Journal of Hazardous Materials 164 (2009) 497–501

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Kinetic study of the degradation of the insecticide pymetrozine in a vegetable-field ecosystem

Guoqing Shen [a,*], Xuan Hu [a], Yinan Hu [b]

[a] Department of Environment and Resource, College of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, PR China
[b] College of Life Sciences, Nanjing Normal University, Nanjing 210097, PR China

ARTICLE INFO

Article history:
Received 14 June 2008
Received in revised form 10 August 2008
Accepted 11 August 2008
Available online 15 August 2008

Keywords:
Pymetrozine
Residue
Degradation
Broccoli

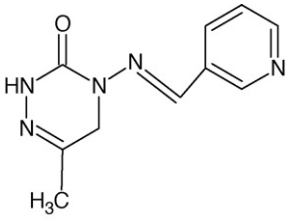
ABSTRACT

The disappearance kinetics of pymetrozine was studied in a broccoli-field ecosystem, and an efficient method for the determination of pymetrozine in broccoli and soil was also developed. Pymetrozine residues were extracted from samples using acetonitrile. The extracts were cleaned up by liquid–liquid partitioning with dichloromethane, followed by purification with ethyl acetate, and were then determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detector. The average recovery was 87–93% from broccoli, and 84–90% from soil. The relative standard deviation (R.S.D.) was less than 4% in broccoli, and in soil less than 11%. These results are all within the accepted range for residue determination. The limit of detection (LOD) of pymetrozine calculated as a sample concentration (S/N ratio of 3) was 0.005 mg kg^{-1}. The minimum detectable quantity (MDQ) was 1×10^{-10} g. The results of the kinetics study of pymetrozine residue showed that pymetrozine degradation in broccoli and soil coincided, with $C = 1.9826\,e^{-0.1965t}$ and $C = 15.352\,e^{-0.4992t}$, respectively; the half-lives were 3.5 and 1.4 days, respectively. The final residue level was lower than the new maximum residue limit (MRL) for pymetrozine on vegetables with a harvest interval of 23 days. A dosage of 300 g a.i. hm^{-2} was suggested, which is considered to be safe for human beings. These results contribute to establishing the scientific basis of the dosage of pymetrozine for use in vegetable-field ecosystems.

1. Introduction

Insecticides are indispensable to the farmers in the fight against plant pests. Although the use of insecticides provides unquestionable benefits in providing a plentiful, low-cost supply of high-quality fruits and vegetables, the indiscriminate and injudicious use of insecticides has resulted in widespread contamination of food and feed. This is related to poor insecticide handling practices and use of more toxic insecticides by farmers [1]. As a consequence, governments and international organizations have established maximum residue limits (MRLs) and pre-harvest intervals for fruits and vegetables. It is well known that such pre-harvest intervals depend, among other factors, on the climatic conditions under which the insecticides are applied. Hence, it is necessary to evaluate the dissipation of residues as a function of time under the specific climatic conditions in which agricultural practice takes place [2–4].

Pymetrozine, or 4,5-dihydro-6-methyl-4(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one (IUPAC), is the first and only substance in the azomethine pyridine group, and is a novel insecticide with selective activity against homopteran insects [5,6]. Its general structure is



Many studies have demonstrated that pymetrozine is effective against aphids, whiteflies and plant hoppers in integrated pest management programmes [7,8]. It acts in a unique way by interfering in the nervous regulation of feeding behavior, which results in death of the insect due to starvation after a few days. Therefore, it is one of the potential insecticides to replace organophosphates [9,10]. However, the United States Environmental Protection Agency (EPA) has recently classified pymetrozine as a "likely" human carcinogen because two types of tumors, benign liver hepatoma and carcinoma, occurred in male and female rats and mice in experimental studies [11]. Many countries, including the USA, EU

* Corresponding author. Tel.: +86 21 34206925.
E-mail address: gqsh@sjtu.edu.cn (G. Shen).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.08.020

member states and Japan, have established MRLs for pymetrozine in vegetables that range between 0.02 and 2 mg kg^{-1}, and the manufacturers propose pre-harvest intervals ranging between 7 and 21 days for vegetables and other crops. As mentioned above, estimates of MRLs and pre-harvest intervals depend on the analysis methods used and on the climatic conditions under which the pesticides are applied, among other factors. Recently, pymetrozine has been widely used on food crops in China. However, there are few publications from this country that report the analysis methods and kinetics of pymetrozine residue in the vegetable and soil environment. This paper describes a high-performance liquid chromatographic (HPLC) method for the analysis of pymetrozine, and determine the kinetics of the disappearance of this pesticide following spraying on to broccoli fields. This provides basic information for developing regulations to ensure the safe use of pymetrozine in pest management strategies on cropland and to prevent any health problems in consumers.

