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Analytical Methods for Cyhalofop-butyl and Its Metabolites in Soil and Water

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Abstract A relatively simple and less expensive method is proposed for the determination of cyhalofop-butyl (XDE-537) and its metabolites: R-(+)-2-[4-(2-fluoro-4-cyanophenoxy) phenoxy] propanoic acid (ACID); R-(+)-2-[4-(4-carboxyl-2-fluoro-4-cyanophenoxy) phenoxy] propanoic acid (DIACID); and 2-[4-(4-carbamoyl-2-fluorophenoxy) phenoxy] propanoic acid (AMIDE). Conversion of the parent compound to its aryl/alkyl/or halide derivative for determination by GC was found unnecessary. Only HPLC was used and under gradient elution all the four compounds separated well. Good recoveries were obtained with the fortified soil and water samples. Low pH was important for the extraction of the four components.

Key words Cyhalofop-butyl; Metabolites; Residue

1 Introduction

Rice is one of the main staple foods consumed all over the world. It is grown in over 100 countries on every continent except Antarctica[1]. By the year 2020, the world population is expected to reach 8 billion. It has been estimated that to meet the consumption needs worldwide rice production of 490 million tones (as for 1988) must increase to 758 million tones by that year 2020, a 65% increase. For the major rice producers of Southeast Asia, the needed increase in rice production by 2020 is about 100%[2].

Weeds account for up to 10.5% loss in crop productivity and is ranked the second after insects[3] Currently, the use of herbicides is on the sharp rise as they are increasingly used to replace manual and mechanical weeding whose labor cost are also increasing. All crops are affected by weeds and rice is no exception.

Cyhalofop-butyl (XDE-537) is the active ingredient of Clincher——a recently developed post-emergency herbicide used in most parts of Asia for controlling a wide range of grass weeds in paddy fields[4].

Cyhalofop-butyl belongs to aryloxyphenoxy propionate (ArPP) type of herbicides. It has three metabolites namely, ACID, DIACID and AMIDE (see Fig. 1). While little is known about the environmental effects of ArPPs there is a more frustrating fact that their analytical methods are limited. Today there are very few analytical methods for the members

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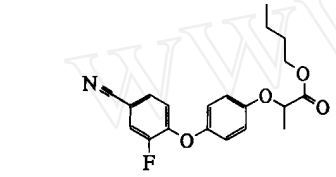
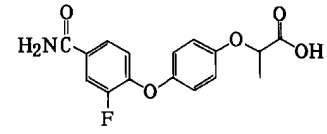
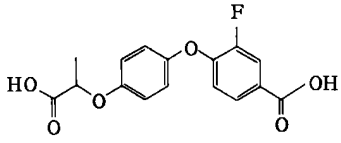
of this group of herbicides. These methods are usually cumbersome involving many difficult steps. Although analytical methods for such ArPPs as quizalofop-butyl, fenoxaprop-butyl, fluazifop-butyl, clodinafop-butyl haloxyfop-butyl and diclofop are well documented[5~7], method for determination of cyhalofop-butyl has not been reported. In many of the reported methods for ArPPs, the analysis is centered on derivation of the acidic group attached to the aliphatic component of the chemical to either its halide or alkyl or aryl derivative[7,8]. For the parent ArPPs it is usually determined by GLC with ionization detector. However, the properties of ArPPs make them more amenable to HPLC than to GC. This is confirmed by the good results reported by Lagana *et al.*[6,7] when they analyzed various herbicides belonging to ArPPs.



