Research Paper

Dissipation kinetics of three toxic fipronil metabolites in water at different pH and GC-MS characterization of hydrolytic transformation products of fipronil sulfide and sulfone.

Devottam Banerjee[1], Hemanta Banerjee[1*] and Srikumar Pal[2]

[1]Department of Agricultural Chemicals, [2]Department of Agricultural Biochemistry
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur – 741252, West Bengal, India.
*Corresponding Author Hemanta Banerjee

Abstract:
*In an effort to examine the persistence and degradation of three toxic fipronil metabolites including fipronil sulfide, fipronil sulfone and desulfinyl fipronil in water as a function of pH, a laboratory study was conducted following treatment of each of these metabolites separately in water maintained to pH 4.0, 7.0 and 9.2. The dissipation of each of these metabolites followed a first order kinetics. The results also indicated that the persistence of fipronil sulfide was much higher followed by fipronil sulfone and desulfinyl fipronil regardless of pH with half life varying from 35- 770, 27.50- 630 and 21.79- 495 days respectively. Moreover, the persistence of these metabolites decreased in the order of pH 4 > 7 > 9.2. GC-MS analysis of experimental solution confirmed the identity of two new additional hydrolytic metabolites, which were characterized as 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4 [(trifluoromethyl)sulfanyl]-1H-pyrazole-3-carboxamide, the amide derivative of fipronil sulphide (**FS-I**) and 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carboxamide, the amide derivative of fipronil sulfone (**FS-II**).*
Keywords: *desulfinyl fipronil, fipronil sulphide, fipronil sulphone, pH 4.0, pH 7.0, pH 9.2, transformed hydrolytic metabolite*

I. INTRODUCTION:

The phenyl-pyrazole insecticide fipronil (5-amino-1-[2,6-dichloro-4- (trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazole-3-carbonitrile); is applied to control diverse insect pests of a number of crops worldwide [Colliot et al. 1992]. It is noteworthy that substances belonging to the phenyl-pyrazole family though reputed for its herbicidal effects, but fipronil, is a potent insecticide. The fipronil gains importance especially in controlling insects that have developed resistance or tolerance to pyrethroid, cyclodiene, organophosphorus, and carbamate insecticides [Colliot et al. 1992; Hosie et al. 1995; Aajoud et al. 2003]. The multidimensional use of fipronil *viz*. crop protection against herbivorous insects and mites, household pest control, veterinary applications and fish farming (Barbee and Stout 2009; Chagnon et al. 2014) apparently looks good but because of the impacts of fipronil on the populations of aquatic organisms, there is likely to be environmental consequences of its use. Recently, in cities of China the occurrence of fipronil and fipronil sulfone in indoor dust samples were detected (Shi et al. 2020). It also binds to GABA receptors (Tingle et al. 2003) and to glutamate receptor coupled to chloride channels (Barbara et al. 2005) and has favorable selective toxicity towards insects rather than mammals (Hainzl and Casida 1996; Ikeda et al. 2004; Hainzl et al. 1998). Its mode of action is antagonistic. Glutamate receptors being insect specific, is more effective on invertebrates than on vertebrates (Narahashi et al. 2007). The toxicity profile of fipronil and its metabolites in the various environmental compartments have been reported (Hainzl and Casida, 1996) and on an acute basis to freshwater invertebrates the sulfone and desulfinyl metabolite found to be 6.6 and 1.9 times more toxic than the parent compound (U.S. EPA 1996). There are several reports on the persistence of fipronil in a number of crops, and other environmental substrates (Gupta et al. 2008; Lin et al. 2009; Lao et al. 2010; Mohapatra et al. 2010; Chopra et al. 2011; Bhardwag et al. 2012; Kumar and Singh 2013). It has been documented that fipronil undergoes degradation involving chemical, biochemical and photochemical mechanism yielding metabolites *viz*. fipronil sulfide, fipronil sulfone and fipronil desulfinyl, which are even more toxic than parent insecticide and

thus considered in residue analysis. The hazards caused to the ecological environment coupled with chronic neurotoxic effects makes fipronil and its metabolites as class C carcinogens (Qian et al. 2020). Fipronil and its metabolites have also shown to occur in treated wastewater effluent (Sadaria et al. 2017; Supowit et al. 2016). Fipronil degrades in water and soil through various metabolic pathways via hydrolysis to the amide metabolite; oxidation to fipronil-sulfone; and reduction to fipronil-sulfide, mainly under anaerobic conditions (Raveton et al. 2007). Under alkaline conditions fipronil is unstable and readily degrades via base-catalyzed hydrolysis and leads to the formation of fipronil-sulfide and fipronil-amide (Bobe et al. 1998b; Ramesh and Balasudramanium 1999). Fipronil sulfide, sulfone and desulfinyl are reported to be more toxic and persistent than the parent molecule to freshwater invertebrates, avian species, freshwater fish and to a variety of animals (Madsen et al. 2003). Thus, it seems reasonable to assume that the traces of this compound and its metabolites, or its transformation products are likely to be found in water (Aajoud et al. 2003). However, hardly is understood relating to persistence of toxic metabolites of fipronil such as sulfide, sulfone and desulfinyl in water as a function of pH. We, therefore, extended our study to examine the persistence and transformation of these metabolites at different pH values viz. 4.0, 7.0 and 9.2 under laboratory condition as pH is a relevant factor determining metabolism. Thus, considering the toxicity of fipronil metabolites the use of fipronil needs more caution in residential and agricultural application.

II. Materials and Methods

2.1 Preparation of water samples:

100 mL distilled water was adjusted to different pH values viz. 4.0, 7.0 and 9.2 using buffer powder for each pH. The pH of the solution was verified by a pH meter.

2.2 Treatment of water with desulfinyl fipronil, fipronil sulfide and fipronil sulfone:

Acetone solution (50 mg L^{-1}) of each of the fipronil metabolites (desulfinyl fipronil, fipronil sulfide and fipronil sulfone) was prepared separately by dilution of secondary stock solution (100 mg L^{-1}) of each metabolites. The water samples (50 mL) of different pH (4, 7 and 9.2) were fortified separately with these fipronil metabolites by adding 1mL of 50 mg L^{-1} or 1 mL of 100 mg L^{-1} to maintain a final concentration of 1 mg L^{-1} (T1) and 2 mg L^{-1} (T2) respectively for each of these metabolites along with an untreated control (T0). Each treatment was replicated thrice. To avoid any loss, the bottles were tightly corked and stored at room temperature (23 ± 2°C).

