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Article

Determination of Benazolin-Ethyl Residues in Soil and Rape Seed by SPE Clean-Up and GC with Electron Capture Detection

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A method has been developed and established for residue determination of benazolin-ethyl in soil and rape seed samples by gas chromatography with electron capture detection (GC–ECD). Limits of quantification of the method are 0.005 mg/kg for both soil and rape seed, which are sufficiently below the maximum residue limit, and the limit of detection is 0.0023 ng. The average recoveries of the analyte range from 85.89 to 105.84% with relative standard deviations (coefficient of variation) less than 5.53% at the three spike levels (0.005, 0.1 and 0.5 mg/kg). The half-life of benazolin-ethyl in soil from the experimental field is 4.62 days. The final residues of benazolin-ethyl in soil and rape seed samples are lower than 0.005 mg/kg at harvest time. Direct confirmation of the analyte in real samples is achieved by GC–mass spectrometry. It is demonstrated that the proposed method is simple, rapid and efficient, and reliable to detect benazolin-ethyl residues in soil and rape seed samples.

Introduction

Herbicide treatments of crops allow economic weed control and provide cost-effective increases in agricultural productivity (1). However, in recent years, application of herbicides has become a controversial issue because of the potential for contributing to human health hazards and causing major environmental contamination problems and unwanted biotoxicity (2, 3). Such concerns have led to increased interest in the dissipation pathways of herbicides (4).

Benazolin-ethyl (CAS Registry No. 25059-80-7), 4-chloro-2-benzothiazole oxide-3-yl ethyl acetate, is a selective, systemic, post-emergence herbicide (5) developed by Boots. It is primarily used in rape fields to kill annual broad-leaved weeds, especially black bindweed, chickweed and cleavers. Figure 1 illustrates the chemical structure of benazolin-ethyl.

To our knowledge, although benazolin-ethyl has been marketed and extensively used in China for several years, few research reports have been published about its residue and degradation. Until now, only Wei (6) and Jiang (7) have reported a macro analysis study of benazolin-ethyl using high-performance liquid chromatography (HPLC), and Yang (8) reported a study using the gas chromatography–flame ionization detection (GC–FID) method. The purpose of this article was to study the dissipation rate of benazolin-ethyl in soil and the terminal residue in rape seed samples, and to provide a guideline for the scientific and safe use of benazolin-ethyl.

Experimental

Reagents and chemicals

Benazolin-ethyl reference standard (purity >99.5%) was obtained from Jinan Kesaijinong Chemical Co. (Shandong, China). Other solvents and chemicals were of analytical grade from Dikma Limited (China), except for *n*-hexane for GC–electron capture detection (ECD) analysis, which was chromatographic grade. Solid-phase extraction (SPE) columns (PSA, 500 mg, 3 mL) were purchased from Agela Technologies (Beijing, China).

Preparation of the herbicide standard solutions

A standard solution (1,000 mg/L) of benazolin-ethyl was prepared in *n*-hexane. Working standard solutions of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L were obtained by volumetric serial dilutions. All solutions were protected against light in brown containers and stored in a refrigerator at 4°C.

Apparatus and chromatographic condition

The analyte was conducted with a gas chromatograph (Shimadzu GC-2014) equipped with a split/splitless injector and an ECD. A capillary column (Rtx-5, 30 m × 0.25 mm × 0.25 μm film thickness) was used as separation column, and the carrier gas was N_2 (>99.999%) with a flow rate of 20 cm/s. Injector and detector temperatures were 280 and 310°C, respectively. The column oven temperature was initially held at 120°C for 1 min, then the temperature was ramped at 20°C/min to 240°C, then at 3°C/min to 270°C, and finally programmed at 20°C/min to 290°C, which was held for 1 min. Injection volume was 1 μL with a split ratio of 20. Under these conditions, the retention time of benazolin-ethyl was approximately 11.1 min.

Confirmatory analysis of the benazolin-ethyl was performed with a Shimadzu GCMS-QP2010 E (EI) system. The GC column and oven temperature program were used as described previously. Injection volume was 1 μL in splitless mode. An Rtx-5 capillary column (30 m × 0.25 mm × 0.25 μm film thickness) was used throughout the entire experiment. Helium (>99.999%) was used as carrier gas at a flow of 37.5 cm/s. Eluent from the GC column was fed into the 80.8 eV electron-impact ionization source, which was maintained at 200°C. The acquisition was performed in full-scan mode in the range m/z 50–450.