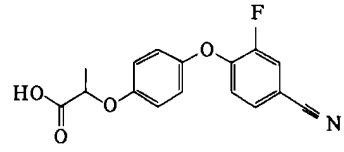
Butyl-2-[4-(2-fluoro-4-cyanophenoxy)phenoxy]propanoate -XDE-537



2-[4-(4-carbamoyl-2-fluorophenoxy)phenoxy]propanoic acid-AMIDE



2-[4-(4-carboxy-2-fluorophenoxy)phenoxy]propanoic acid DIACID



2-[4-(2-fluoro-4-cyanophenoxy)phenoxy]propanoic acid -ACID

Fig. 1　Structures of XDE-537 and its metabolites

Usage of herbicides has resulted in findings of them in soil, surface and groundwater[9,10]. All agricultural chemicals found in the environment may be said to be environmental pollutants. This might not necessarily mean that they are a threat to the environment or living organisms. However, there are known cases of environmental disturbances caused by herbicides or they may have influenced other organisms than the target ones[11,12].

The need of water and food quality control makes it necessary to develop methods that can be used to determine the amounts of the compounds so as to check them against exceeding no-observed-effect concentration (NOEC). The method should be reliable and easy to be adopted in most developing rice producing countries where accurate but expensive facilities are not easily available. This paper presents the determination method for cyhalofop-butyl (XDE-537) and its three metabolites in soil and water.

2　Material and Methods

2.1　Chemicals

Analytical reference compounds viz. cyhalofop-butyl, ACID, DIACID, AMIDE were

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kindly supplied by Dow Elanco. Co. (American). Acetonitrile and methanol (HPLC grade) were products of Fisher chemicals, USA. All other chemicals were purchased from local commercial sources and were of analytical grade.

2.2 Instrumental conditions

HPLC (Hewlett Packard 1100) was used to determine cyhalofop-butyl and its three metabolites. The machine was fitted with a YWG-C18 column with inner diameter of 4.6 mm and a length of 25 cm. The mobile phase was composed of 0.2% phosphoric acid (phosphoric acid/water, 0.2∶100, *w*/*w*)-[A] and acetonitrile (HPLC grade)-[B]. The elution was of gradient type as described in Table 1. The concentrations of the analytes in the final extracts were calculated by measuring the peak area and subsequent calculation of recoveries was done with reference to peak areas obtained from a standard solution.

Table 1 Gradient elution for the determination of XDE-537, AMIDE, ACID and DIACID by HPLC*

Time /min	Mobile phase	Flow /mL·min^{-1}
0	A = 100	0.9
1.0	A∶B = 90∶10	0.9
2.0	A∶B = 80∶20	0.9
3.0	A∶B = 70∶30	0.9
5.0	A∶B = 60∶40	0.9
6.0	A∶B = 50∶50	0.9
7.0	A∶B = 40∶60	1.0
10.0	A∶B = 10∶90	1.0

* Injection volume: 20 μL; Detection: DAD, 248 nm.
Retention times (typical chromatograms in Fig. 1):
AMIDE 9.4 min; DIACID 10 min; ACID 11 min; XDE-537 (cyhalofop-butyl) 13 min.

2.3 Preparation of standard solutions

Standard solutions were prepared by dissolving appropriate weight of each compound (XDE-537, ACID, DIACID and AMIDE) in methanol to make 1000 mg/L from which appropriate dilution was done to make such lower concentrations as 0.1, 0.5, 1, 2, 5, and 10 mg/L. It was noted during the experiment that some of the compounds degraded to some unidentified metabolites in spite of keeping the standard solutions under dark and low temperature in the refrigerator. In this experiment we had to make fresh standard solutions whenever we wanted to analyze the samples.

2.4 Water/soil sample fortification

Samples were fortified by spiking appropriate aliquots of standard solution of each compound in distilled water and virgin soil samples. For soil, samples were air-dried and sieved (100-mesh). Twenty grams of soil were measured and put into 200 mL Erlenmeyer flask. For water, 200 mL distilled water was measured and transferred into each of 500 mL separatory funnels. After fortification the samples were thoroughly mixed and left for 24 h

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before extraction.

2.5 Extraction and cleanup procedure

2.5.1 Water　Samples were put into 500 mL separatory funnels. The pH was checked by universal indicator and accordingly adjusted to 2 by adding a few drops of 1 mol/L hydrochloric acid. Extraction was achieved by the use of 50 mL dchloromethane and repeated two times. At each time shaking was for 1 min. The dichloromethane layers were eluted into 250 mL round flask and evaporated to ca. 1 mL with a rotary evaporator in water bath at 40℃ and to dryness by slight jet of nitrogen gas. The residues were dissolved in 2 mL methanol (HPLC grade) for HPLC determination in the same day.