2.3 Sampling

The water samples with a gradient of pH from each treatement replications, were collected periodically at 0 (1 hr after application), 3, 7, 15, 30, 60, 90 and 120 days after treatment and was subsequently analyzed for each of these parent metabolites and identification of degradation products.

Each treated water samples was extracted thrice with 50 mL portions of ethyl acetate and 10 g sodium chloride each time in a 500 mL separatory funnel. The upper organic phase was collected over anhydrous Na_2SO_4 in a pear shaped flask and subsequently dried in a rotary vacuum evaporator (40°C). The residues were reconstituted with acetone (10 mL) for estimation by GC-MS. The recovery experiment was carried out to find out the efficiency and reliability of the analytical methods adopted in the present study after fortification each of these metabolites at a concentration of 0.01, 0.05 and 0.10 mg L^{-1}. The parameters used for GC-MS analysis were summarized in Table 1.

III. RESULTS AND DISCUSSION

3.1 Recovery study and Detector Linearity : Multi-residue recovery of fipronil metabolites following treatment with respective metabolites at the level ranging from 0.01-0.10 mg L^{-1} in water of different pH values (4, 7 and 9.2) led to overall recovery from 88.0- 97.20% with RSD of 2.84- 10.0% and signal to noise ratio > 10 suggesting the suitability and efficiency of the method. Thus, the quantification limit for each of these compounds was set at 0.01 mg L^{-1} irrespective of pH from the recovery study.

Linearity of detector response for each metabolite was established in GC-MS from the five point calibration curves ranging from 0.01-1.0 mg L^{-1} based on correlation coefficient > 0.997. The ions present in the mass spectrum of desulfinyl fipronil, fipronil sulfide and fipronil sulfone were comparable to earlier reports (Kaur et al. 2015; Jimenez et al. 2007; Bichona et al. 2008).

3.2 Fate of fipronil metabolites in water of different pH under laboratory conditions

The results relating to persistence and dissipation pattern of desulfinyl fipronil, fipronil sulfide and fipronil sulfone in aqueous solution kept in dark for 120 days (Figure 1 & 2) indicated that 15.17-16.83% of desulfinyl fipronil, 10.67-12.00 % of fipronil sulfide and 12.11-13.50% of fipronil sulfone persisted under acidic pH even after120 days. The degradation of fipronil metabolites followed a first order kinetics with the predicted

half life values ranging from 462 – 495, 693-770 and 577.50-630 days for desulfinyl fipronil, fipronil sulfide and fipronil sulfone depending on treatment. Thus, similar to fipronil, its metabolites were quite stable under acidic pH (Bobe et al. 1998b; Ramesh and Balasudramanium 1999). Under neutral conditions, the degradation of desulfinyl fipronil, fipronil sulfide and fipronil sulfone increased marginally over acidic condition with their persistence ranging from 26.20-28.00, 20.00-24.09, 22.17-26.00 % respectively after 120 days. The half life values, worked out based on first order dissipation kinetics, were found to be 238.97-266.54, 301.30-364.73 and 277.20-301.30 days for desulfinyl fipronil, fipronil sulfide and fipronil sulfone respectively (Table 2). In contrast to acid and neutral pH, the degradation rate of these metabolites was much faster under alkaline condition that registered half life values ranging from 21.79 – 22.57, 31.50-35.53 and 24.57-27.50 days for desulfinyl fipronil, fipronil sulfide and fipronil sulfone respectively. Similar to parent molecule, fipronil metabolites also undergo rapid degradation under alkaline condition (Bobe et al. 1998; Ramesh and Balasudramanium 1999) but without characterization of additional hydrolytic transformed products. The rank order of persistence of these metabolites were very similar under different pH values and stability increased in the order desulfinyl fipronil < fipronil sulfone < fipronil sulfide. The experimental results fitted well with first order kinetics for all the three compounds as the R^2 values for desulfinyl fipronil, fipronil sulfide, fipronil sulfone were in the range of 0.98-0.99, 0.98-0.99 and 0.96-0.99 respectively.

A careful scrutiny of the total ion chromatograms for each of these compounds at different time intervals revealed that two additional peaks with the retention time of 27.202 (Figure 3) and 38.057 minutes (Figure 5), designated as FS-I and FS-II, were observable in water samples treated with fipronil sulfide and fipronil sulfone respectively but not in samples treated with desulfinyl fipronil. It was further evident that an additional peak was detectable on 15 days onwards in the water samples at different pH with its abundance at pH 9.2. The gradual increase in height of this peak throughout the experimental period supported the corresponding increase in its concentration. Bobe et al. 1998b reported that fipronil in water in absence of light undergoes hydrolysis involving nucleophilic attack of the hydroxide ion to a polar nitrile bond of fipronil to yield an unstable hydroxyimine intermediate, which on total tautomerization produces the corresponding fipronil amide. Similarly, all the fipronil metabolites such as desulfinyl fipronil, fipronil sulfide and fipronil sulfone bears a polar nitrile bond and thus prone to nucleophilic attack by one of the ionic components of water, the hydroxide ion, which leads to the formation of the corresponding amide derivative.

3.4 Characterization of FS-I and FS-II based on GC-MS/MS

The mass spectrum of **FS-I** is shown in Figure 4. The mass spectrum showed molecular ion peak $(M^{+\cdot})$ at *m/z* 438 as base peak with four moderately intense diagnostic peaks at *m/z* 369, 352, 255 and 225. The molecular ion peak at m/z 438 seemed to result from the addition of a water molecule across the cyano group of fipronil sulfide. The ion peak at m/z 369 might arise from the loss of a trifluoromethyl group (CF_3) from the M+ peak at m/z 438, while loss of a $-NH_2$ group from ion fragment at m/z 269 could be ascribed to radical cation with m/z 352 (Scheme 1). The ion fragment at m/z 255 appeared to arise from m/z 438 by the cleavage of pyrazole ring, while the ion fragment at m/z 225 formed by the cleavage of C-N bond of the phenylpyrazole moiety. Based on the mass fragmentation pattern, the compound can be characterized as 5 – amino – 1 - [2,6-dichloro-4-(trifluoromethyl)phenyl] – 4   (trifluoromethyl)sulfanyl] - 1H – pyrazole – 3 - carboxamide (FS-I), the amide derivative of fipronil sulfide.