Field trials

The field trials, including the dissipation experiments and terminal residue experiments, were implemented in Sichuan, which is located in the southwest of China. A random block scheme was used with three replications for each test. Each plot had a dimension of 30 m^2, and a control plot was separated by guard rows to avoid contamination by drift.

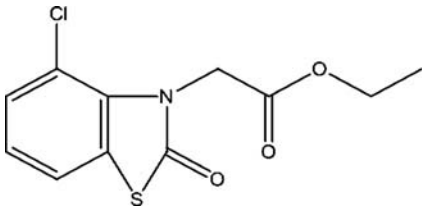

Figure 1. Chemical structure of benazolin-ethyl.

Benazolin-ethyl (20% EC) was sprayed in the rape field after the emergence of the first three leaves of the broadleaf weeds at the dose of 2,250 g/a.i./ha^{-1} (1.5 times the recommended dosage), which was diluted 3,000 to 4,000 times with water. The terminal residue experiments were carried out with a dosage level of 1,500 g/a.i./ha^{-1} (recommended dosage) and a higher dosage level 2,250 g/a.i./ha^{-1} (1.5 times the recommended dosage), respectively. A plot with the same size but no benazolin-ethyl application was simultaneously compared.

Representative soil samples were collected from different depths ranging from 0–10 cm with a tube auger at 0 (2 h after spraying), 1, 3, 5, 7, 14, 21 and 28 days after spraying. To determine the final residue of benazolin-ethyl, both rape seed and soil samples were collected at harvest time. After picking, the samples were put into polyethylene bags and transported to the laboratory, where they were chopped, thoroughly mixed, and divided into three sub-samples each. All samples were stored in a freezer at −20°C before further analysis.

Sample preparation

Rape seed and soil samples were collected from the experimental field located in Sichuan, and all of the samples were stored at −20°C.

Analytical method

A 10-g sample of homogenized soil was extracted with 40 mL methanol–water (1/1, v/v) in a 250-mL conical flask by shaking thoroughly for 30 min on a mechanical horizontal shaker. The sample was filtered through a 12-cm Buchner funnel into a 250-mL side-arm flask under vacuum, and the residue was washed twice with additional 20 mL of methanol–water (1/1, v/v). The combined filtrate was then transferred to a 250-mL separatory funnel in the presence of 30 mL 2% Na_2SO_4 aqueous solution, and the analyte was subsequently extracted with 20 mL dichloromethane by vigorously shaking for approximately 1 min, respectively. The organic phase was dehydrated on anhydrous sodium sulfate and collected in a 250-mL flask, then evaporated to near dryness using a vacuum rotary evaporator at 45°C. After the extract was dried under a gentle nitrogen stream, 10 mL of petroleum ether was added to dissolve the sample for purification by the PSA cartridge, which was preconditioned with 2 mL petroleum ether. Immediately, a 2-mL extract solution was loaded onto the cartridge at a flow rate of approximately 1 mL/min and the eluate was discarded. The cartridge was re-washed with 2 mL petroleum ether–ethyl acetate (95/5, v/v), the elute was discarded, and then the benazolin-ethyl analyte was eluted with 4 mL petroleum ether–ethyl acetate (9/1, v/v), and the eluate was concentrated to near dryness at 45°C by a vacuum rotary evaporator and completely dried under a nitrogen purge. The resulting residue was re-dissolved in 2 mL *n*-hexane for GC–ECD analysis.

A 10-g rape seed sample was extracted by adding 40 mL ethyl acetate to a 250-mL conical flask by shaking thoroughly for 30 min on a mechanical horizontal shaker. The sample was filtered through a 12-cm Buchner funnel, and the residue was extracted with additional 20 mL of ethyl acetate by ultrasonic extraction for 3 min. The combined filtrate was transferred into a 250-mL flask and then evaporated to near dryness with a rotary vacuum evaporator at 45°C. The extract was dried under a gentle stream of nitrogen and then dissolved with 10 mL of petroleum ether for further cleanup. Extract purification was performed on a PSA cartridge, and the purification procedure is the same as previously described for the soil sample. Finally, the residue was re-dissolved in 2 mL *n*-hexane for GC–ECD analysis.

Recovery experiments were performed, in three replicates, at three fortification levels (0.005, 0.1 and 0.5 mg/kg) by adding known volumes of benazolin-ethyl standard solutions to different matrices (soil and rape seed). Blank analyses were performed to check interference from the matrices.