2.5.2　Soil　Twenty miniliters of 1 mol/L hydrochloric acid and 80 mL methanol were added to the flasks (2.3) followed by shaking for 1 h. The mixture was then filtered using Buchnner funnel and Whatman filter paper. The bottle cap and filter cake were rinsed with 30 mL methanol and the filtrate was combined. The filtrate was then transferred into 500 mL separatory funnels and mixed with 150 mL of 6% sodium chloride solution. Cyhalofop-butyl (XDE-537) and its metabolites (AMIDE, ACID and DIACID) were extracted from the aqueous layer by 50 mL methylene chloride after shaking for at least 1 minute. Partitioning by 50 mL dichloromethane was repeated twice for each flask and the dichloromethane layers were combined into 250 mL round bottomed flak. The dichloromethane layer was evaporated to 1 mL with a rotary evaporator in water bath at 40℃. It was then evaporated to dryness using slight jet of nitrogen gas.

2.5.3　Cleanup for soil samples　The residues were re-dissolved by adding 5 mL of eluting solvent mixture (acetic acid / methanol / dichloromethane 1: 69: 30, *v*/*v*/*v*). After slight and careful swirling, the mixture was transferred to the silica gel column containing 5 g silica gel prepared earlier and pre-washed with 30 mL of methanol/dichloromethane (7: 3, *v*/*v*). Cyhalofop-butyl and its three metabolites were eluted by 70 mL of the eluting mixture. The eluate was collected and evaporated under vacuum (vacuum pump was used) at 50℃. The remaining residues were dissolved in 2 mL methanol (HPLC grade) for HPLC determination.

3　Results and Discussion

3.1 Fortification recoveries

Fig. 2 shows the typical chromatograms for cyhalofop-butyl and its metabolites (all reference standards). Under isocratic conditions the parent compound takes much longer time to appear and the metabolites may not be easily separated. The gradient elution however, enabled to minimize the total time as XDE-537 was eluted earlier and at all the metabolites separated clearly. The results of the fortified sample were compared to those of reference standard solutions. The four compounds separated well at the operating conditions and good recoveries were obtained with the fortified samples (Table 2). Under these conditions the few peaks representing impurities in control samples did not interfere with the desired peaks as they appeared at different retention times (Fig. 2～6). In this aspect this

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method differs slightly with the ones presented by Lagana *et al.*[5,6] and Negre *et al.*[13], for determination of other ArPPs.

Table 2 Percentage of recoveries for the fortified water and soil samples

	Fortification level/mg·L^{-1}	Recovery* (%) ±*SD*			
		AMIDE	DIACID	ACID	XDE-537
Water	0.01	92±2.6	89±2.5	94.5±2.2	95±2.7
	0.1	93±2.1	95±2.3	99.2±2.1	96±2.3
	1.0	92±2.2	99±2.2	96.2±2.3	101±3.1
Soil	0.01	80±4.2	90±2.1	99±2.3	99±2.4
	0.1	89±2.5	98±2.3	91±2.5	95±2.9
	5.0	92±2.8	91±2.0	90±2.1	92±2.7

*Mean of three replicates. Lowest detectable limit: 4×10^{-9} g. Lowest detectable level: Water 2 μg/L, Soil 0.01 mg/kg.