Moreover, the higher retention time (27.202) of **FS-I** than that of parent compound, fipronil sulfide (20.946 minutes) indicated that **FS-I** is more polar than fipronil sulfide. The presence of a $-CONH_2$ group in place of the $-CN$ group at 3 position of the phenyl pyrazole moiety of fipronil sulfide can possibly increase the polarity of the compound with higher retention on the chromatographic column. Thus, it seemed reasonable to assume that fipronil sulfide undergoes a nucleophilic attack by hydroxide ion resulting into the formation of corresponding amide. The plausible mechanism for formation of amide derivative of fipronil sulfide was presented in Scheme 2.

The mass spectrum of FS-II with retention time of 38.057 minutes was shown in Figure 6 with fipronil sulfone. **FS-II** showed the molecular ion peak at *m/z* 470, which probably result by the addition of a water molecule to the cyano group of fipronil sulfone and the base peak at *m/z* 401 by the loss of a trifluoromethyl group (CF_3) from *m/z* 470. The cleavage of pyrazole ring from *m/z* 470 led to the formation of ion fragment at *m/z* 255. The plausible mass fragmentation pattern of compound **FS-II** was shown in Scheme 3. Based on the mass spectral fragmentation pattern, the structure of FS-II could be characterized as 5-amino-1-[2, 6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carboxamide (**FS-II**), the amide derivative of fipronil sulfone. Moreover, the retention time of **FS-II** was higher than that of the parent compound fipronil sulfone (29.445 ± 0.2 min) suggesting it's more polar than the corresponding sulfone. Increase in polarity of the transformed product which further supports the suggested structure of **FS-II**. Similar to **FS-I,** a plausible mechanism for the formation of **FS-II** was shown in Scheme 4. Jones et al. 2007 described FS-I as a microbial transformation product of fipronil sulfide in anoxic sediments. Our result, however,

documented the formation of FS-I in water over a wide range of pH. Contrary to microbial degradation, the formation of FS-I in water via chemical degradation can not be ruled out. Interestingly, the formation of **FS-II** as a degradation product of fipronil appears to be first report.

IV. CONCLUSION:

Fipronil metabolites such as sulphide, sulfone and desulfinyl, although reported to be present in food commodities, also finds its way to natural ecosystem and are more toxic than the parent molecule. The present study demonstrates the rapid degradation of fipronil metabolites in alkaline pH to produce metabolites viz. FS-I and FS-II which needs for their toxicity evaluation. However, in water bodies with acidic to neutral pH, fipronil metabolites are more stable and can be a threat to aquatic organisms. The result observed in the present study might be expected to occur in natural ecosystem. Thus, both the persistence and toxicity of fipronil metabolites are to be taken into consideration while recommending it in agriculture and public health programme.

ACKNOWLEDGMENTS

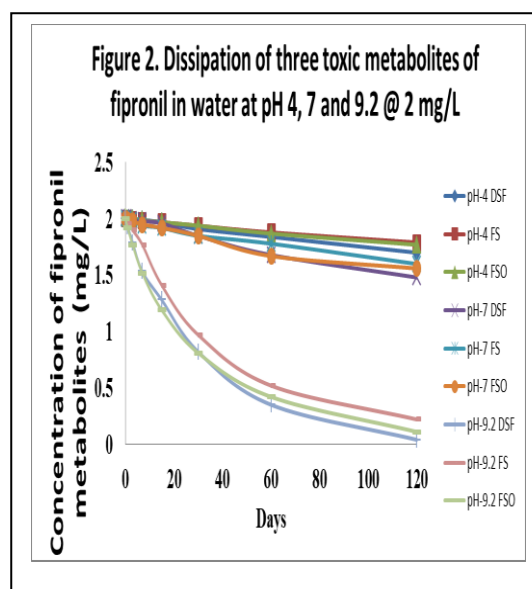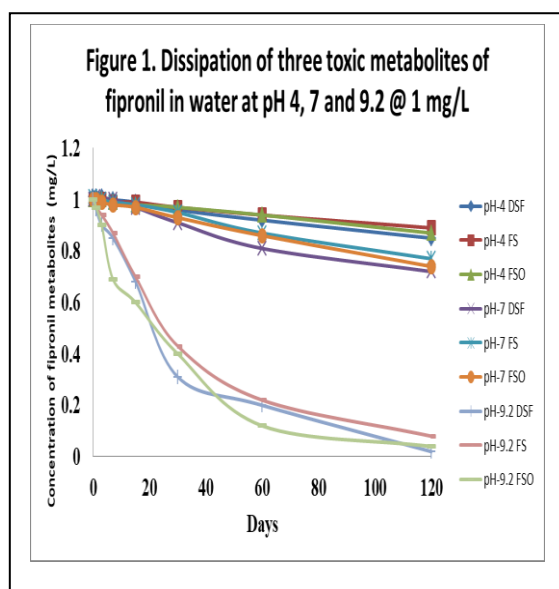## REFERENCES:

[1]. Aajoud A, Ravanel P, Tissut M (2003) Fipronil Metabolism and Dissipation in a Simplified Aquatic Ecosystem. J. Agric. Food Chem. 51: 1347-1352.

[2]. Barbara GS, Zube C, Rybak J, Gauthier M, Grünewald B (2005) Acetylcholine, GABA and glutamate induce ionic currents in cultured antennal lobe neurons of the honeybee, Apis mellifera. J Comp Physiol A. 191:823–836.

[3]. Barbee GC, Stout MJ (2009) Comparative acute toxicity of neonicotinoid and pyrethroid insecticides to non-target crayfish (Procambarus clarkii) associated with rice-crayfish crop rotations. Pest Manag Sci. 65:1250–1256.

[4]. Bhardwaj N, Kumar R, Kaur S, Sahoo SK, Mandal K, Battu RS (2012) Persistence of fipronil and its risk assessment on cabbage (Brassica oleraceae var. capitata L. Ecotoxicology & Environ Safety. 79(1): 301-308.

[5]. Bichona E, Richard CA, Bizeca BL (2008) Development and validation of a method for fipronil residue determination in ovine plasma using 96 well plate solid-phase extraction and gas chromatography–tandem mass spectrometry. Journal of Chromatography A. 1201: 91–99

[6]. Bobe A, Meallier P, Cooper JF, Coste CM (1998b) Kinetics and mechanisms of abiotic degradation of fipronil (hydrolysis and photolysis). Journal of Agriculture and Food Chemistry. 46: 2834-2839

[7]. Chagnon M, Kreutzweiser D, Mitchell EAD, Morrissey CA, Noome DA, et al (2014) Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environ Sci Pollut Res:1–16. doi:10.1007/s11356-014-3277-x

[8]. Chopra I, Chauhan R, Kumari B, Dahiya K (2011) Fate of fipronil in cotton and soil under tropical climatic conditions. Bull. Environ. Contam. Toxicol. 86: 242-245.

