

Determination of Tolfenpyrad residue and degradation on the cabbage surface

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Abstract: This article describes the comparison of different versions of preliminary steps for the determination of Tolfenpyrad in cabbage by concurrent use of Gas chromatography(GC) and Liquid chromatography (LC) for detection. The two compared versions were based on the solid phase extraction. After extraction with acetonitrile, a SPE cleanup procedure was conducted. The method was validated using cabbage samples fortified with pesticide at three concentration levels. As expected, the experimental results were satisfactory (average recoveries ranged from 77.3 to 100.9%, with relative standard deviations between 3.1 and 9.4%). The detection and quantification limits for cabbage of different instruments were 0.05, 0.05mg/kg, respectively. Also, the field trails of 15% Tolfenpyrad suspension residue and dissipation in cabbage were carried out in period of Tolfenpyrad in cabbage, also which was concluded by using GC corresponding with FTD detector. In the additional levels of 0.05, 0.5, 5mg/kg, the average recoveries were from 99.0 to 100.1%, with relative standard deviations from 3.1 to 5.7% in cabbages, while the average recoveries in soils were from 96.5 to 101.3% and relative standard deviations were from 2.4 to 9.0%. The dissipation rate of Tolfenpyrad followed the first-order kinetics and the half-lives were from 2.5 to 2.7 days in cabbage and from 2.0 to 2.9 days in soil. The final residue experimental was conducted by spraying low and high dosage. After sprayed the low dosage pesticide 14 days, the final residues in the cabbages and soils were both lower than that of detection limits.

Key words: Tolfenpyrad; cabbage; residue; field trails

1. Introduction

Tolfenpyrad, as an emerging pyrazole hetero-cyclic pesticide, has its special chemical structure, currently, which plays a critical role in protecting vegetables and fruit crops.

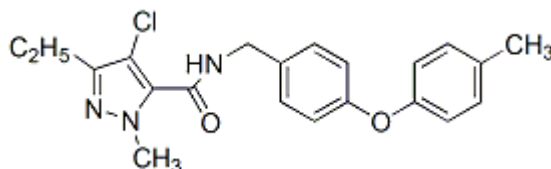


Fig1. The chemical structure of Tolfenpyrad

Besides, it has been utilized to control insects for a wide variety of vegetables because of their broad-spectrum insecticidal effects, especially for the lepidoptera larvae small diamondbackmoth^[1]. Tolfenpyrad has a relatively low acute toxicity, and it requires following characteristics: efficient, fast degradation, contact action, therefore, its markets at home and abroad are analyzed and predicted. Also, it is characterized by insensitizing effects, quick acting high ability of forming slag^[2]. Nevertheless, the over-dose usage of Tolfenpyrad will lead to phytotoxicity. On one side it will cause the injury of skin, it even appeared the first lethal case in Japan^[3], on the other side it will pollute the surrounding environment^[4]. Although this pesticide is moderately or low toxicity to mammals, it has been drawn wide attention due to the food safety and quality in recent years, several authorities around the world have established maximum residue limits (MRLs) or tolerance in different vegetables and fruits to guarantee the food safety and protect the environment and consumers^[5].

In order to protect public health, we have established the acceptable daily intake(ADI), which is 0.006mg/kg^[6]. Meanwhile, the Tolfenpyrad was encoded as a quasi-evaluation pesticide in the preferred list in 2016, and the eggplant and cruciferae plant were regarded as the registration crops of which in China^[7].

The current analysis methods of determining the residue of Tolfenpyrad in vegetables were based on chemical analysis, such as Liquid Chromatography -tandem Mass spectrometry (LC-MS/MS)^[8-10], Gas Chromatography (GC)^[11], Liquid Chromatography (LC) with ultraviolet detector(UV)^[12], etc. The

determination of pesticide in cabbage usually involves preliminary steps including sampling, extraction and cleanup.