2. Materials and methods

2.1. Materials

A formulation containing 25% of pymetrozine was purchased from the Jiangsu Anpon Company, PR China. Broccoli was used as the test vegetable in this experiment.

2.2. Chemicals

The pymetrozine reference standard (purity, 99.9%) was purchased from Dr. Co. (Augsburg, Germany). Ultrapure water used for HPLC detection was obtained from an Aquapro Hi-end water treatment solution provider (Ever Young Enterprises Development Co. Ltd.). Acetonitrile, dichloromethane, ethyl acetate and all the other solvents were of residue analysis grade. Anhydrous sodium sulfate, active carbon, silica gel (80–120 mesh), and all other chemicals used were also analytical grade (Shanghai Experiment Reagent Co. Ltd., Shanghai, China). All solvents and samples were filtered through a membrane filter (0.45 μm) before HPLC runs.

2.3. Field experiment

The field experiment was carried out at Chongming Island Farm, which is located in Shanghai, China. Broccoli plants were transplanted into nine field plots, each with an area of 15 m^2. A complete randomized block design (CRD) was applied with three replicates. Pymetrozine formulations were sprayed at 300 g a.i. hm^{-2} (recommended), and three untreated pots were sprayed with water as a control. The solution was sprayed at 800 l hm^{-2} until the tested broccoli was fully covered with pymetrozine. Representative samples were taken approximately 2 h, and 1, 3, 5, 7, 14, 21, 30 and 45 days after application of pymetrozine.

The ultimate residue field test was designed as above, but pymetrozine formulations were sprayed at two doses, 300 and 600 g a.i. hm^{-2} (double the recommended dose). Succession spraying was applied three times with a 7-day interval. The samples were collected and placed in a freezer at −4 °C until analysis.

2.4. Sample preparation

2.4.1. Broccoli plants

A cut broccoli sample (10 g) was placed in a cone flask with 100 ml acetonitrile. The cone flask was capped and shaken on a shaker (THZ-C, Taicang Experiment Equipment Co., China) for 1 h. The extracts were filtered through filter paper, followed by evaporation using a vacuum rotary evaporator (RE-52, Yarong Biochemistry Equipment, Shanghai) at 50 °C until the final volume reached 10 ml. The sample was transferred to a separating funnel containing 1 ml of 4% sodium chloride, and this was followed by liquid–liquid partitioning with dichloromethane three times using a volume of 50 ml. The organic phases were combined and further concentrated to about 0.1 ml with the vacuum rotary evaporator at 40 °C.

2.4.2. Soil

Surface soil (0–15 cm depth) was used in the following experiments. The soil sample was air-dried, crushed with a hammer and passed through a 40-mesh screen. 10 g of soil was packed with filter paper and then soaked with 150 ml dichloromethane in Soxhlet's apparatus, followed by extraction for 3–4 h at 60 °C. The volume of the extracts was decreased to about 0.1 ml with the vacuum rotary evaporator at 40 °C.

2.4.3. Clean up

A column (20 cm × 15 mm ID) was packed with a glass wool plug at the bottom and covered with anhydrous sodium sulfate. Then 3 g silica gel and another 1 cm layer of anhydrous sodium sulfate were placed on top. The two sides of the column were compacted uniformly. The column was pre-wetted with 10 ml ethyl acetate. The 1 ml concentrated extract was then applied to the column and eluted with ethyl acetate. The eluate was evaporated to dryness with the vacuum rotary evaporator at 40 °C. The residue was diluted to 5 ml with acetonitrile. The extract was filtered through a 0.45-μm membrane for HPLC analysis.

2.5. HPLC conditions

All extracts were investigated using HPLC apparatus (Shimadzu liquid chromatography, LC-10ATVP) equipped with an SPD-10AVP UV–vis detector. Separation was carried out on a shim-pack VP-ODS column (150 mm × 4.6 mm ID, particle size 4.6 ± 0.3 μm, Shimadzu Corporation, Japan). The mobile phase was acetonitrile–water (85:15 v/v) with 1 ml min^{-1} flow rate. The column oven was kept at 20 °C. The best detection was attained at a wavelength of 299 nm and the volume injected was 20 μl.

