | 0.817 ± 0.011 | 81.7 |
| | Grain | Ethyl acetate | 0.50 | 0.413 ± 0.022 | 82.6 |
| | | | 1.00 | 0.829 ± 0.019 | 82.9 |
| Clodinafop acid | Straw | Ethyl acetate | 0.50 | 0.411 ± .011 | 82.2 |
| | | | 1.00 | 0.809 ± .008 | 80.9 |
| | Grain | Ethyl acetate | 0.50 | 0.423 ± .017 | 84.6 |
| | | | 1.00 | 0.829 ± .020 | 82.9 |

Average of three replicates.

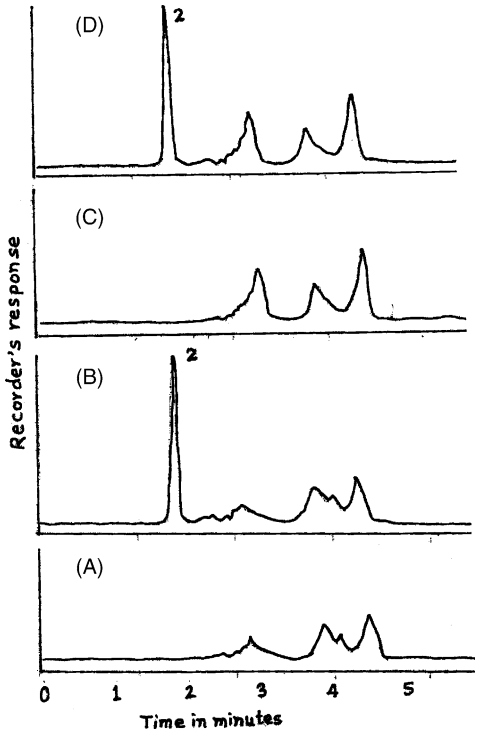


Fig. 3. HPLC chromatogram showing recovery: (A) control wheat straw; (B) wheat straw fortified with clodinafop acid (**2**); (C) control wheat grain; (D) wheat grain fortified with clodinafop acid (**2**).

clodinafop acid is the translocated form of the herbicide. Hence, for metabolic studies, the method extracting both acid and its parent ester form will be useful. Thus for wheat and *P. minor* plant samples, ester quantification method using ethyl acetate as the extracting solvent was standardized (Fig. 4). The recovery of ester and acid ranged 76–78%. Out of three adsorbents used for plant material, neutral alumina gave the best cleanup with a recovery range of 76–78% in comparison to charcoal and florisil. (Table 5).

The method is simple, sensitive and can be used for determination of residues of clodinafop in soil and wheat crop. The instrument detection limit was found to be 0.1 $\mu g\,ml^{-1}$ indicating a sensitivity of 2 ng. The limit of determination (method detection limit ranged between 1 and 1.2 ng $g^{-1}$ The method standardized for wheat and *P. minor* plant is useful in studying the metabolic fate of this herbicide in order to understand the mechanism of selectivity.

In the field samples for persistence of clodinafop ester, zero day soil samples were collected after 4 h of application and the analysis was done after extraction within 6 h. But clodinafop ester was not detected even after 6 h of herbicide application indicating that clodinafop ester rapidly degrades in soil (Table 6).

The acid was detected upto 10 days in field soil samples. The initial concentration of clodinafop acid in soil (0–15 cm) was 0.209 $\mu g\,g^{-1}$ of soil at the recommended dose, which dissipated in soil to 0.018 $\mu g\,g^{-1}$ and BDL after 10 and 15 days of application, respectively. The residue of clodinafop

Table 4
Recovery for clodinafop ester and acid from plant samples

| Compound (method) | Substrate | Extracting solvent | Amount added ($\mu g\,g^{-1}$) | Amount recovered ($\mu g\,g^{-1}$) mean ± s.d. | Percent recovery |
|---|---|---|---|---|---|
| Ester (ester quantification) | Wheat | Ethyl acetate | 1.00 | 0.766 ± 0.012 | 76.6 |
| | | | 0.50 | 0.386 ± 0.015 | 77.3 |
| | *Phalaris minor* | Ethyl acetate | 1.00 | 0.782 ± 0.009 | 78.2 |
| | | | 0.50 | 0.379 ± 0.005 | 75.8 |
| Clodinafop acid | Wheat | Ethyl acetate | 1.00 | 0.832 ± 0.002 | 83.2 |
| | | | 0.50 | 0.417 ± 0.013 | 83.4 |
| | *Phalaris minor* | Ethyl acetate | 1.00 | 0.819 ± 0.007 | 81.9 |
| | | | 0.50 | 0.413 ± 0.015 | 82.6 |

Average of three replicates.