Table1 The maximum residue limits of Tolfenpyrad in China and Japan (mg/kg)

The names and types of plants		China (3)	Japan (36)	grouping
Vegetables	cabbage	0.5	0.3	
	celery cabbage	0.5	2	
	broccoli		1	
	Japanese radish root (including radish)		10	cruciferae
	Japanese radish achilles (including radish)		0.2	
	turnip tops (including rutabaga)		25	
	turnip root (including rutabaga)		1	
	Red bell pepper (pimiento)		3	
	tomato		2	solanaceae
	eggplant	0.5	2	
	celery		3	umbelliferae
	cucumber		1	
	watermelon		0.05	cucurbitaceae
	Cucurbitaceous vegetables		0.2	
	lettuce		10	compositae
	potato		0.05	moremillet
	onion (including scallion)		5	
	Chinese chive		10	
	garlic		0.05	liliaceae
	asparagus		0.7	
Fruit	pea (immature, zone with beanpot)		2	leguminosae
	valencia orange		3	
	orange (including navel orange)		3	
	grapefruit		3	
	lemon		3	citrus
	Citrus aurantium L		3	
	Wenzhou tangerine flesh		0.1	
	other citrus kinds of fruit		3	
	Japanese pear		2	
	pear		2	pome
	Japan lie (including preserved plum)		2	
	nectarine		5	stone fruit
	peach		0.2	
	strawberry		3	berry
	perfume	Other kinds of perfume		15
Tea			20	tea

In this paper, we presented a covering about the differences of two detection methods of determination pesticide residues in fruit and vegetables using acetonitrile extraction and determination by means of LC equipped with UV detector or GC equipping with FTD detector. The residue levels and degradation dynamics of Tolfenpyrad cabbage and soil after application were also determined. The two compared versions were based on the solid phase extraction. Solid phase extraction (SPE) was based on the principle of the similarity and consistency, via selectively absorption and selectively elution, thus achieved and realized purity. Though microparticle may be due to the adsorbent blockage, thus reduce the adsorption efficiency^[13-14]. A series of facts proved that this effect on the experimental results was small^[6]. Just because of it has a series of properties, such as easy operating, economical effectiveness performance and high purity efficiency, it has been widely applied to sample pretreatment^[15-16]. After extraction with acetonitrile, a SPE cleanup procedure was conducted. The method was validated by using cabbage and soil samples fortified with pesticide at three concentration levels. Also, the field trials of 15% Tolfenpyrad suspension residue and dissipation in cabbage were carried out in

period of Tolfenpyrad in cabbage, and which were concluded by using GC corresponding with FTD detector.

2. Materials and Methods

2.1 Reagents and materials

Certified standards of Tolfenpyrad were purchased from Qingdao hailier Pharmaceutical Co.Ltd. The purity of the Tolfenpyrad analytical standard was 98.02%. Ultrapure water was obtained by purification of distilled water through a Milli-Q gradient system (Bedford,MA,USA), and sodium chloride(NaCl) was analytical grade, so were the acetonitrile, n-hexane, methanol, and acetone(Beijing Reagent Company). Also, it still needed acetone that chromatographic grade. The application of SPE column was Florisil, which bought from Qingyun company. Standard stock solutions of Tolfenpyrad(1000mg/L) were prepared in pure methanol. Standard working solutions were prepared at various concentrations by diluting the standard stock solutions in methanol, and all standard solutions were stored in amber bottles at 4°C.

2.2 Field trails

The field trails including dynamic resolution and final residues study were conducted in cabbage plot in Shandong, Tianjin, Nanjing in China in 2015. Experimental area was determined as 15-m² for separating each plot, and every treatment was repeated three times.

Tolfenpyrad 15% suspension concentrate was conducted at the experimental plot one time with the dosage of 75 g/667 m² for the dynamic digestion. The dynamic digestion sample was collected randomly from each plot at 1, 2, 3, 5, 7, 10, 14, 21, 30 d after application. All samples were putted into the plastic bags and transported into the laboratory, where the cabbage sample were comminuted and the soil sample were sieved. Each individual sample was mixed thoroughly and stored at -20°C for analysis.

Tolfenpyrad 15% suspension concentrate of the finally residual implement of experiment, however, used two different dosage, the low (50g/667 m²) and the high dosage(75g/667 m²). The finally residual sample, sampled with uniform random from each plot at 5, 7, 14d. It was sprayed two or three times on cabbage at rosette stage. The interval of each application was 7 days. All samples were processed as same as the dynamic digestion experiment.