3. Results and discussion

3.1. HPLC chromatograms of pymetrozine

Reverse-phase HPLC, with UV detection, has proven to be a good alternative for pymetrozine determination because the peak of pymetrozine is obvious and separated. Chromatographic separation in C18 columns provides good results. Detection at 299 nm offers suitable chromatograms for the quantification of pymetrozine in field samples. Under conditions described above, pymetrozine showed a retention time of 3.691 min. Fig. 1 shows typical chromatograms of standard pymetrozine, broccoli and soil spiked with or without pymetrozine. No peaks were found in the chromatograms of the broccoli and soil without pymetrozine at retention times corresponding to the pymetrozine peaks (Fig. 1C and E). The background level for broccoli was more complicated than that at soil, but it didnot disturb the determination of pymetrozine, the other peak was 4.246 min. This demonstrated the validity of the method when used in the kinetic study of pymetrozine in broccoli under field conditions.

3.2. Linearity

An external standard method was adopted in this experiment. For most chromatographic procedures a linear relation is achieved

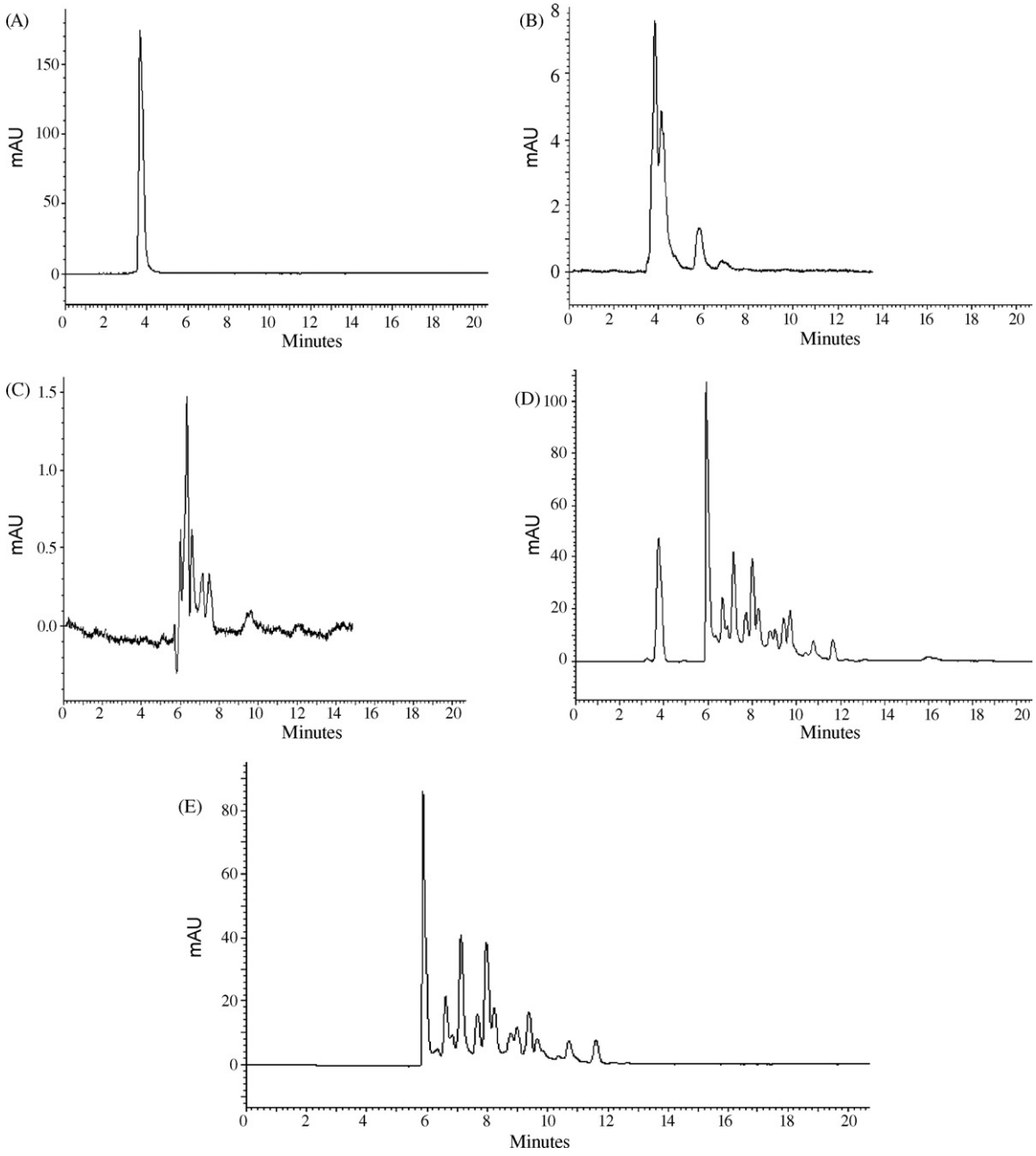


Fig. 1. Typical chromatograms for the analysis of (A) pymetrozine standard; (B) broccoli spiked with pymetrozine; (C) broccoli without pymetrozine; (D) soil spiked with pymetrozine; (E) soil without pymetrozine.

between the peak area (y) and the analyte concentration (x), but in this experiment, y was the height of the peak, not the area. In the chromatograms of broccoli samples, pymetrozine and its metabolite were so difficult to separate that the whole peak of pymetrozine itself could not be obtained. However, there were two obvious high spots of adjoining peaks, which could be used in the peak height quantitative method [12]. A five-point calibration curve (peak height versus concentration) was constructed by spiking blank samples with the standard solution, and the regression equation obtained was $Y = 17382X + 411.4$ ($R^2 = 0.9999$). The linearity of a method is a measure of range within which the results are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [13].

3.3. Recovery test

Pymetrozine was added to untreated control samples at three levels: 0.01, 0.1, and 1.0 mg kg^{-1}, and each sample was analyzed based on the described procedure to evaluate the precision of the analytical method. The recovery tests were carried out on three replicates at each spike level. The results reported are the mean of three replicates. The recoveries and precision values obtained in the validation portion of the study are shown in Table 1. The average recoveries ranged from 87% to 93% in broccoli, and in soil from 84% to 90%, while the relative standard deviation (R.S.D.) in broccoli was less than 4%, and in soil less than 11%. These results are all within the accepted range for residue determination.

