Bull Environ Contam Toxicol (2011) 87:653–656
DOI 10.1007/s00128-011-0421-x

Determination of Hymexazol in Cucumber and Soil Samples by Derivatization Using GC-FPD

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Received: 1 June 2011 / Accepted: 19 September 2011 / Published online: 30 September 2011

Abstract A sensitive and effective analytical method for the determination of hymexazol in cucumber and soil samples by gas chromatography with a flame photometric detector was developed. This method was validated with fortified at three different levels of 0.2, 1.0 and 5.0 mg/kg. Average recoveries obtained from cucumber and soil samples at three fortified levels were 94.0%–107.8% with relative standard deviations (RSDs) of less than 11.4%. Limits of quantification (LOQ) in cucumber and soil were 0.2 mg/kg. The method was successfully applied to determine hymexazol in real samples of cucumber and soil under open fields.

Keywords Hymexazol · Cucumber · Soil · Derivatization

Cucumber is an important vegetable crop in China, which suffers yield losses due to diseases. Hymexazol, 3-hydroxy-5-methoxzaolum, is an effective fungicide (Fig. 1) recommended for use to against various diseases in cucumber such as damping-off, anthracnose, blight, dry-rot, phytophthora blight that caused by fungus, which lead great losses of cucumber yields. Because hymexazol is a systemic fungicide, translocated to most plant tissues, there is potential risk to consumer health if the pesticide is still present at harvest (Qian et al. 2011). Therefore, it is imperative to set up an effective and efficient analytical method to evaluate and monitor hymexazol residues. The maximum residue limits (MRLs) in Japan for hymexazol in cucumber is 0.5 mg/kg. However, no MRLs have been set by China and the WHO/FAO.

There are limited reports available in literature about the analytical methods of hymexazol. The difficulty in hymexazol analysis results from its high polarity and spectral properties. Its maximum-absorption is at 200 nm, which results in severe solvent interference during HPLC analysis. Furthermore, hymexazol is unstable at high temperature, making direct GC analyzing unsuitable. Pilar et al. (2008) developed a method for the analysis of multi-residues of oxazole fungicides including hymexazol in wines and juices by UPLC with two novel sample preparation procedure (i) stir bar sorptive extraction (SBSE) and (ii) membrane-assisted solvent extraction (MASE). Tamura et al. (2008) reported a method for the determination of hymexazol in agricultural products by GC-NPD with a high-polarity capillary column and highly deactivated inlet liner. Their results showed that the recovery of hymexazol from spiked agricultural products ranged from 65.0% to 84.7%, and the limits of detection (LOD) was 0.02 mg/kg. The present work was carried out to establish a simple and efficient analytical method for determination of hymexazol. Hymexazol was applied in cucumber and soil to evaluate its dissipation and residue levels under field conditions, and afford evidence for registration in China.

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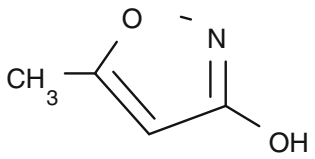

Fig. 1 The chemical structure of hymexazol

Materials and Methods

Hymexazo1 standard (purity at 98%) was provided by the Quality Supervision and Inspection Center of Pesticide (China). A stock solution of hymexazol (1,000 mg/kg) was prepared in acetone, and working standards were obtained after further diluted to the required concentration. The stock and working standard were stored in dark at 4°C. O, O-diethylthiophosphoryl chloride (DECTP) with purity at 98% (Acros Organics Co. USA) was dissolved in acetone (0.2 mL DECTP was added to 100-mL volumetric flask). Macrogol-400 (PEG) (First Reagents Factory of Shanghai China) was dissolved in acetone (1 g PEG-400 was added to 100-mL volumetric flask). Acetone, sodium hydrogen carbonate, sodium chloride, hydrochloric acid, potassium carbonate, ether and sodium sulphate anhydrous were of analytical grade and were purchased from Beijing Chemical Reagents Company (China). Sodium sulfite was added in ether overnight and redistilled. Anhydrous sodium sulphate was baked at 650°C for 4 h before use.