[9]. Colliot F, Kukorowski KA, Hawkins DW, Roberts DA (1992) Fipronil: A new soil and foliar broad spectrum insecticide. In Brighton Crop Protection Conference: Pests and Diseases. British Crop Protection Council, Bear Farm, UK, pp 29–34.

[10]. Gupta S, Gajbhiye VT, Gupta RK (2008) Persistence of fipronil in or on Okra, brinjal and gram. In First Int. Conf. on agrochemicals protecting crop health and natural environment. 8-11 Jan, 2008. New Delhi, pp. 193.

[11]. Hainzl D, Casida JE (1996) Fipronil insecticide: Novel photochemical desulfinylation with retention of neurotoxicity. Proc Natl Acad Sci 93:12764–12767.

[12]. Hainzl D, Cole LM, Casida JE (1998) Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chem Res Toxicol. 11:1529–1535.

[13]. Hosie AM, Baylis HA, Buckingham SD, Sattelle DB (1995) Actions of the insecticide fipronil on dieldrin sensitive and resistant GABA receptors of Drosophila melanogaster. Br J Pharmacol 115:909–912.

[14]. Ikeda T, Nagata K, Kono Y, Yeh JZ, Narahashi T (2004) Fipronil modulation of GABA receptor single-channel currents. Pest Manag Sci. 60(5):487–492.

[15]. Jimenez JJ, Bernal JL, Nozal MJD, Martın MT, Mayo R (2007) Comparative study of sample preparation procedures to determine fipronil in pollen by gas chromatography with mass spectrometric and electron-capture detection. Journal of Chromatography A. 1146: 8–16.

[16]. Jones WJ, Mazur C, Kenneke JF, Garrison AW (2007) Enantioselective microbial transformation of the phenylpyrazole insecticide fipronil in anoxic sediments. Environmental Science and Technology. 41: 8301–8307

[17]. Kaur R, Mandal K, Kumar R, Singh B (2015) Analytical Method for Determination of Fipronil and its Metabolites in Vegetables Using the QuEChERS Method and Gas Chromatography/Mass Spectrometry Journal of AOAC International 98 (2): 464-71.

[18]. Kumar R, Singh B (2013) Persistence and metabolism of fipronil in rice (Oryza sativa Linnaeus) field. Bull. Environ. Contam. Toxicol 90(4): 482-486.

[19]. Lao W, Tsukada D, Greenstein D, Bay SM, Maruya KA (2010) Analysis, occurrence and toxic potential of pyrethroids and fipronil in sediments from an urban estuary. Toxicol & Chemistry. 29(4): 843-851.

[20]. Lin K, Haver D, Oki L, Gan J (2009) Persistence and sorption of fipronil degradates in urban stream sediments. Environ. Contam.& Toxicol. 28(7): 1462-1468.

[21]. Madsen J, Sandstrom M, Zaugg S (2003) Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory – A Method Supplement for the Determination of Fipronil and Degradates in Water by Gas Chromatography/Mass Spectrometry: Open File Report 02-462.

[22]. Mohapatra S, Deepa M, Jagdish GK, Rashmi N, Kumar S, Prakash GS (2010) Fate of fipronil and its metabolites in/on grape leaves, berries and soil under semi arid tropical climatic conditions. Bulletin of Environ Contamination Toxicology. 84:587–591

[23]. Narahashi T, Zhao X, Ikeda T, Nagata K, Yeh JZ (2007) Differential actions of insecticides on target sites: basis for selective toxicity. Hum Exp Toxicol. 26:361–366.

[24]. Qian C, Dai J, Tian Y, Duan Y, Li Y (2020) Efficient degradation of Fipronil in water by microwave-induced argon plasma: Mechanism and degradation pathways. Science of The Total Environment 725: 138487.

[25]. Ramesh A, Balasudramanian M (1999) Kinetics and hydrolysis of fenamiphos, fipronil and trifluralin in aqueous buffer solutions. Journal of Agriculture and Food Chemistry. 47: 3367-3371

[26]. Raveton M, Aajoud A, Willison J, Cherifi M, Michel T, Ravanel P (2007) Soil distribution of fipronil and its metabolites originating from a seed-coated formulation. Chemosphere 69(7):1124-9

[27]. Sadaria AM, Sultan AR, Moran KD, Teerlink J, Brown JV, Halden RJ (2017) Passage of fiproles and imidaclorprid from urban pest control uses through wastewater treatment plants in Northern California, USA. Environ. toxicol. Chem. 36(6): 1473-1482.

[28]. Shi L, Jiang Y, Wan Y, Huang J, Meng Q, He Z, Xu S, Xia W (2020) Occurrence of the insecticide fipronil and its degradates in indoor dust from South, Central, and North China. Science of The Total Environment Volume 741: 140110

[29]. Supowit SD, Sadaria AM, Reyes EJ, Halden RU (2016) Mass balance of fipronil and total toxicity of fipronil related compounds in process streams during conventional wastewater and wet land treatment. Environ Sci. &Toxicol. 50(3): 1519-1526.

[30]. Tingle CCD, Rother JA, Dewhurst CF, Lauer S, King WJ (2003) Fipronil: environmental fate, ecotoxicology and human health concerns. Rev Environ Contam Toxicol. 176:1–66.

[31]. US EPA (United States Environmental Protection Agency) (1996) New Pesticide Fact Sheet. PB96-181516. EPA737-F-96-005. U.S. EPA Office of Prevention, Pesticides and Toxic Substances. May 1996.

Table 1: GC-MS parameters for analyzing desulfinyl fipronil, fipronil sulfide and fipronil sulfone

Model	Gas Chromatograph CP 3800 (make Varian)				
Column	VF-1701, 30m × 0.25mm × 0.25µm				
Detector	Mass spectrometric detector: Saturn – 2200				
Carrier gas & flow	Helium (99.999% pure) 1mL min^{-1}				
Injector temperature	260ºC				
Injection mode	split				
Oven temperature	170ºC hold for 1min - increased @ 3.5ºC min^{-1} to 240ºC and hold for 5min - increased @ 5ºC min^{-1} to 265ºC hold for 10 min – increased @ 20º C min^{-1} to 280ºC hold for 5 min				
Trap temperature	200ºC				
Manifold temperature	40ºC				
Transfer line temperature	280ºC				
Emission current	15 µamps				
Ionization mode	Electron Impact (EI)				
Ion preparation	SIS and TIC				
Details of SIS method					
Segment description	Start (min)	End (min)	Ionization mode	Ion preparation	Quantifying ion	Qualifying ions
Solvent delay	0	17.00	-	-		
Desulfinyl fipronil	17.00	20.00	EI	SIS	333	388,390
Fipronil sulfide	20.00	27.00	EI	SIS	351	353,420
Fipronil					369	367,420
Fipronil sulfone	27.00	35.00	EI	SIS	383	385,255

DSF: Desulfinyl fipronil. FSO: Fipronil sulfone. FS: Fipronil sulphide.