Table 5
Recovery of clodinafop ester from plant samples using different cleanup agents

| Substrate | Cleanup agent | Amount added ($\mu g\,g^{-1}$) | Amount recovered ($\mu g\,g^{-1}$) mean ± s.d. | Percent recovery |
|---|---|---|---|---|
| Wheat | None | 1.00 | 0.832 ± 0.003 | 83.2 |
| | None | 0.50 | 0.420 ± 0.013 | 84.0 |
| | Charcoal | 1.00 | 0.662 ± 0.017 | 66.2 |
| | Charcoal | 0.50 | 0.348 ± 0.009 | 69.7 |
| | Florisil | 1.00 | 0.716 ± 0.013 | 71.6 |
| | Florisil | 0.50 | 0.349 ± 0.007 | 69.8 |
| | Alumina | 1.00 | 0.766 ± 0.012 | 76.6 |
| | Alumina | 0.50 | 0.386 ± 0.015 | 77.3 |
| *Phalaris minor* | None | 1.00 | 0.817 ± 0.008 | 81.7 |
| | None | 0.50 | 0.419 ± 0.019 | 83.8 |
| | Charcoal | 1.00 | 0.637 ± 0.011 | 63.7 |
| | Charcoal | 0.50 | 0.339 ± 0.002 | 67.8 |
| | Florisil | 1.00 | 0.733 ± 0.007 | 73.3 |
| | Florisil | 0.50 | 0.355 ± 0.020 | 71.0 |
| | Alumina | 1.00 | 0.782 ± 0.009 | 78.2 |
| | Alumina | 0.50 | 0.379 ± 0.005 | 75.8 |

Average of three replicates. Ethyl acetate as an extracting solvent.

in soil exhibited declining pattern as a function of time. It can be concluded from the dissipation curve that the rate of dissipation was rapid during initial period but declined thereafter as time proceeded. The logarithmic plots of herbicide residues versus time obtained by fitting the regression equations indicated that the rate of dissipation fitted a first order kinetics decay curve well ($r = 0.95 - 0.97$). The half-life of the compound in soil under field condition was calculated as 3.44 days.

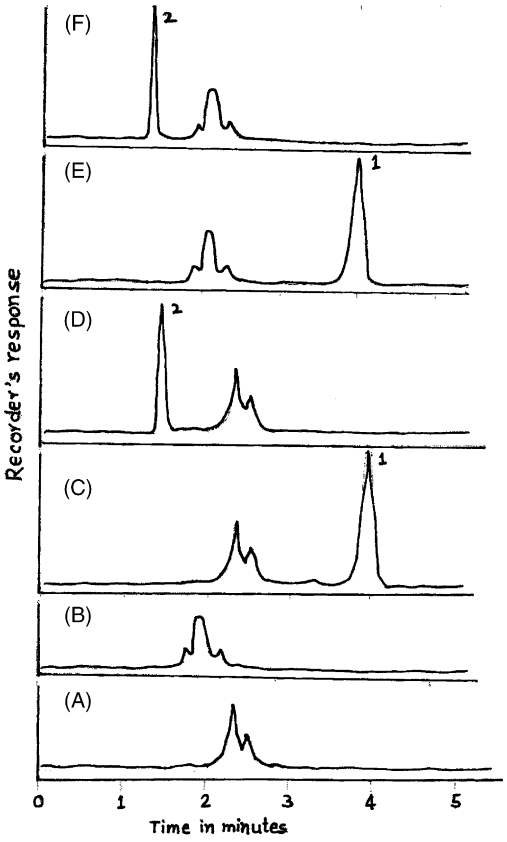


Fig. 4. HPLC chromatogram showing recovery: (A) control wheat leaves; (B) control *P. minor* leaves; (C) wheat leaves fortified with clodinafop-propargyl (**1**); (D) wheat leaves fortified with clodinafop acid (**2**); (E) *P. minor* leaves fortified with clodinafop-propargyl (**1**); (F) *P. minor* leaves fortified with clodinafop acid (**2**).

Table 6
Dissipation of clodinafop in field soil

| Time (days after application) | Herbicide amount remaining ($\mu g\,g^{-1}$) at 60 g a.i. $ha^{-1}$ |
|---|---|
| 0 | 0.209 ± 0.012 (0) |
| 1 | 0.127 ± 0.005 (39.4) |
| 3 | 0.088 ± 0.007 (57.8) |
| 5 | 0.045 ± 0.009 (78.4) |
| 10 | 0.018 ± 0.002 (91.0) |
| 15 | BDL |

Figure in parenthesis indicates percent dissipation. BDL: below detectable limit. Half-life: 3.44 days.

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