

# Determination of Fipronil Residue in Soil and Water in the Rice Fields in North of Iran by RP-HPLC Method

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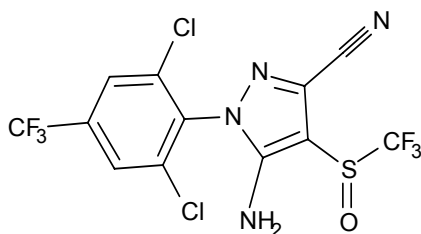
## Abstract

A method for the determination of amount of residue of insecticide fipronil in paddy water and soil was developed based on RP-HPLC. Trials were carried out on rice fields in Iran using a fipronil granular formulation at a rate of 40 g ai/ha just after transplanting, which corresponded to 20 kg/ha of formulated product. Liquid-Liquid Extraction (LLE) and Soxhlet extraction were applied for extraction of fipronil from paddy water and soil respectively. In paddy water, Fipronil levels declined from 0.018 to 0.0028  $\mu\text{g/ml}$  from one day to 40 days after application. No analyte was observed after 40 days at levels exceeding limit of detection. Soil analysis also showed that the amount of fipronil declined from 0.032 to  $<0.009$  mg/kg from one day to 14 days after application and after 28 days, no analyte was left. The hydrolysis of Fipronil was also studied in aqueous buffer solution of pH 7.8 at temperature of 20 and 30  $^{\circ}\text{C}$ . Results showed that the Fipronil degradation was increased at 30  $^{\circ}\text{C}$ .

**Keywords:** insecticide, fipronil, RP-HPLC

## 1. Introduction

Fipronil ( $\pm$ )-5-amino-1-(2,6-dichloro- $\alpha,\alpha,\alpha$ -trifluoro-p-tolyl)-4-trifluoromethyl sulphinyl pyrazole-3-carbonitrile (Figure 1) is a phenylpyrazole insecticide discovered in 1985 by the company Rhone-Poulenc Agro. This insecticide is applied at low dose, and is active against wide range of insect pests of crops, notably rice insect, thrips (citrus, cotton, mango, ...), termites (sugar cane, corn) and click beetles (cereals, corn, sunflower, beets, ...).<sup>1</sup> It was recommended for use in locust control.<sup>2</sup> It's major agricultural use include granular applications at 30-100 g ai/ha (regent 3GR) on rice fields for the control of rice stem borers (*chilo* spp. *Tryporyza* spp.), brown plant hopper (*nilarvata* lagens) and rice water weevil (*lissorhoptas orzyophilus*).<sup>3</sup>



**Figure 1.** Structural formula of Fipronil( $\pm$ )-5-amino-1-(2,6-dichloro- $\alpha,\alpha,\alpha$ -trifluoro-p-tolyl)-4-trifluoromethyl sulphinylpyrazole-3-carbonitrile

Few analytical methods have been reported for determination of this compound. Bobe et al. proposed gas chromatography (GC) method for the determination of Fipronil residue in soil.<sup>4</sup> Viches et al. were developed a simple and practical GC-MS method in combination with Solid Phase Micro Extraction (SPME) for determination of Fipronil in water, soil and human urine samples.<sup>5</sup> Hainzl et al. also used HPLC with a reversed phase ultra sphere  $\text{C}_{18}$  column using Methanol/water as the mobile phase to determine metabolites in aqueous fractions from mice faeces.<sup>6</sup> Since regent 2GR (0.2% Fipronil) is used in Iranian rice fields to control rice stem borers, because of its environmental effect and human health, due to its increasing consumption, it is important to determine the residue of this compound in water and soil. In this study a RP-HPLC method was used to determine the Fipronil residue in water and soil of rice fields in north of Iran.