An Agilent technologies 4890 GC system equipped with a flame photometric detector (FPD) under phosphorus mode was used with a HP 1701 capillary column (30 m × 320 mm × 0.25 μm). Temperatures of injector and detector were held constant at 250 and 230°C, respectively. The column-oven temperature was 180°C. High purity nitrogen was used as carrier gas and makeup gas with flow rates of 1.2 and 15 mL/min, respectively. Hydrogen and air were used as detector gases at 75 and 100 mL/min, respectively. Injections were carried out in the splitless mode, and the injection volume was 2 μL.

Twenty grams of cucumber or soil were extracted with 50 mL acetone in a 250-mL conical flask with stopper, and shaken for 30 min on an oscillator. The extracts were transferred to another 150-mL flat bottom flasks containing 1 mL of 1% PEG acetone solution, and then the flat bottom flasks were washed twice with 20 mL acetone. The mixtures were evaporated to near dryness in a rotary evaporator at 40°C, washed with 40 mL of 2% $NaHCO_3$ aqueous solution (20 mL × 2 times), and then transferred into a 250-mL separating funnel to (Eight grams of NaCl were added for cucumber samples firstly) The residues were extracted by liquid–liquid partition with ether two times at the same volume of 25 mL. The organic layer was discarded. Twenty milliliter of 20% NaCl aqueous solution and 4 mL of 2 N HCl were added to the aqueous layer, and then the pH was adjusted below 2. The mixtures were extracted by liquid–liquid partition with 75 mL of ether (25 mL × 3 times), shaking for 2 min each time. The ether fractions were combined and filtered through anhydrous sodium sulphate. One milliliter of 1% PEG was added to the extracts and the volume reduce to 1 mL by evaporation at 40°C, and then mixed with 0.05 mL of 25% K_2CO_3 aqueous solution and 2 mL of 0.2% DECTP acetone solution. After incubation for 30 min at 45°C, the mixtures were evaporated to near dryness at 40°C and redissolved in 2 mL of methanol.

Field trials including the dissipation and final residue experiments were carried out at Beijing, China during 2010. The experiment field consisted of three replicated plots with each plot being 20 m^2. Another three plots were sprayed with water and maintained as controls. During the experiment period, cucumber plants received routine horticultural treatment. In order to study the dissipation trends of hymexazol in cucumber and soil, 65% wettable power (WP) formulation of hymexazol was sprayed on cucumber at the dosages of 50 mg active ingredient per plant. Cucumber and soil samples were collected on 0 (2 h after spraying), 1, 2, 3, 4, 5, 7, 10, and 14 days. In order to study the final residues of hymexazol, 25 mg active ingredient per plant (low dosage) and 50 mg active ingredient per plant (high dosage) were sprayed on cucumber. Cucumber and soil samples were collected randomly from each treatment plot at 7, 14 and 21 days after the hymexazol application. Soil was sampled to a depth of 0–10 cm in each plot using a tube auger. Control samples were obtained from control plots. All samples were put into polyethylene bags and transported to the laboratory where they were chopped, thoroughly mixed, and divided into sub-samples. The sub-samples were kept at −20°C until analysis.

Results and Discussion

Acetone, dichloromethane, and methanol were tested as extraction solvents, and acetone was the most efficient. Different amount of acetone (30, 50, 70 mL) was also studied and the results indicated that the volume of 50 mL was the most suitable. Because hymexazol has relatively high volatility and adsorptivity, 1 mL of 1% PEG acetone solution was added as protective agent to prevent the loss of hymexazol during evaporation steps.

