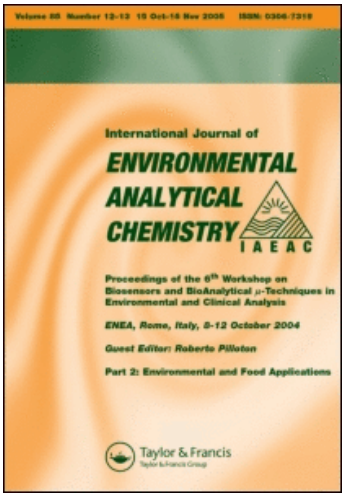

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Analysis of pyraflufen-ethyl residues in apples and soil by high-performance liquid chromatography

Huili Wang[a]; Jiye Hu[a]; Hongxun Zhang[a]; Changlong Chen[a]; Xuyan Chen[a]; Jianzhong Li[a]
[a] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Haidian District, Beijing 100085, China

To cite this Article Wang, Huili , Hu, Jiye , Zhang, Hongxun , Chen, Changlong , Chen, Xuyan and Li, Jianzhong(2007) 'Analysis of pyraflufen-ethyl residues in apples and soil by high-performance liquid chromatography', International Journal of Environmental Analytical Chemistry, 87: 2, 99 — 109
To link to this Article: DOI: 10.1080/03067310600922089
URL: http://dx.doi.org/10.1080/03067310600922089

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 2, 15 February 2007, 99–109

Taylor & Francis
Taylor & Francis Group

Analysis of pyraflufen-ethyl residues in apples and soil by high-performance liquid chromatography

HUILI WANG, JIYE HU, HONGXUN ZHANG, CHANGLONG CHEN, XUYAN CHEN and JIANZHONG LI*

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing 100085, China

(*Received 8 March 2006; in final form 20 June 2006*)

A new method was developed to analyse pyraflufen-ethyl residues by high-performance liquid chromatography (HPLC). The UV detector was used for routine analysis and the ion-trap MS was used to confirm the identity of the compound. The residue levels of the pesticide and its dissipation rate in apples and soil in an apple orchard of Beijing were also studied. Primary secondary amine (PSA) and octadecyl (C_{18}) solid-phase extraction (SPE) cartridges were applied for the determination of pyraflufen-ethyl residues in apples and soil, respectively. The limit of detection was estimated to be 1.6 ng, and the limit of quantification of pyraflufen-ethyl in the samples was 0.01 mg kg^{-1}. Average recoveries were between 90.1 and 102.1% at three spiking levels of 0.01, 0.1, and 1 mg kg^{-1}, and relative standard deviations were less than 10% throughout the whole recovery test. A PSA column was found to provide effective cleanup for apples extract in the determination of pyraflufen-ethyl, and C_{18} could remove the greatest number of sample matrix interferences in soil. A dissipation study showed that the half-life obtained for pyraflufen-ethyl in soil was approximately 11.89 days at 1.5 times of the recommended dosage, and no pyraflufen-ethyl residues were detected in apples at harvest.

Keywords: Pyraflufen-ethyl; Apple; Soil; Dissipation; Solid-phase extraction; HPLC

1. Introduction

Pyraflufen-ethyl [ethyl2-chloro-5-(4-chloro-5-difluoromethoxy-1-methyl-1*H*-pyr-azol-3-yl)-4-fluorophenoxyacetate; figure 1] is a selective post-emergence herbicide for cereals and is highly effective against several important broad-leaf weeds [1, 2]. It has been developed as a desiccant for potatoes and a defoliant for cotton [3, 4]. In China, pyraflufen-ethyl as a herbicide was first registered in 1998 for use on

*Corresponding author. Fax: +86-010-62849385. Email: whlzblv@263.net

International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2007 Taylor & Francis
DOI: 10.1080/03067310600922089

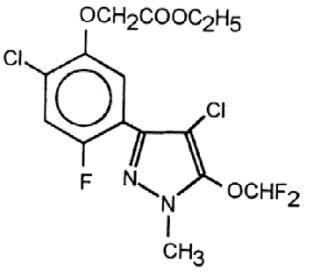

Figure 1. Chemical structure of pyraflufen-ethyl.

wheat and then registered in 2002 for use in orchards in combination with glyphosate-trimesium salt.

Nowadays, the technique most widely used for isolation and preconcentration of pesticides from environmental samples is solid-phase extraction (SPE), which has a number of well-documented advantages [5]. SPE is still the dominant method for soil-extract purification [6], and it is one of the most popular techniques used in sample preparation prior to analysis by HPLC and gas chromatography (GC), being used for environmental, food, pharmaceutical, and biological analysis [7, 8]. Solid-phase extraction (SPE) can be used either off-line or on-line to recover herbicides from water [9–11], soil [10, 12–13], vegetables and fruits [14–16], and biological samples [17–20].

The most common methods of herbicide detection are gas chromatography coupled with mass spectrometry detection (GC/MS) and high-performance liquid chromatography (HPLC) [21, 22]. Immunochemical methods, such as ELISA are also available for herbicide detection [23–25]. The application of enzyme-linked immunosorbent assays (ELISA) in the determination of herbicides has also been reported [26, 27]. Herbicides that are thermally unstable or have low volatility are determined by liquid chromatography with ultraviolet [28] or fluorescence detection [29]. LC–MS is a fast developing technique because MS detection offers the possibility of achieving high sensitivity and selectivity. During the last few years, atmospheric pressure ionization (API) techniques, electrospray ionization (ESI) [30, 31], and atmospheric pressure chemical ionization (APCI) [32–34] have become the more popular interfaces. ESI is more suited to polar and ionic compounds, whereas APCI is used for moderately non-polar compounds. An important aspect when performing residue analysis at the low concentrations relevant to soil is to ensure a high degree of confidence in the identification of the compounds, in order to avoid false positives. The MS fragmentation pattern is a powerful tool for obtaining such confidence in compound identification.