Table 1
Accuracy (%) and precision (%) of pymetrozine measurement in broccoli and soil (n = 5)

Spiking level (mg kg^{-1})	Broccoli		Soil	
	Recovery	R.S.D.	Recovery	R.S.D.
0.01	87.29	0.78	90.08	9.70
0.10	93.60	1.93	84.18	10.13
1.00	91.57	3.14	87.24	7.34

The limit of detection (LOD) calculated as a sample concentration (S/N ratio of 3) was 0.005 mg kg^{-1}. The minimum detectable quantity (MDQ) was 1×10^{-10} g.

3.4. Kinetic study

The kinetic study of pymetrozine in broccoli and soil was performed by plotting residue concentration against time, and the maximum squares of the correlation coefficients were used to determine the equations of the best fitting curves. For all samples studied, exponential relations were found to apply, corresponding to first-order rate equations. The first-order kinetics were calculated from the first-order rate equation: $C_t = C_0 e^{-kt}$, where C_t is the concentration of pesticide at any time t, C_0 is the initial concentration and k is the rate constant in day^{-1}. The pesticide persistence in an environmental compartment can be characterized by the pesticide half-life. The half-life is a measure of the time required for the pesticide concentration to be reduced to half the original value through biological or chemical degradation processes. The half-life ($t_{1/2}$) was determined from the k value for each experiment, being $t_{1/2} = \ln 2/k$.

3.4.1. Pymetrozine residue in broccoli

Fig. 2 shows the residue of pymetrozine in broccoli after spraying. The estimated residues were 2.03, 1.57, 1.18, 0.30, 0.12, 0.02, and 0.008 mg kg^{-1} at 0, 1, 4, 8, 15, 22, and 29 days after application, respectively. As expected, a gradual and continuous deterioration of the pesticide residue in the broccoli was observed as a function of time after application. The half-life of pymetrozine in broccoli was 3.5 days and the dynamics could be described by the equation $C = 1.9826\, e^{-0.1965t}$, with $R^2 = 0.9895$. The pymetrozine residue in broccoli was undetectable for 31 days after application. The results showed that a sharp decline in residue level occurred for pymetrozine in the first week, compared with the slow rate of degradation in the succeeding days. Tablei and Ahsaii [14] observed that no residues were detected for 4 days after spraying of pymetrozine in cucumber under field condition. Walash et al.