The derivatization reaction proved to be a good method to convert hymexazol into a more stable compound with lower polarity. According to the structure of hymexazol, we hypothesized that a hydroxyl should be relatively easy to react with phosphorus oxychloride. Some studies

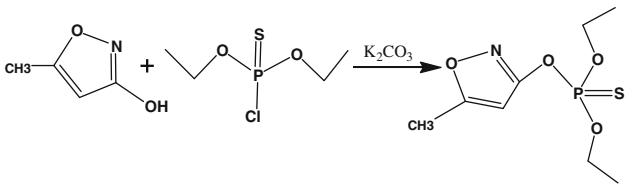

Fig. 2 The reaction procedure of derivatization

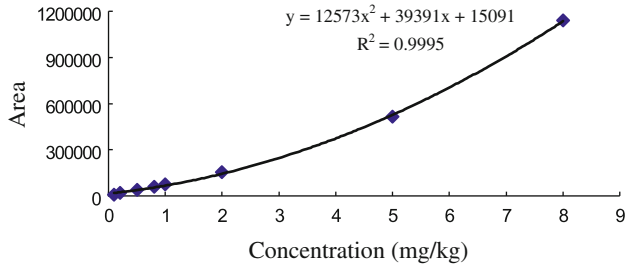

Fig. 3 The standard curve of hymexazol in cucumber at the concentrations ranged from 0.1 to 8.0 mg/kg

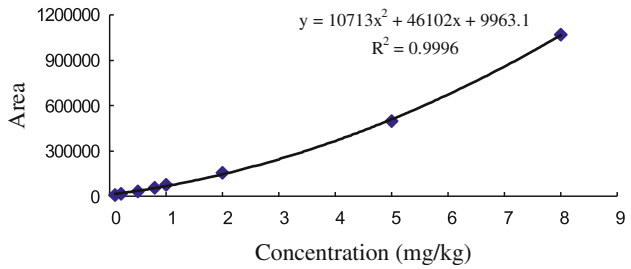

Fig. 4 The standard curve of hymexazol in soil at the concentrations ranged from 0.1 to 8.0 mg/kg

reported that organic phosphate could be obtained by the reaction of phosphorus oxychloride and hydroxyl (Argauer 1969, Butler and Madonough 1968). Figure 2 showed the reaction procedure of derivatization. The obtained hymexazol derivative contained a phosphorus element and responded well on FPD, which indicated this procedure suitable to be analyzed by GC-FPD. This reaction could be easily conducted with relatively high productivity, few reagents and no special instrument.

Quantification was performed by comparing sample peak areas to a standard curve derived from hymexazol-spiked samples at eight different concentrations. For chromatographic procedures, a relation could be observed between detector response (y) and analyte concentration (x). The stock solution of hymexazol (1,000 mg/kg) was diluted stepwise with acetone to make a series of standard solutions (2.0, 4.0, 10.0, 16.0, 20.0, 40.0, 100.0, 160.0 mg/kg). One milliliter of the standard solutions was added to 20 g of cucumber or soil samples. The standard series obtained were 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, 5.0, 8.0 mg/kg, correspondingly. Spiked-samples were treated following the sample preparation and detection mentioned above. Figures 3 and 4 showed the standard curve of hymexazol in cucumber and soil samples.

The efficiency of the analytical method was evaluated by spiking hymexazol into cucumber and soil samples at 0.2, 1.0, and 5.0 mg/kg with five repetitions at each level. The recovery of hymexazol from cucumber and soil samples ranged from 94.0% to 107.8% and the RSD range was 8.1% to 11.4% (Table 1).

The recovery and accuracy of the our analytical method were within the acceptable parameters described in the Guideline on Pesticide Residue Trials issued by the Ministry of Agriculture of the People's Republic of China, 2004.

Limit of detection (LOD) was obtained as signal-to-noise (S/N) ratios of 3, and limit of quantification (LOQ) was defined as the minimum of the spiked samples. The presence of potential interference in the chromatograms from the analyzed samples was monitored by running control blank samples in each calibration. Blank samples were extracted and derivatized by the same procedure. The LOD by this method on our apparatus was 1×10^{-10} g and LOQ for hymexazol in cucumber and soil was 0.2 mg/kg.