2.3 Extraction procedure

The pretreatment procedures of utilizing GC were as follows. A representative portion of the sample was chosen. Ten grams of cabbage sample was placed in a 100-mL centrifuge tube. Then 20 mL acetonitrile was added into the tube, and conducted high speed pounding 1min. Adding 3 g NaCl into the tube, then treated violently for a minute. Afterwards, the extract was centrifuged at 3,000 rpm for 5min, which made the acetonitrile phase and the water for stratified. Then, 10mL of the upper layer was transferred into a beaker waiting for nitrogen-blow and evaporated to dryness with a water bath at 80°C. The obtained residue was redissolved in 2 mL n-hexane and waiting for purification. Certain ratio of acetone and n-hexane (10+90) was applied to washing off the impurity during the clarification of Florisil. The purified elutriant solution was removed, then imported into clarifying solution which was waiting for cleaning. A 15-mL calibration centrifugal tube was used to purify the refined pesticide residue. The centrifugal tube which has elutriant was mounted on the water bath at 80°C and carried out nitrogen-blow until the amount was under 5mL. The measurements were performed at constant volume 5mL by acetone.

The sample pretreatment by using LC detection method were mostly identical. The different department lied in the centrifugal tube which has elutriant was mounted on the water bath at 80°C and carried out nitrogen-blow until completely drying. Then injected into the LC After the residues was reconstituted by 5 mL methanol.

2.4 Detection conditions

The determination of Tolfenpyrad was done by using GC combining with FTD detector. Separation was performed through using HP-5 column (30.0 m×0.25 mm×0.25 μm), with a temperature programme raised that the temperature was elevated by 30 °C/min to 300 °C remaining for 8 minutes at initial temperature of 180 °C. The nitrogen as carrier gas, and the flow was 2.0mL/min; the injector temperature 250 °C; the detector temperature was 315 °C; 3μL samples were injected to GC splitlessly; the combustion gas including hydrogen at the flow of 1.5mL/min and air at the flow of 145.0mL/min.

LC experiments were performed on ODS C18 column (4.6mm×250mm, 5μm) corresponding with ultraviolet detector(UV), composed of two Prostar pumps, a column oven was 25°C, The mobile phase consisted of methanol- water (80+20) with the flow rate of 1.0mL/min; the detection wave length was 245nm with injection volume of 20μL.

3. Results and Discussion

The sensitivity of the method was evaluated by determining the instrumental LOD. The instrumental LODs of the LC and the GC were both 0.05mg/kg. Under the given conditions, LODs and LOQs of the proposed method were determined at the same concentration producing a peak height 3 and 10 times that of the baseline noise. Recoveries of Tolfenpyrad at different concentrations were calculated. The results of recovery experiments of LC and GC were presented in Table2 and Table3. From the Table2, it was found that the recoveries of LC and GC were between 77.3% and 100.9%. The results showed that the two methods accorded with detecting demands. In particular, adopting the GC method and its pretreatment technology had a high and stable recoveries, meanwhile it had a lower relative standard deviations, it embodies a good reproducibility, to some extent, it indicated that this method was accurate, efficient, reliable.

Table2 The result of recovery with fortified concentration by using LC

fortified concentration (ppm)	recovery (%)						RSD (%)
	1	2	3	4	5	Ave	
0.1	102	107	104	101	90.3	100.9	6.3
0.5	75	78	76	79	80	77.3	2.7
5	89.6	88.8	87.5	72	78	83.2	9.4

Table3 The result of recovery with fortified concentration by GC

fortified concentration (ppm)	recovery (%)						RSD (%)
	1	2	3	4	5	Ave	
0.05	100.4	95.2	101.3	101.7	96.2	99	3.1
0.5	89.9	95.6	104.1	94.4	100.7	96.9	5.7
5	105.2	96.9	98.1	100.1	100.4	100.1	3.2

The field trails experiments including dynamic resolution and final residues study, they were both performed by using GC, corresponding with FTD detector. The recovery results of Tolfenpyrad on cabbages and soils at different fortification levels were given as Table 4, which ranged from 96.5% to 101.3% . The relative standard deviations were ranged from 2.4% to 9.0%. Both the recoveries and the relative standard deviations were within acceptable range. Also, it had determined a strong linear correlation. Calibrations were performed using five calibration standards(0.05, 0.5, 1.0, 2.0, 5.0 mg/kg), prepared and determined in GC, which as described in Section 2.4. The correlation coefficients(r^2) of the calibration curves were 0.9996.