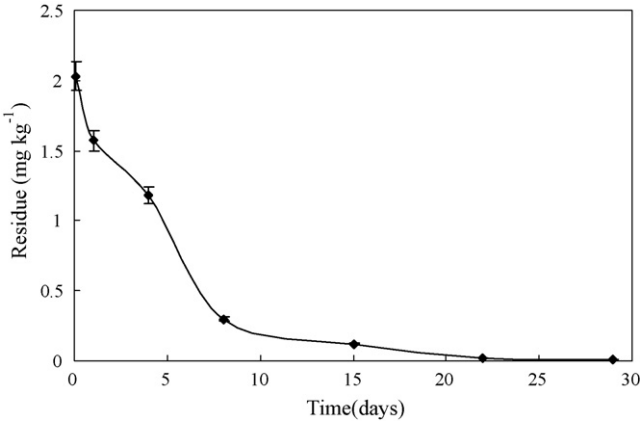


Fig. 2. Kinetics of pymetrozine in broccoli.

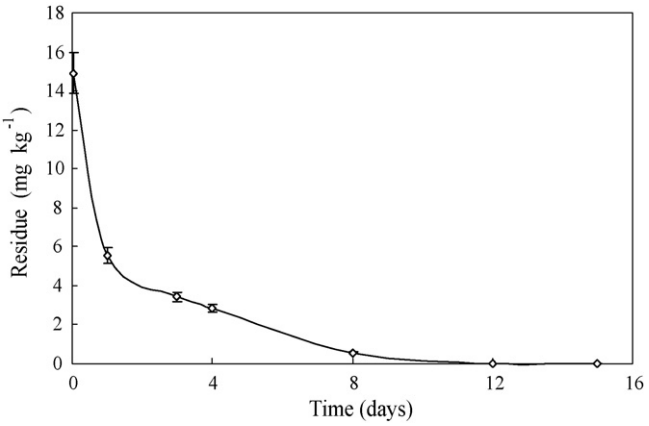


Fig. 3. Kinetics of pymetrozine in soil.

[15] and Valverde-Garcia et al. [16] reported that the rate of growth of the vegetable fruit will affect the observed disappearance rate constant of applied pesticides. Cucumber fruit grows at a faster rate than any other fruit and its weight doubles within 36 h. This increase in the fruit weight will cause a dilution of the observed pesticide concentration even if the pesticide does not degrade on the fruit at all [17]. From these findings, it was concluded that the observed faster degradation rate of pymetrozine on cucumber could be due partially to the higher growth rate of this fruit relative to broccoli.

3.4.2. Pymetrozine residue in soil

Fig. 3 shows the residue of pymetrozine in soil over the time period of the experiment. The estimated residues were 14.09, 5.56, 3.43, 2.86, 0.55, 0.03, and 0.007 mg kg^{-1} at 0, 1, 3, 4, 8, 12, and 15 days after application, respectively. The half-life of pymetrozine in soil was 1.4 days and the dynamics could be described by the equation $C = 15.352\, e^{-0.4992t}$, $R^2 = 0.9815$. The pymetrozine residue in soil was undetectable for 21 days after application. After the application of pymetrozine, the initial deposit of the residue in the soil was higher than that in the broccoli, which demonstrates that the residue of pymetrozine exists in the surface layer of the soil.

3.5. Ultimate pymetrozine residue

The ultimate residue of pymetrozine in soil was undetectable at both the recommended (300 g a.i. hm^{-2}) and double the recommended (600 g a.i. hm^{-2}) dosage of 45 days after application. However, in broccoli, the ultimate residue levels of pymetrozine for 45 days after application were undetectable and 0.008 mg kg^{-1} at the recommended dose and double dose when sprayed twice, and 0.011 and 0.017 mg kg^{-1} at the recommended dose and double dose when sprayed three times, respectively. The MRL of pymetrozine in broccoli is 0.02 mg kg^{-1} based on the new regulations established in Japan. The residues following both the recommended and double dosage were all lower than 0.02 mg kg^{-1}. According to the equation describing the kinetics pymetrozine in broccoli, the harvest interval that could be considered safe for human beings and other animals is 23 days.