Table I
The Recoveries of Benazolin-ethyl in Soil and Rape Seed ($n = 3$)

Sample type	Amount added (mg/kg)	Recovery (%)				CV (%)
		1	2	3	Average	
Soil	0.5	91.31	91.14	88.40	90.28	1.81
	0.1	86.10	85.32	86.24	85.89	0.58
	0.005	101.40	104.55	101.3	102.42	1.80
Rape seed	0.5	98.41	101.2	100.11	99.91	1.41
	0.1	101.62	107.91	108.00	105.84	3.46
	0.005	90.31	98.72	100.20	96.41	5.53

Results and Discussion

Method validation

The standard calibration curve of benazolin-ethyl was constructed by plotting the analyte concentration against peak areas under the proposed chromatographic conditions. A good linearity was achieved from 0.01 to 1.0 mg/L with $R^2 = 0.999$. The standard curve equation was $y = 2881878.403x + 50778.892$, where y was peak area and x was benazolin-ethy concentration.

The mean recoveries of benazolin-ethyl at the three spiking levels (0.005, 0.1 and 0.5 mg/kg) are shown in Table I. Satisfactory results were found in the three levels, with recoveries between 85.89 and 105.84% in soil and rape seed samples, and the coefficient variations (CV) of the method ranged from 0.58 to 5.53%. All recoveries and CV values were within the permissible range for pesticide residue analysis. Figure 2 shows the representative chromatograms of the herbicide. The results showed no peak in blank samples at the retention time, so there was hardly any signal interference from the matrix.

Optimization of extraction method

In this study, for the soil sample, benazolin-ethyl was extracted with methanol–water (1/1, v/v), by liquid–liquid partition with dichloromethane, and then the extract was further cleaned by the PSA cartridge. In the method, the recoveries were above 85.89%. For the rape seed sample, the fortified