## 2. Results and discussion

Fipronil in soil extracted using different solvent such as methanol, acetonitrile and dichloromethane-acetone (1:1, v/v). Figure 2 shows recovery percentage of Fipronil, which were extracted with these solvents and indicated that dichloromethane-acetone (1:1, v/v) was the best solvent. Figure 3 illustrates effect of extraction time on recovery percentage. As can be seen, there is no significant different between results obtained after

8 hours, therefore 8 hours was chosen as appropriate time of extraction.

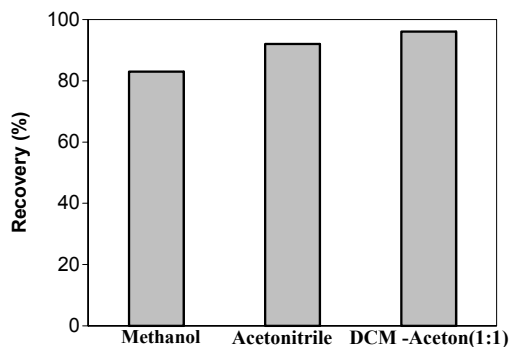


Figure 2. Effect of solvents type on (%) recovery of fipronil

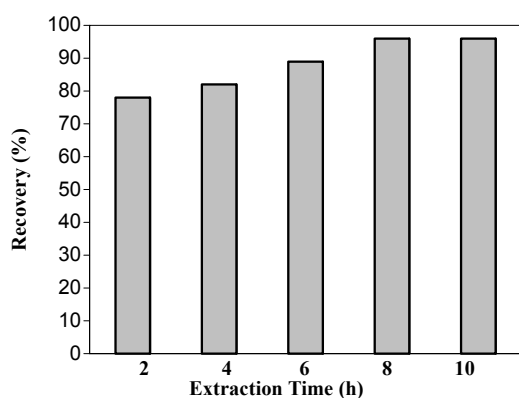


Figure 3. Effect of extraction time on (%) recovery with dichloromethane-acetone (1:1) in soil

Figure 4 shows typical chromatograms of Fipronil standard and soil samples.

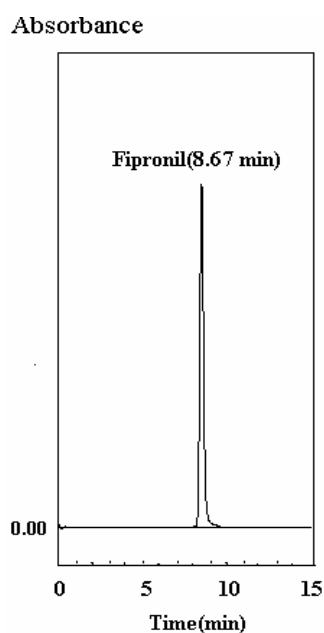


Figure 4. (a) Chromatogram of Fipronil standard solution

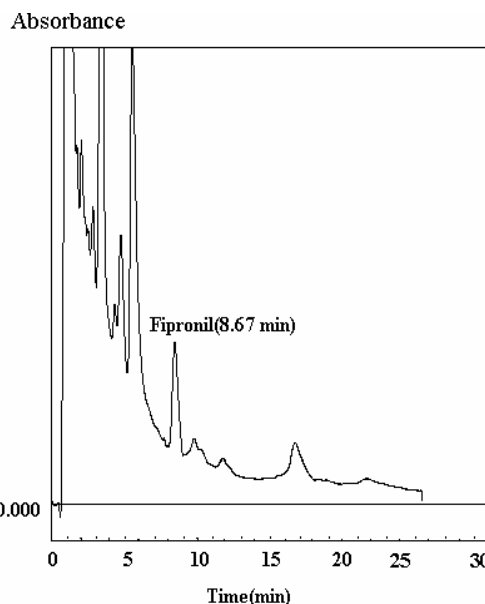


Figure 4. (b) chromatogram of Fipronil residue in soil samples, Conditions: column,  $C_{18}$ ,  $\mu$ Bondapak,  $250 \times 4.6$  mm,  $10 \mu$ m; Flow rate 1.5 ml/min;  $\lambda$  280 nm; Temperature  $35^\circ\text{C}$

Recoveries were determined by adding known amounts of Fipronil to portions of control samples and analyzing by the method detailed. In soil spiked with 0.1–2  $\mu\text{g}/\text{ml}$  the recoveries ranged from 91 to 102% for low spike levels and from 93 to 99% for high spike levels. In water fortified with 0.1–2  $\mu\text{g}/\text{ml}$ , recoveries were between 87 and 98% for low spike levels and between 90 and 99% for high spike levels.