4. Conclusions

This study was designed to investigate the residues of pymetrozine in broccoli and soil. The results showed that pymetrozine degradation in broccoli and soil corresponded to $C = 1.9826\, e^{-0.1965t}$ and $C = 15.352\, e^{-0.4992t}$, while the half-lives were

approximately 3.5 and 1.4 days, respectively. The final residue of pymetrozine on broccoli was lower than the new MRL; therefore, a dosage of 300 g a.i. hm^{-2} is suggested. This dosage is considered to be safe for human beings, and a 23-day interval between application and harvest was determined for safe use of the pesticide. These results contribute to establish adequate monitoring of the residue of pymetrozine and its incorporation in pest management strategies in vegetable fields, and to prevent health problems in consumers.

Acknowledgment

The work was supported by the Standard Program of Shanghai Science & Technology Committee, China (No. 06DZ05128).

References

[1] M. Bhanti, A. Taneja, Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern India, Chemosphere 69 (2007) 63–68.
[2] L. Trinidad, L.M. José, D.G. María, M. María, A.R. José, Benzoylphenylurea residues in peppers and zucchinis grown in greenhouses: determination of decline times and pre-harvest intervals by modeling, Pest Manage. Sci. 60 (2003) 183–190.
[3] P. Zhou, Y.T. Lu, B.F. Liu, J.J. Gan, Dynamics of fipronil residue in vegetable-field ecosystem, Chemosphere 57 (2004) 1691–1696.
[4] X. Yi, Y.T. Lu, Residues and dynamics of probenazole in rice field ecosystem, Chemosphere 65 (2006) 639–643.
[5] B. Sechser, B. Reber, F. Bourgeois, Pymetrozine: selectivity spectrum to beneficial arthropods and fitness for integrated pest management, J. Pest Sci. 75 (2002) 72–77.
[6] H. Mercana, E. Yılmaza, R. Inam, Determination of insecticide pymetrozine by differential pulse polarography/application to lake water and orange juice, J. Hazard. Mater. 141 (2007) 700–706.
[7] J.E. Polston, T. Sherwood, Pymetrozine interferes with transmission of tomato yellow leaf curl virus by the Whitefly, *Bemisia tabaci*, Phytoparasitica 31 (2003) 490–498.
[8] M.R. Lashkari, A. Sshragard, M. Ghadamyari, Sublethal effects of imidacloprid and pymetrozine on growth parameters of cabbage aphid, *Brevicoryne brassicae* on rapeseed, *Brassica napus* L., Insect Sci. 14 (2007) 207–212.
[9] W.F. Nicholson, R. Senn, C.R. Flueckiger, D. Fuog, Pymetrozine—a novel compound for control of whiteflies, in: D. Gerling, D. Mayer (Eds.), Bemisia: Taxonomy, Biology, Damage, Control and Management. Intercept, Andover, Hants, United Kingdom, 1995, pp. 635–639.
[10] http://www.epa.gov/opprd001/factsheets/pymetrozine.pdf.
[11] X. Zhang, X. Cheng, C. Wang, Z. Xi, Q. Li, Efficient high-performance liquid chromatography with liquid–liquid partition cleanup method for the determination of pymetrozine in tobacco, Ann. Chim. 97 (2007) 295–301.
[12] J. Wang, Z. Shang, HPLC Methods, Chinese Environment Science Press, 1992.
[13] E. Francotte, A. Davatz, P. Richert, Development and validation of chiral high-performance liquid chromatographic methods for the quantitation of valsartan and of the tosylate of valinebenzyl ester, J. Chromatogr. B 686 (1996) 77–83.
[14] K. Talebi, H.G. Ahsaii, Determination of pymetrozine residues in cucumber Commun, Agric. Appl. Biol. Sci. 71 (2006) 75–78.
[15] M.I. Walash, F. Belal, M.E. Metwally, M. Hefnay, Spectrophotometric determination of maneb and zineb and their decomposition products in some vegetables and its application to kinetic studies after greenhouse treatment, Food Chem. 47 (1993) 411–416.
[16] A. Valverde-Garcia, E. Gonzalez-Pradas, A. Aguilera-Del Real, D.M. Urena-Amate, F. Camacho-Ferre, Determination and degradation study of chlorothalonil in cucumbers, peppers and cherry tomatoes, Anal. Chim. Acta 276 (1993) 15–23.
[17] M.E.S. Metwally, M.S. Osman, A high-performance liquid chromatographic method for the determination of cypermethrin in vegetables and its application to kinetic studies after greenhouse treatment, Food Chem. 59 (1997) 283–290.