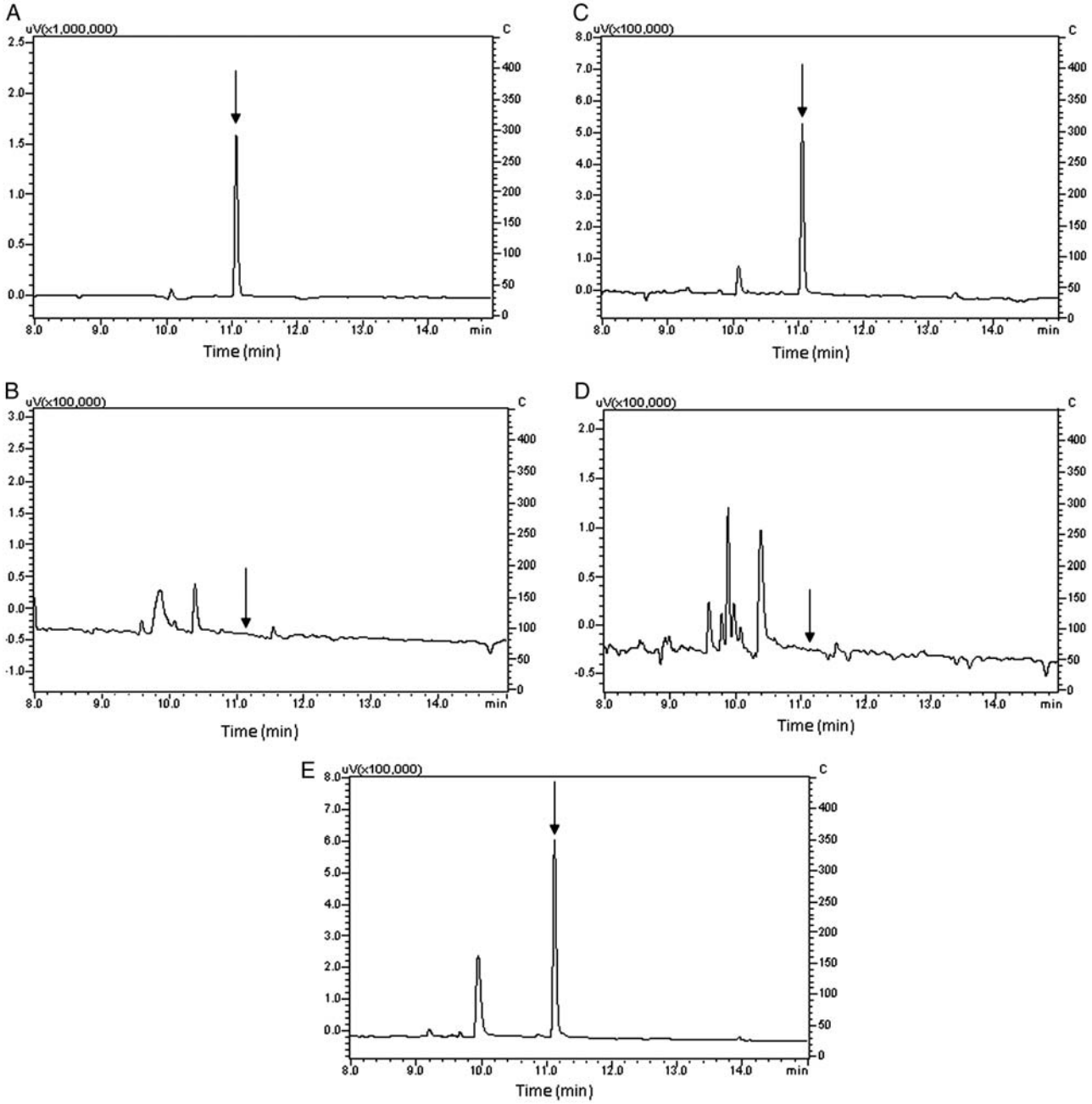

Figure 2. Chromatograms obtained from the target herbicide: benazolin-ethyl standard solution (A); blank soil sample (B); soil spiked with benazolin-ethyl at 0.1 mg/kg (C); blank rape seed sample (D); rape seed spiked with benazolin-ethyl at 0.1 (E).

recoveries were below 73.00% when it was extracted with methanol–water solution, so the rape seed sample was instead extracted with ethyl acetate by shaking and ultrasonic, and then the fortified recoveries were above 96.41%. The results showed that this extraction method was reliable for the residue analysis of benazolin-ethyl in soil and rape seed samples.

SPE procedure

To improve the selectivity and reproducibility of the method, a cleanup procedure was implemented after the extraction process. SPE was used in this method. We have investigated PSA cartridges for soil and rape seed sample purification in this study. The herbicide was not retained by the PSA cartridge when eluted with 4 mL of petroleum ether–ethyl acetate (9:1 v/v). This indicated that the PSA cartridge provides sufficient cleanup for the crude extracts of soil and rape seed samples, because it eliminated most interfering peaks and allowed good recoveries at low fortification levels.

Residue dissipation of benazolin-ethyl in soil sample

The proposed method was applied to a dissipation study of benazolin-ethyl in an experimental rape field. The dissipation data of benazolin-ethyl in soil are listed in Table II. The dynamics regression line equation for the concentration (C) related

to time (t) was $C = 1.469e - 0.15t$ ($R^2 = 0.893$). The half-life time ($t_{1/2}$) of the herbicide in soil was 4.62 days, and 90.51% of benazolin-ethyl residue dissipated after 2 weeks. This demonstrated that the degradation of the herbicide was rapid under the field conditions. Figure 3 shows the dissipation curves of benazolin-ethyl in soil.

Table II
Dissipation of Benazolin-ethyl Residues in Soil in Sichuan, China in 2011

Days after spraying	Residue (mg/kg)					CV (%)	Dissipation rate (%)
	1	2	3	4	Average		
2h	2.14	2.23	2.25	2.06	2.17	4.04	—
1	1.27	1.33	1.33	1.34	1.32	2.43	39.29
3	0.62	0.66	0.58	0.67	0.63	6.50	70.86
5	0.68	0.56	0.66	0.63	0.63	8.30	70.86
7	0.44	0.38	0.46	0.40	0.42	8.69	80.65
14	0.21	0.21	0.22	0.19	0.21	6.06	90.51
21	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	—	—

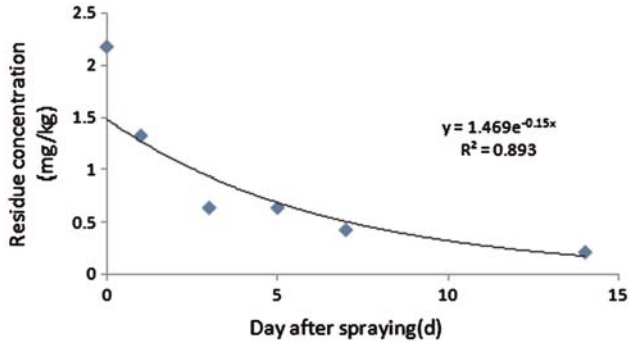

Figure 3. Dissipation rate of benazolin-ethyl in soil.

Final residues of benazolin-ethyl in soil and rape seed samples

The concentration levels of benazolin-ethyl in real samples at harvest time were detected. Results showed that the terminal residues of benazolin-ethyl in soil and rape seed samples in Sichuan were below the limit of quantification (LOQ) (0.005 mg/kg).