Dissipation curve of Tolfenpyrad residue under the natural conditions on cabbage and soil were shown as Fig2 and Fig3. Basic conclusion can be drawn through the figure: the initial deposite of Tolfenpyrad were 3.22, 2.53, 5.00mg/kg in cabbages and 0.522, 0.637, 0.720mg/kg in soils in Shandong, Jiangsu and Tianjin.

From the three models of the curve for the Tolfenpyrad dissipation, we got that the residues was significantly declined along with the time passed by. On the 21thday, the degraation rate was up to 98%. The half-life of Tolfenpyrad were 2.5d, 2.6d, 2.7d in cabbages, and 2.0d, 2.9d, 2.3d in soils in Shandong, Jiangsu and Tianjin, respectively. Many factors can be involved in the pesticide degradation, including biological and non-biological fators, SOM(soil organic material), temperature and moisture are all important abiotic fators that could affect degradation rate. The information we can be derived from the resolution rate that the Tolfenpyrad in cabbages was faster than that in soils. On the 35th day, the Tolfenpyrad was nearly degraded completely in soil.

Table4 Recoveries of Tolfenpyrad on cabbage and soil at different fortification levels by using GC

substrates	Level of fortification	Recovery(%)	RSD(%)
Cabbage	0.05	99	3.1
	0.5	96.9	5.7
	2.0	100.1	3.2
Soil	0.05	98.0	9.0
	0.5	101.3	5.5
	2.0	96.5	2.4

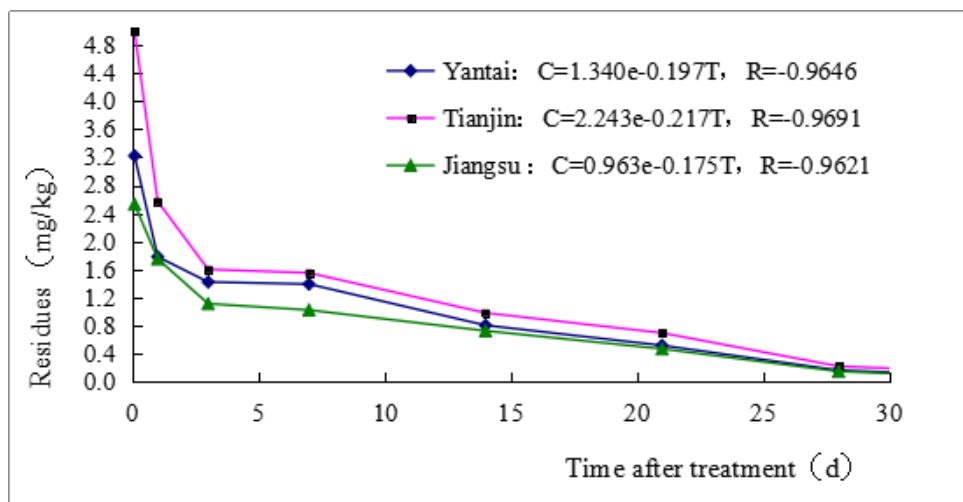


Fig.2 Dissipation curve of Tolfenpyrad residue on cabbage(2015)