Average pH of water samples of rice fields was 7.8. Fipronil degradation in aqueous buffer solution in this pH at temperatures 20 and  $30^\circ\text{C}$  was examined. Results (table 1) showed that Fipronil degradation at  $30^\circ\text{C}$  was much higher than  $20^\circ\text{C}$ . After 40 days at  $30^\circ\text{C}$ , the initial concentration (4  $\mu\text{g}/\text{ml}$ ) decreased to 0.29  $\mu\text{g}/\text{ml}$  (7%) while at  $20^\circ\text{C}$  decreased to 94%.

Fipronil residue in water and soil of rice fields determined using standard addition method, which is reported in table 2. Results showed that the amount of Fipronil in paddy water, declined from 0.018 to 0.0028  $\mu\text{g}/\text{ml}$  one to 40 days after application respectively. No analyte was observed after 40 days at levels exceeding limit of detection. The limit of detection (LOD) was 0.1  $\mu\text{g}/\text{ml}$  using  $3S_b/m$  equation.

Table 1. Degradation of Fipronil in aqueous buffer solution (pH 7.8) at temperature  $20^\circ\text{C}$  and  $30^\circ\text{C}$

Time (Day)	Fipronil residue ( $\mu\text{g}/\text{ml}$ ) at $20^\circ\text{C}$	Fipronil residue ( $\mu\text{g}/\text{ml}$ ) at $30^\circ\text{C}$
0	3.83	3.77
10	3.78	2.68
20	3.70	1.79
30	3.64	1.42
40	3.59	0.29

**Table 2.** Amount of Fipronil residue in Water and soil samples in rice fields of the north of Iran

Time (Day)	Fipronil residue in water sampels ( $\mu\text{g}/\text{ml}$ )	Fipronil residue in soil samples ( $\text{mg}/\text{kg}$ )
-1	-	-
1	0.0180	0.0320
3	0.0160	0.0280
7	0.0110	0.0128
14	0.0061	<0.0090
28	0.0055	-
40	0.0028	-
56	-	-

Studies on leaching and adsorption/desorption of Fipronil showed that the technical grade Fipronil and its metabolites have low mobility in soil.<sup>7</sup> Since amounts of Fipronil at soil depths of higher than 5 cm is very low, its analysis was impossible at depth of more than 5 cm. The analysis on the outer surface layer (lower than 5 cm) showed that the amount of fipronil declined from 0.032 mg/kg to <0.009 mg/kg one day to 14 days after application.

Repeatability Precision Values, expressed as the mean Relative Standard Deviation (R.S.D), was calculated from six independent extraction of Fipronil from water and soil samples. At the spike levels considered, the repeatability ranged 2.8 to 4.9% for all type of samples, indicating good performance of the method developed in this work.

### 3. Experimental

#### 3.1. Chemicals

Acetonitrile (HPLC grade), Dichloromethane, acetone and Sodium dihydrogen phosphate were obtained from Fluka (Buches, Switzerland). Water used was doubly distilled deionized and filtered through a 0.22 $\mu\text{m}$  Millipore filter. The analytical reference Standard of Fipronil (99.4% purity) was obtained from Rhone-Poulenc Company.