The limit of detection (LOD) and LOQ were determined to be the sample concentration of benazolin-ethyl at peak heights of 3 and 10 times the baseline noise, respectively. The LOD was estimated to be 0.0023 ng, and the LOQ of benazolin-ethyl in soil and rape seed was 0.005 mg/kg in this method. The maximum residue limit (MRL) of benazolin-ethyl set by the Guideline for Safety Application of Pesticides for rape seed is 0.1 mg/kg. Obviously, it would be acceptable to spray the benazolin-ethyl under the recommended dosage or 1.5 times the recommended dosage due to its low residue and short half-life. Therefore, applying benazolin-ethyl (20% EC) in a rape seed field was safe.

The benazolin-ethyl in a real soil sample was also identified, as were the specific ion fragments $(M\text{-}H)^+$ at m/z 271; $(M\text{-}H\text{-}COOCH_2CH_3)^+$ at m/z 198; $(M\text{-}H\text{-}C_4H_7O_2N)^+$ at m/z 170; $(170\text{-}Cl)^+$ at m/z 134 and $[(C_6H_4)S]^+$ at m/z 108 in GC–mass spectrometry (MS), according to the proposed conditions (Figure 4).

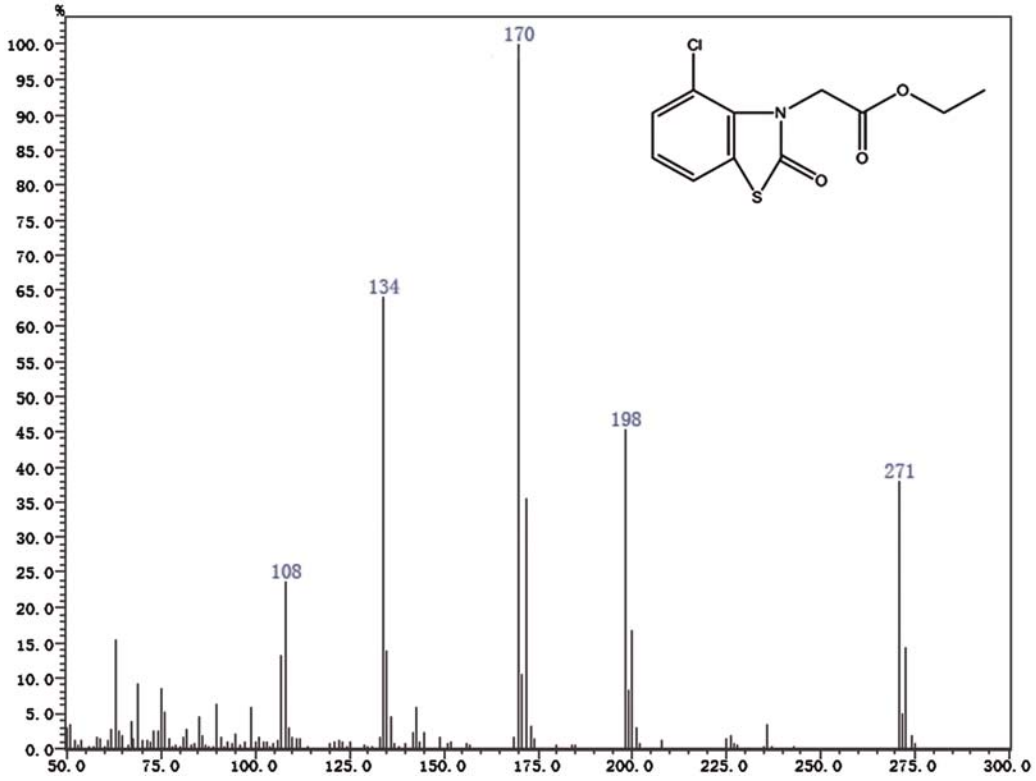

Figure 4. Mass spectra of benazolin-ethyl.

Conclusion

In this paper, a rapid and simple GC–ECD method was developed and validated for the determination of benazolin-ethyl residues in soil and rape seed. The procedures were characterized by recoveries $> 85.89\%$ and CVs $< 5.53\%$. LOQ of benazolin-ethyl was found to be 0.005 mg/kg. Results from this study showed that the half-life of benazolin-ethyl in soil was 4.62 days, and the final residues of benazolin-ethyl in soil and rape seed samples were lower than 0.005 mg/kg at harvest time. As a result, this analytical procedure has been successfully applied to regular monitoring of benazolin-ethyl residues in rape field.

Acknowledgments

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