The method was validated with three spiked levels which showed good accuracy and sensitivity. It was successfully employed for the determination of hymexazol in cucumber and soil samples collected from treated fields. The chromatograms of hymexazol in cucumber and soil samples were shown in Figs. 5 and 6. Compared to others

Table 1 Recovery and RSD of hymexazol in samples at different levels

Sample	Fortified level (mg/kg)	Recovery (%)						RSD (%)
		1	2	3	4	5	Average	
Cucumber	0.2	102.8	88.3	91.6	86.0	112.0	96.1	11.4
	1.0	104.2	99.1	81.9	90.5	107.6	96.7	10.8
	5.0	101.8	100.7	95.7	86.7	85.6	94.0	8.1
Soil	0.2	97.1	100.0	112.2	110.0	119.7	107.8	8.6
	1.0	99.6	91.4	110.9	86.1	106.7	98.9	10.4
	5.0	107.9	89.4	89.5	87.6	107.1	96.3	10.7

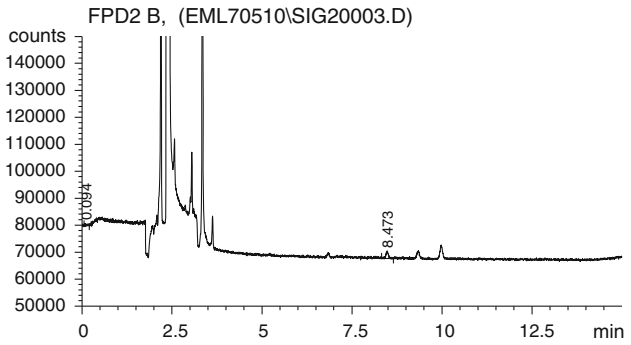

Fig. 5 Chromatogram of hymexazol in cucumber (0.2 mg/kg)

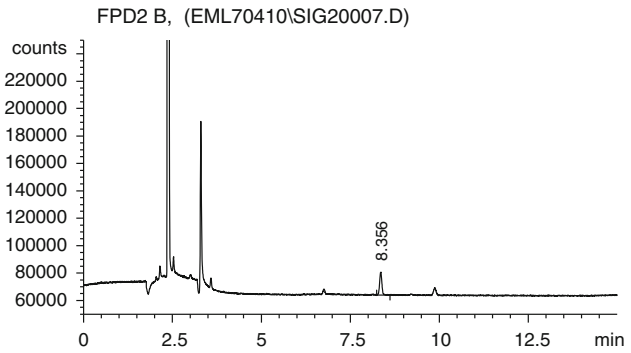

Fig. 6 Chromatogram of hymexazol in soil (1.1 mg/kg)

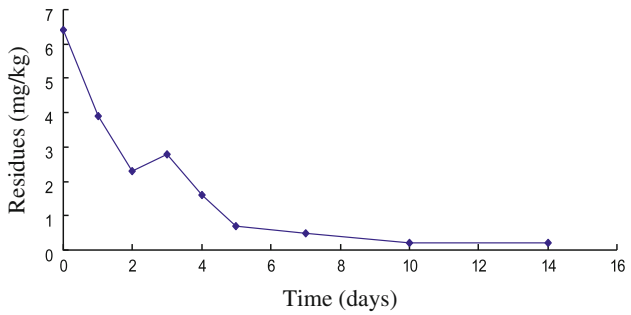

Fig. 7 Dissipation of hymexazol in soil

studies, in which UPLC or GC-NPD was used to analysis hymexazol, this method showed satisfied recovery and precision, high sensitivity and low LOD and LOQ.

The residue dynamics of hymexazol in soil was shown in Fig. 7. The initial concentration of hymexazol in soil was 6.4 mg/kg after 2 h of the application. Five days after application, the dissipation rate of hymexazol was 89%. The dynamic regression equation and the half-life of hymexazol in soil was $C=16.0218e^{-0.3667T}$, $T_{1/2}= 1.9$ days. The dissipation of pesticides in soil was affected by variety of complex physical, chemical and biological processes, including sorption–desorption, volatilization, chemical and biological degradation, uptake by plants, run-off and leaching under field conditions (Zhang and Cooper 1996). Hymexazol residues in cucumber were lower than the Japanese maximum residue limits (MRLs) of 0.5 mg/kg on 7, 14 and 21 days after the treatment of this pesticide. With respect to hymexazol, the cucumbers would be considered safe to human consumption.

Acknowledgments This work was supported by grants from the Institute for the Control of Agrochemicals, Ministry of Agriculture of the People's Republic of China.

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