#### 3.2. Instrumentation and chromatographic conditions

The HPLC system comprised of a series 10 liquid chromatograph pump module, a model LC – 95 UV detector (Perkin Elmer, Norwalk, CT, USA), a STG software for data processing (Teifgostar Industry, Iran) and column C<sub>18</sub>, 250  $\times$  4.6 mm, 10 $\mu\text{m}$  (Waters Assoc. Milford, MA, USA). A Jenway pH meter 3030 (Jenway Ltd., UK) was used for determination of pH. A rotary evaporator equipped with a thermostated water bath and vacuum system was supplied by Büchi (plawil,

switzerland). Disposable PTFE syringe filter units, 0.5  $\mu\text{m}$  pore size, were purchased from Microfiltration system (Dublin, CA, USA).

Appropriate conditions for determination of fipronil by HPLC, were obtained using its standard, were 60% acetonitrile in mobile phase with pH of 5 (using phosphate buffer) and column temperature of 35 °C. At these conditions no interferences was observed with Fipronil peak using extracted materials from water and soil samples. At least 25 min was necessary to elute all non-polar compounds which were present in the acetonitril extract for next injection. Flow rate, wavelength detection ( $\lambda$ ) and injected volume were 1.5 ml/min, 280 nm and 10  $\mu\text{l}$  respectively.

#### 3.3. Procedure

Ten equal parts of rice fields area choosed and fipronil granular formulation was applied at a rate of 40 g ai/ha just after transplanting, which corresponds to 20 kg/ha of formulated product. Water and soil samples for analysis were taken one day prior to treatment, and 1, 7, 14, 28, 40 and 56 days after application. All samples were placed in ice flask and transferred to laboratory.

Water samples were filtered through a Whatman filter (pore size 0.45  $\mu\text{m}$ ) and washed with dilute HCl and deionized water and stored at 4 °C. The samples then extracted with dichloromethane four times (total volume 300 ml). The organic layers were collected and evaporated under reduced pressure at 35 °C to give a dry residue. This residue was dissolved in acetonitrile and used for analysis by HPLC system.

Methanol, acetonitrile and dichloromethane-acetone (1:1 v/v) were tested as solvents for Extraction of fipronil from soil using soxhlet apparatus for 8 hours. Results showed that dichloromethane-acetone (1:1, v/v) was the best solvent. Air-dried soil samples (25 g) were refluxed in 300 ml of dichloromethane-acetone (1:1, v/v) for 8 hours on a soxhlet extraction device. The extract was concentrated to near dryness in rotary evaporator at 35 °C, and then redissolved in acetonitrile. Solution filtered by syring filter and was used for analysis by HPLC system.

### 4. Conclusion

A rapid and sensitive isocratic reversed-phase HPLC method has been developed herein for the determination of fipronil in soil and water of rice field in north of Iran. Liquid –Liquid extraction proved to be an adequate way, for the separation of the analyte from matrix interferences. The recovery of analyte from soil and water were over 90% which means that its extraction efficiency is very satisfactory. Detection limit, the good sensitivity and resolution and the short analysis time (approximately 25 min) as well as the

simplicity of the procedure should make this method a useful tool for the routine analysis of the examined compound.

## 5. References

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## Povzetek

Razvili smo metodo za določanje ostankov fipronila z uporabo tekočinske kromatografije z obrnjeno fazo. Opravili smo poskus z uporabo fipronilnega granulastega preparata (40 g ai/ha) na riževih poljih, kar odgovarja 20 kg produkta/ha. Uporabili smo ekstrakcijo tekoče-tekoče in po Soxhletu. V odcednih vodah so koncentracije fipronila v 40 dneh padle z 0,018 na 0,0028  $\mu\text{g/ml}$ . Po 40 dneh so bile manjše od meje določitve. Analiza prsti je pokazala, da vsebnost fipronila pade z 0,031 na <0,009 mg/kg v 14 dneh po uporabi. Preverili smo tudi hidrolizo v vodnih razopinah (pH 7,8) pri temperaturah 20 in 30 °C.