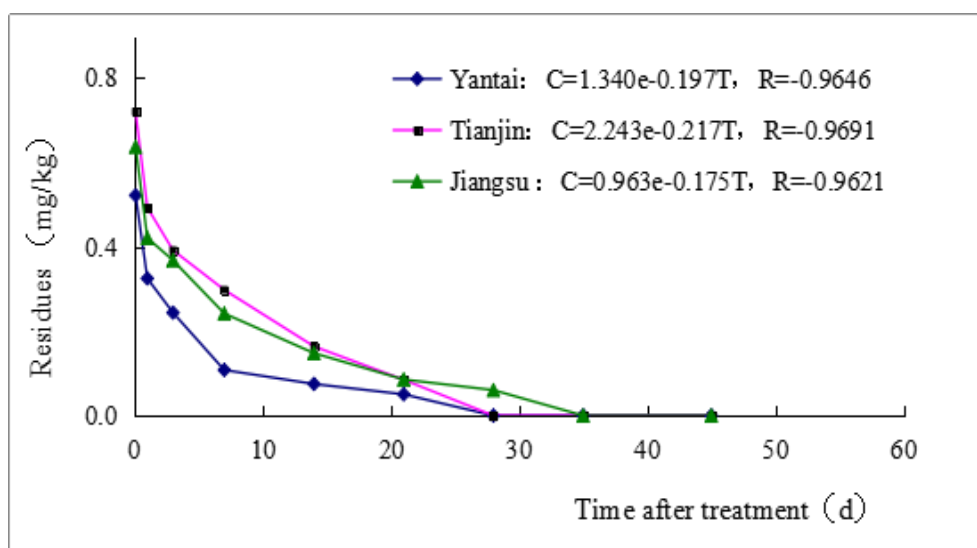


Fig.3 Dissipation curve of Tolfenpyrad residue on soil(2015)

The final residue experimental was conducted by spraying low and high dosage. Finally the paper has described the final result of Tolfenpyrad degradation (Tab 5). The data displays that under the conditions of low dose (112.5 g a.i./hm²), after sprayed the pesticide 14 days, the final residues of Tolfenpyrad in the cabbage samples and soil samples were lower than that of detection limit. However, for the high dosage, the final residue in the cabbages were 0.282, 0.250, 0.0551 mg/kg in Shandong, Jiangsu, Tianjin after sprayed three times, respectively. The others were below 0.05 mg/kg. Whether the high dosage or not, the final residues of Tolfenpyrad in soils were both below the detection limits after the 14th day. Compared with the final residues statistics of the cabbage samples and the soil samples, the closely positive correlation was found between the spraying dosage and the spraying times and the residues of Tolfenpyrad.

In this study, we selected acetonitrile as the extraction solvent, then carried out using SPE purification. Also, the detection determination was chosen by GC. The statistics shows that it had a good effect indeed. This method affords us a useful avenue to perform the experiment of 15% Tolfenpyrad suspension residue and dissipation in cabbage. As an emerging pesticide, the application of Tolfenpyrad becomes more and more extensive in the produce of vegetables. The National Standards of Food Safety (GB2763-2014) has only stipulated the maximum residue limits of Tolfenpyrad in cabbage, celery cabbage and eggplant, which were 0.5 mg/kg^[17]. According to the issues of the pesticide residues in harvest products, many other countries had set up a perfect detection system, thus forming a series of well quality and safety system of agricultural products^[18]. In China, we have using eggplant and cruciferae as the registration crops to enlarge the usage scope of Tolfenpyrad, moreover, specification the dosage.

Table 5. Average residues of Tolfenpyrad in cabbage and soil BDL^b

Sample	Dosage g a.i./hm ²	Sprayin g times	Days after spraying	Residues (mg/kg) Mean ^a		
				Shandon g	Tianjin	Jiangsu
Cabbage	112.5	2	5	0.411	0.543	0.287
			7	0.111	0.127	0.0921
			14	BDL ^b	BDL	BDL
		3	5	0.708	0.948	0.173
			7	0.164	0.247	0.151
			14	BDL	BDL	BDL
	168.75	2	5	1.28	1.38	0.623
			7	0.295	0.296	0.148
			14	BDL	BDL	BDL
		3	5	1.49	1.72	0.851
			7	0.352	0.439	0.265
			14	0.282	0.250	0.0551
Soil	112.5	2	5	0.0748	0.166	0.143
			7	BDL	BDL	BDL
			14	BDL	BDL	BDL
		3	5	0.130	0.326	0.282
			7	0.0547	0.138	0.135
			14	BDL	BDL	BDL
	168.75	2	5	0.104	0.247	0.254
			7	BDL	BDL	BDL
			14	BDL	BDL	BDL
		3	5	0.177	0.515	0.454
			7	BDL	0.175	0.149
			14	BDL	BDL	BDL