Table 2. Half life values and rate equations for degradation of desulfinyl fipronil, fipronil sulfide and fipronil sulfone at different pH

Compound	pH of water	Fortification Level(mg L^{-1})	First order rate equation	Determination coefficient (R^2)	Half life T½ (days)
Desulfinyl fipronil	4	1	$C = 1.01e^{-0.0015t}$	0.9942	462.00
	4	2	$C = 2e^{-0.0014t}$	0.9944	495.00
	7	1	$C = e^{-0.0029t}$	0.9774	238.97
	7	2	$C = 2.01e^{-0.0026t}$	0.9898	266.54
	9.2	1	$C = e^{-0.0307t}$	0.9881	22.57
	9.2	2	$C = 2e^{-0.0318t}$	0.9965	21.79
Fipronil sulfide	4	1	$C = e^{-0.0009t}$	0.9982	770.00
	4	2	$C = 2e^{-0.001t}$	0.9937	693.00
	7	1	$C = 1.01e^{-0.0023t}$	0.9935	301.30
	7	2	$C = 2e^{-0.0019t}$	0.9779	364.73
	9.2	1	$C = e^{-0.022t}$	0.9823	31.50
	9.2	2	$C = 2e^{-0.0195t}$	0.9842	35.53
Fipronil sulfide	4	1	$C = e^{-0.0012t}$	0.9895	577.50
	4	2	$C = 2.01e^{-0.0011t}$	0.9946	630.00
	7	1	$C = e^{-0.0025t}$	0.9988	277.20
	7	2	$C = 2e^{-0.0023t}$	0.9549	301.30
	9.2	1	$C = e^{-0.0282t}$	0.9688	24.57
	9.2	2	$C = 2e^{-0.0252t}$	0.9908	27.50

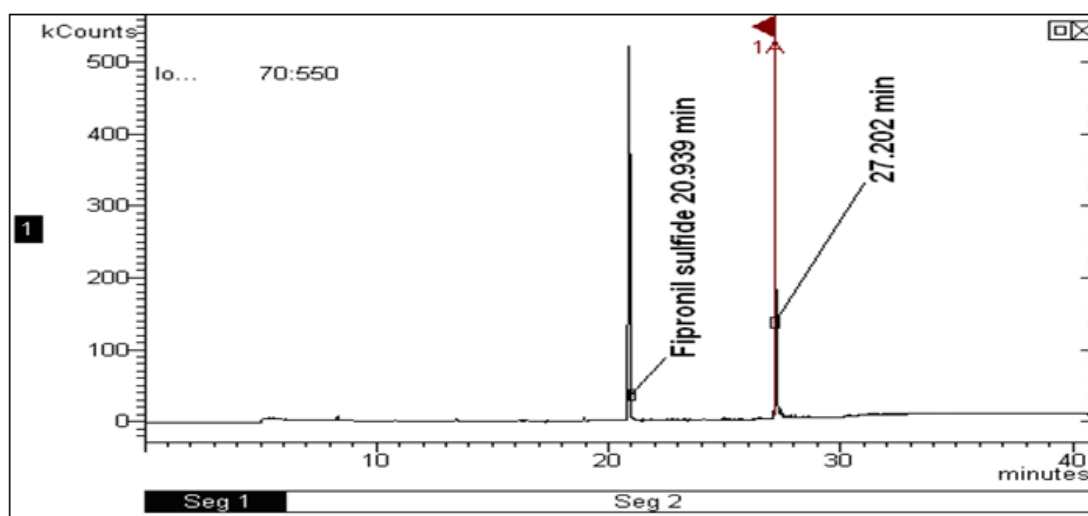Here, C = concentration (in mg mL^{-1}) and t = time in days