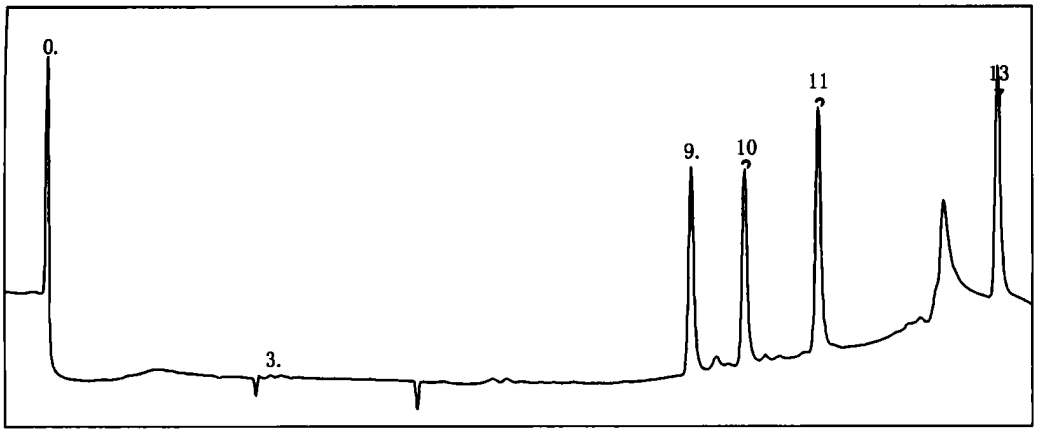

Fig. 2 Standard chromatogram at 5 mg/L in the order: AMIDE, DIACID, ACID and XDE-537

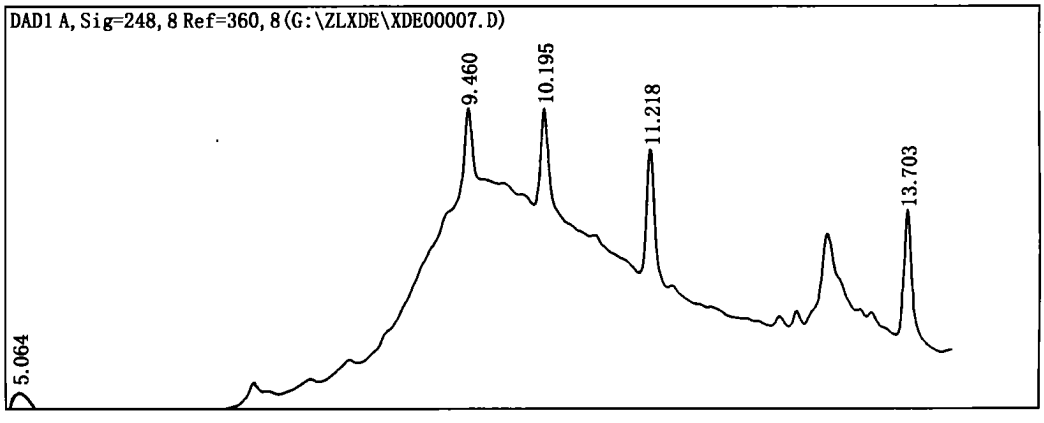

Fig. 3 Retention times for fortified soil sample in the order: AMIDE, DIACID, ACID, XDE-537

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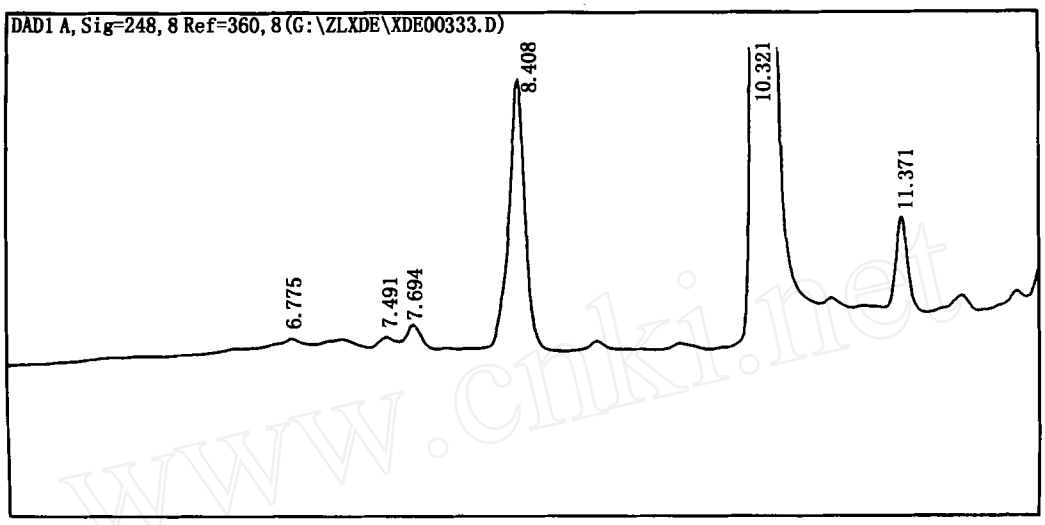