a n=3

b BDL below determination limit of 0.05mg/kg

References

- [1] Tolfenpyrad[J]. Pesticide Science and Administration, 2010, 31(6): 58
- [2] ZHANG Yibin. Novel insecticidal acaricide-Tolfenpyrad[J]. World Pesticides, 2003, 25(6): 45
- [3] WAKAKO H, KOJI Y, KANJU S, et al. Acute Fatal Poisoning with Tolfenpyrad[J]. Journal of Forensic and Legal Medicine, 2013, 20(8): 962-964
- [4] SOCEANU A, DOBRINAS S, COATU V, et al. Pesticide Residues Determination in Vegetables from Romania by GC-ECD[J]. Springer Netherlands, 2009: 423-430
- [5] FAO/WHO. Establishment on the CODEX schedules and priority lists of pesticides prepared by Australia (CX/PR 15/47/12) [R]. The 47th session of Codex Committee on Pesticides Residues, China: Beijing, 2015: Agenda Item 10, 2015- 06- 26
- [6] ZENG M-S, LIU F-J, HUANG H-S, et al. Residues and Safety of Tolfenpyrad Used in Tea Plantations[J]. Fujian Journal of Agricultural Sciences, 2014, 29(8): 774-778
- [7] Chen Zongmao. Rare earth elements in tea standard is expected to be revoked[J]. China Tea, 2012, (3):4-5
- [8] HUANG Chao-Qun, XIE Wen, YU Zhuo-Ran, et al. Determination of 10 amid pesticides in fruits and vegetables by liquid chromatography-tandem mass spectrometry[J]. Journal of Food Safety and Quality, 2015, 3(6)
- [9] ZHU Jian-hua, ZHAO Li. Determination of Tolfenpyrad, Flonicamid, Chlorantraniliprole and Flubendiamide in vegetables and fruits by Liquid Chromatography-tandom Mass Spectrometry[J]. Journal of Instrumental Analysis, 2011, 30(6): 660-661
- [10] ASHOK K M, CRISTINA B, YOLANDA P. Liquid Chromatography-Mass Spectrometry in Food Safety[J]. Journal of Chromatography A, 2010, 1217(25): 4018-4040
- [11] QIN Xu, SUN Yang, QIN Dong-mei, et al. Analysis of Tolfenpyrad in Soils and Cabbage by GC[J].

AGROCHEMICALS, 2009, 48(9): 660-661

[12] Lan H-Y, LIANG Y-X, WEI W, et al. High performance liquid chromatographic analysis of Tolfenpyrad 15% EC[J]. Modern Agrochemicals, 2013, 12(3)

[13] MA Ji-ping, XIAO Rong-hui, LIN Jin-hua, et al. Determination of 16 Polycyclic Aromatic Hydrocarbons in Environmental Water Samples by Solid-phase Extraction Using Multi-Walled Carbon Nanotubes as Adsorbent Coupled with Gas Chromatography-Mass Spectrometry[J]. J Chromatogr A, 2010, 1217(34) : 5462-5469

[14] Ma Ji-ping, Li Mo, Zhao Xiu-hua et al. Solid phase Extraction coupled with Reversed-phase high performance liquid chromatography for the determination of phthalic acid ester in water[J]. Modern Agrochemicals, 2009

[15] Ma Ji-ping, Xiao Rong-hui, et al. Headspace Solid-phase Microextraction with On-fiber Derivatization for the Determination of Aldehydes in Algae by Gas Chromatography-mass Spectrometry[J]. J Sep Sci, 2011, 34(12): 1477-1483

[16] Ma Ji-ping, Wang Han-wen, Guan Ya-feng, et al. New Technology of Solid-phase Microextraction[J]. Chinese Journal Chromatography, 2002, 20(1): 16-20

[17] GB2763-2014 The National Standards of Food Safety— the maximum residue limits for pesticide in food[S]. Beijing: the People's Republic of China national health and family planning commission and ministry of agriculture of the People's Republic of China, 2014

[18] PANG Zhuo, CHEN Jun-ke, SU Min-li, et al. HUUHOT vegetable pesticide residues pollution and response measures[J]. Journal of Inner Mongolia Agricultural University (Natural Science Edition), 2007, 28(3)