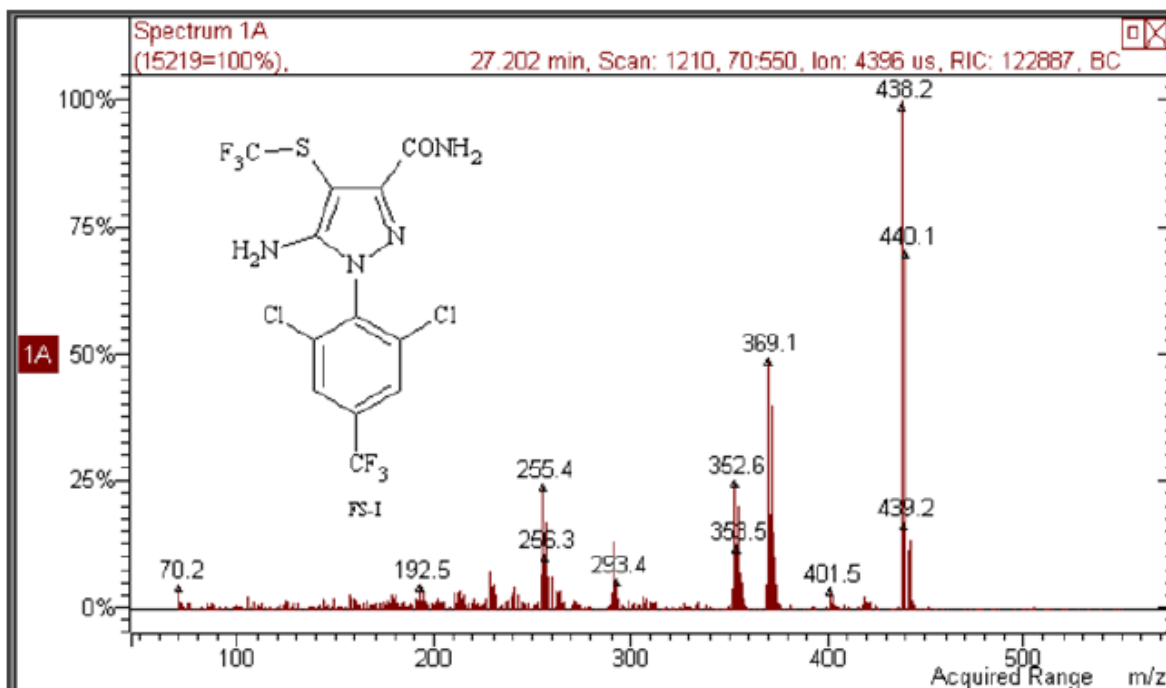**Figure 3.** Total ion chromatogram of fipronil sulfide including an additional peak at retention time 27.202 minute
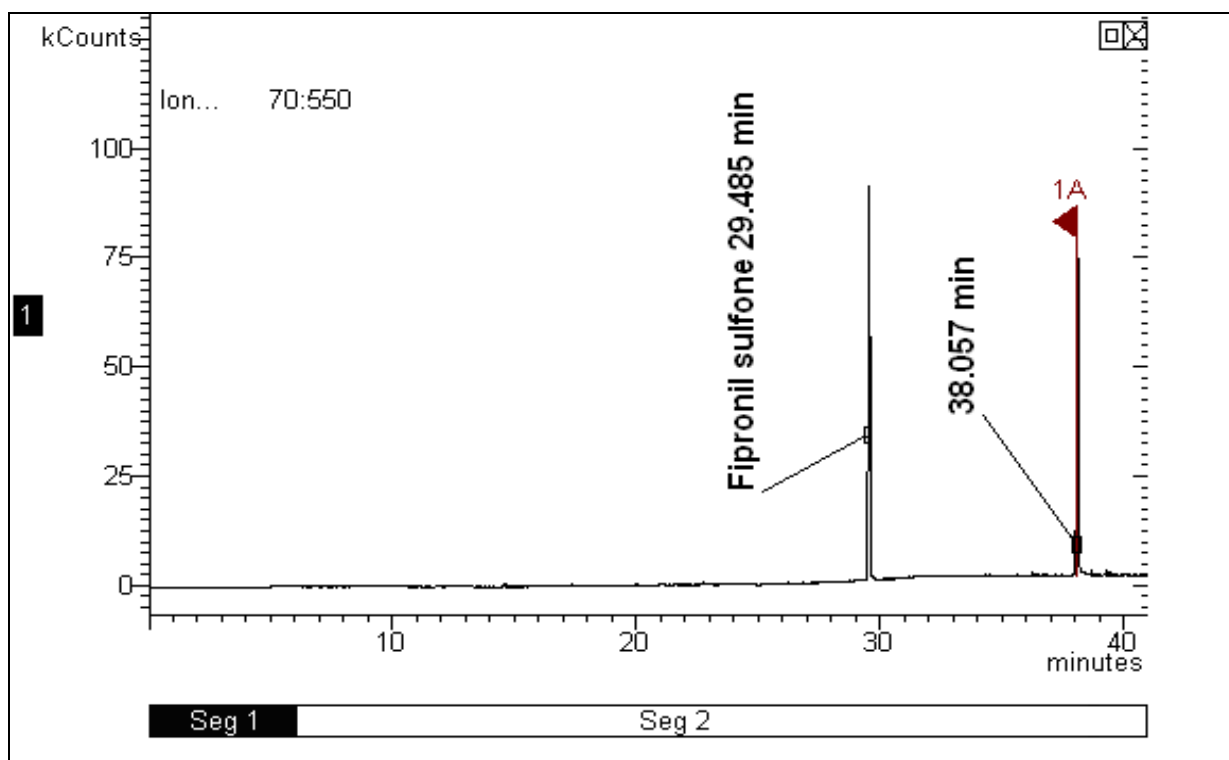
Figure 4. Mass spectrum of **FS-I**