Fig. 4　Chromatogram for water control sample

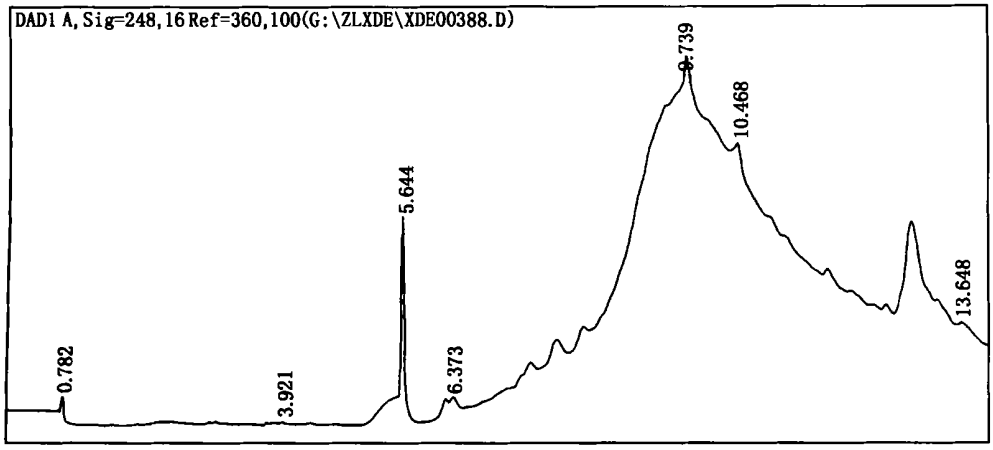

Fig. 5　Chromatogram for soil control sample

3.2　Multi residue determination

Contrary to other methods for the determination of ArPPs[5, 6, 13], this method is much easier and more convenient. Lack of expensive facilities and the problem of workforce in many laboratories will prefer this method to all the others. In water and soil cyhalofop-butyl is metabolized into AMIDE, ACID and DIACID and all of them together with the parent compound need to be quantified if a comprehensive work is to be done on the residue contents in water and soil. A keynote to this method that makes it much different from the other ones is the fact that all the three metabolites and the parent compounds are determined at the same time. This method is reliably accurate to μg/L level for water and mg/kg levels for soil. While the lowest amounts detected can not be compared with those of other ArPPs, it can confidently be used to monitor the water quality regarding the contents of XDE-537 and

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its metabolites.

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土壤和水中氰氟草酯及其代谢物的分析方法

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摘 要 建立了一种简易而实用的残留分析方法, 用于检测土壤与水中氰氟草酯及其 3 种代谢产物, 即(R)-2-[4-(4-氰基-2-氟苯氧基)苯氧基]-丙酸(ACID)、(R)-2-[4-(4-氨基甲酰基-2-氟苯氧基)苯氧基]-丙酸(AMIDE)、(R)-2-[4-(4-羧基-2-氟苯氧基)苯氧基]-丙酸(DIACID)。该方法仅用高效液相色谱法的梯度洗脱程序即可将 4 种化合物有效分离, 省去了需将氰氟草酯(母体)衍生化反应再用气相色谱法检测这一复杂步骤。同时, 该方法的添加回收率高。较低的 pH 值对提高 4 种化合物的提取效率很重要。

关键词 氰氟草酯; 代谢物; 残留

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