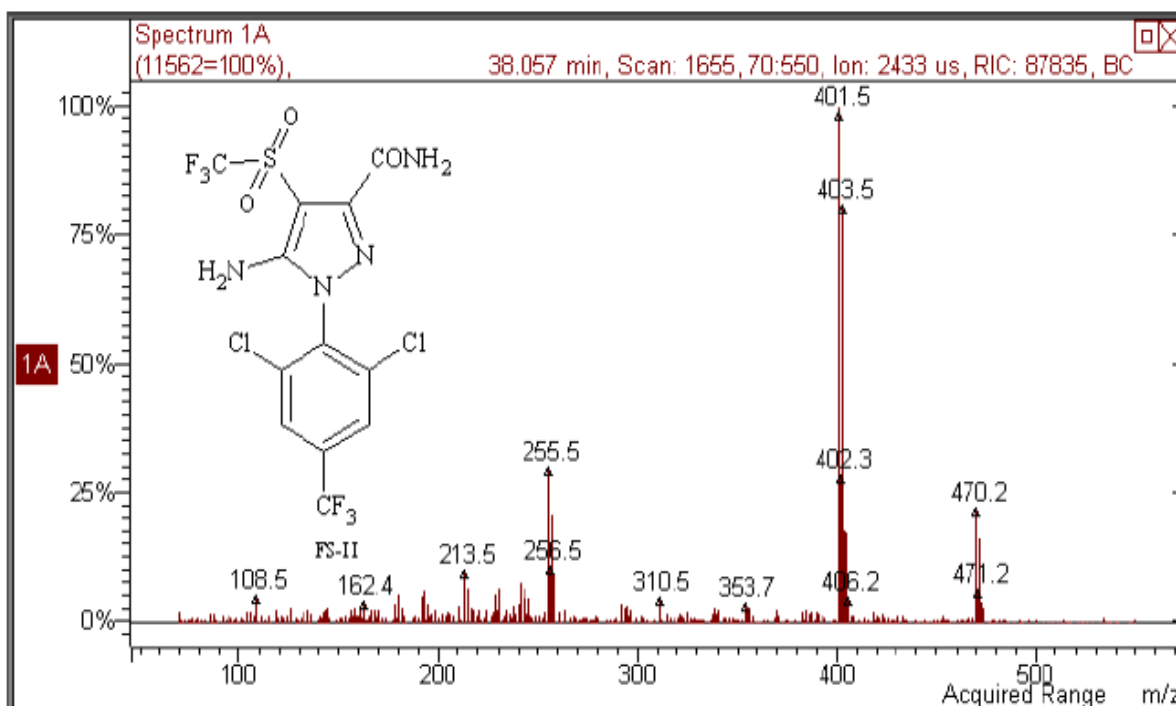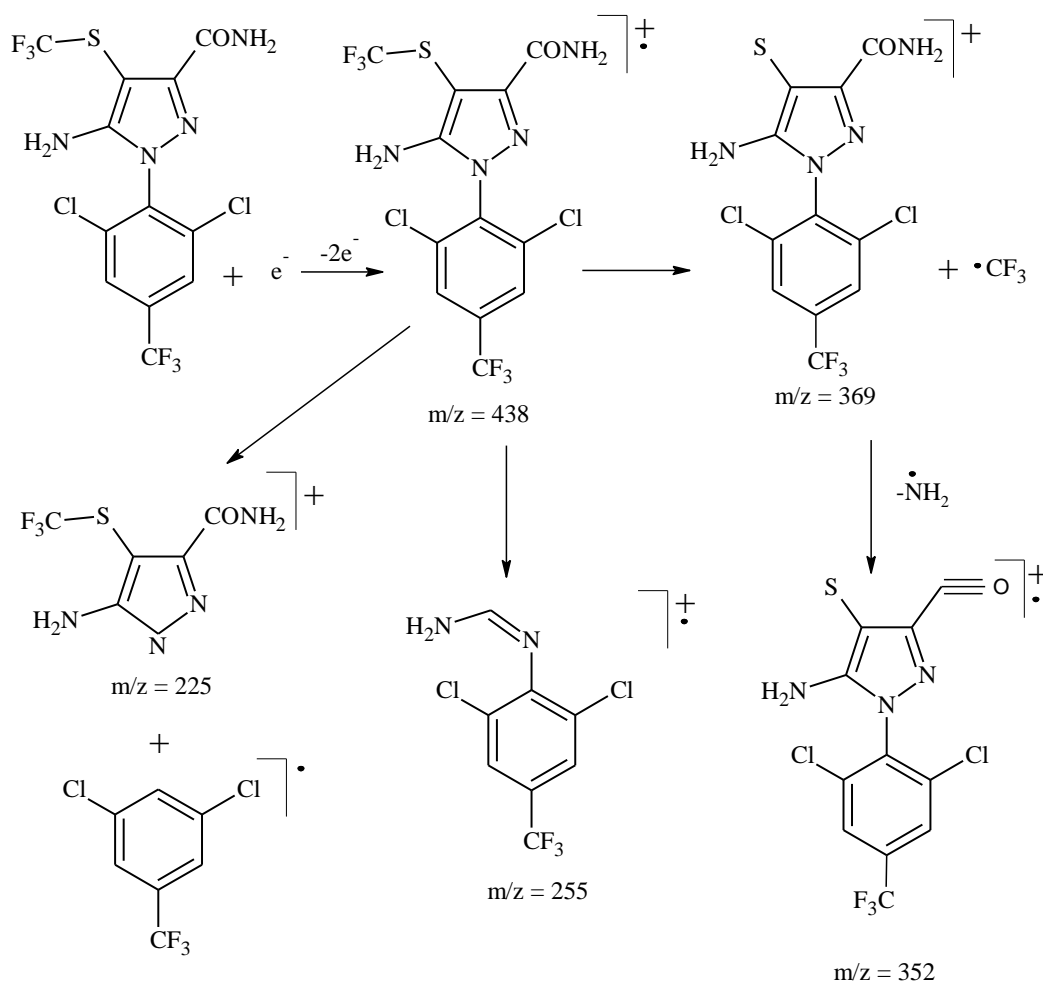**Figure 5.** Total ion chromatogram of fipronil sulfone including an additional peak at 38.057 min. RT
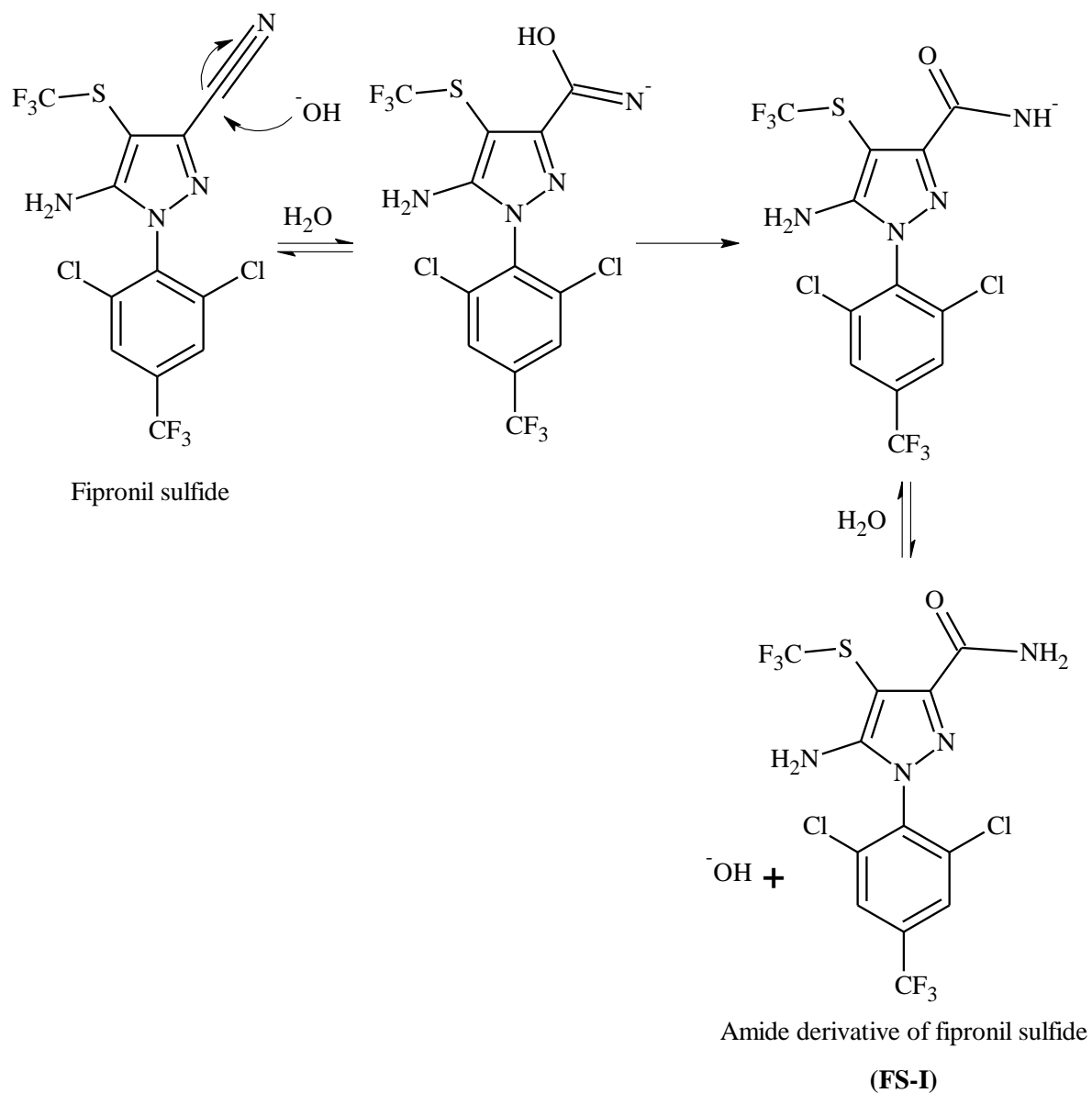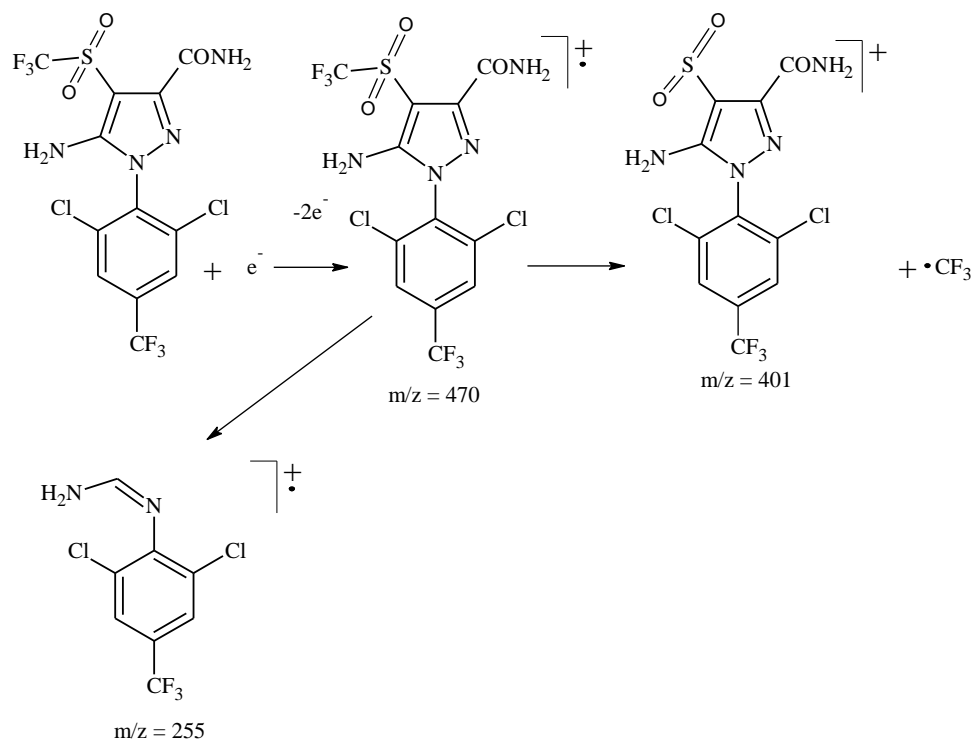
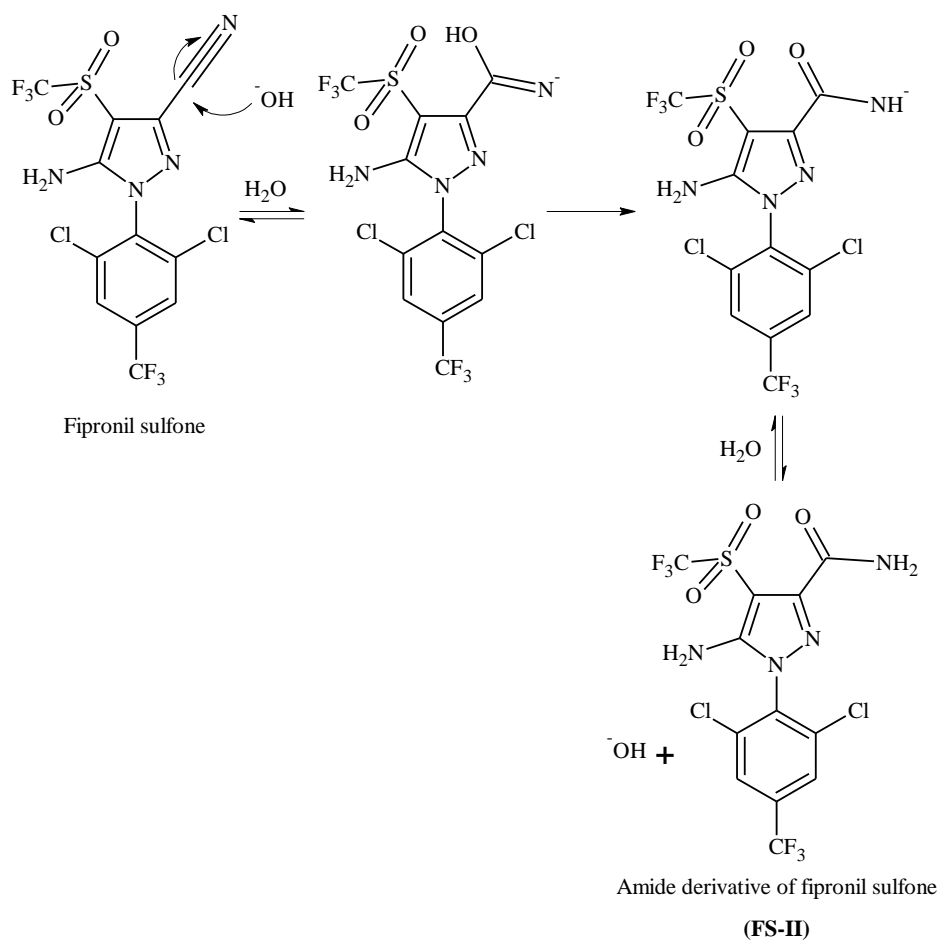**Figure 6.** Mass spectrum of compound **FS-II**

Scheme 1. Plausible mass fragmentation pattern of compound **FS-I**

Scheme 2: Plausible mechanism for formation of amide derivative of fipronil sulfide

Scheme 3. Plausible mass fragmentation pattern of compound **FS-II**

Scheme 4. Plausible mechanism for formation of amide derivative of fipronil sulfone