No analytical method for determination of pyraflufen-ethyl residues in crops and soil has been reported so far, and no work has been found in the literature on the dissipation rate of pyraflufen-ethyl in soil and apples. Thus, the objective of our work was to establish a method for analysing the residue of pyraflufen-ethyl in soil and apples, and to evaluate pyraflufen-ethyl residue levels in apples and its dissipation rate in soil in an apple orchard to provide scientific evidence for both environmental monitoring studies and systematic determination of the environmental fate of pyraflufen-ethyl.

2. Experimental

2.1 *Chemicals and reagents*

Standard pyraflufen-ethyl (98.8%) and a commercial formulation (NNH-950 SC) were provided by Nohyaku Co., Ltd (Tokyo, Japan). Acetone, acetonitrile, hexane, sodium chloride, anhydrous sodium sulphate, and anhydrous magnesium sulphate were of residue grade and obtained from Beijing Chemical Factory (Beijing, China). HPLC-grade acetonitrile was purchased from Dikma Limited (Beijing, China). The PSA cartridge (500 mg/3 mL) was from Varian Sample Preparation Products (Walnut Creek, USA); the C_{18} cartridge (500 mg/3 mL) was obtained from Supelco (Bellefonte, USA).

2.2 *Instrumentation and experimental conditions*

HPLC analysis was carried out on an Agilent 1100 series (Agilent Technologies, Palo Alto, CA) chromatograph, equipped with a Quat-Pump delivery system, an injector with a 20 μL loop and an HP UV-Vis absorbance detector. A C_{18} analytical column 250 mm × 4.6 mm i.d., 5 μM ODS-3 (GL Science, Japan) was employed. All solvents were filtered through a 0.45 μM filter disk (Millipore). Data acquisition and treatment were performed using Agilent ChemStation Version Rev.A.10.02. The chromatographic conditions used for the analysis of pyraflufen-ethyl residues were as follows: the mobile phase was acetonitrile : water (65 : 35 v/v) at a flow rate of 1 mL min^{-1}. Detection was performed at 207 nm. All measurements were carried out at room temperature.

An Agilent 1100 LC/MSD Trap VL (Agilent Technologies, Ltd, Germany) was used under the following conditions: Mass Range Mode, Std/Normal; gas drying temperature, 250°C; APCI temperature, 325°C; drying gas flow, 5 L min^{-1}; nebulizer pressure, 60 p.s.i.; cutoff, 27%; amplitude, 1.0 V; smart mode, 30–200%; the mass spectrometer was operated in the APCI positive ion mode, and the scan range was 50–400 amu for determination of pyraflufen-ethyl.

2.3 *Analytical method*

2.3.1 Analytical standards and working solutions. A stock solution (100 mg L^{-1}) of pyraflufen-ethyl was prepared in HPLC-grade acetonitrile. Working solutions (0.1–20 μg mL^{-1}) were prepared by appropriate dilution of the stock solution in HPLC-grade acetonitrile. All solutions were protected against light with aluminium foil and were stored in a refrigerator at 4°C.

2.3.2 Extraction and purification procedure. Extracts were taken from chopped apples (30 g) with 95 mL of acetonitrile by ultrasonic extraction (30 min). The extracts were collected into a polypropylene centrifuge tube and washed with a small amount of acetonitrile. The extract was made up to 100 mL with acetonitrile. Anhydrous magnesium sulphate (15 g) and sodium chloride (5 g) were added into the centrifuge tube. The mixture was vigorously shaken for 5 min and then centrifuged for a further 15 min at 3000 rpm. The organic extract (80 mL) was quantified and transferred to a 250 mL round-bottomed flask and concentrated under vacuum at 40°C to dryness

with a rotary evaporator. The pyraflufen-ethyl residue was dissolved with acetonitrile (3 mL) for purification by PSA cartridges. The PSA cartridges were preconditioned with acetonitrile (2 mL), and then the extracts (2 mL) were added. Collection of the eluate started immediately after applying the extract. The elution continued with acetonitrile (3 mL) and this eluate was collected in the same tube. The total eluate was concentrated under a slow nitrogen stream. The residue obtained was redissolved in acetonitrile (1 mL), and the extract was filtered through a 0.45 μm Teflon filter for HPLC analysis.

Soil samples (30 g, passed through a 2 mm sieve) were extracted twice by ultrasonic extraction for 30 min with a mixture of acetone–water (80 : 20, v/v, 2×60 mL). The combined extracts were filtered through Whatman No. 1 filter paper and then concentrated under vacuum with a rotary evaporator at a bath temperature of 50°C until the final volume reached about 10 mL. The resultant mixture was dehydrated by passing through a bed containing anhydrous magnesium sulphate and eluted with acetone. The eluate was then concentrated under vacuum at 40°C to dryness with a rotary evaporator. The residue of the extracts was redissolved with 3 mL acetonitrile-water (30 : 70, v/v) and centrifuged for 5 min at 5000 rpm for purification by C_{18} cartridges. The C_{18} cartridges were connected to a Visiprep 12-port SPE manifold and conditioned with acetonitrile (5 mL), followed by distilled water (5 mL). The extract (2 mL) was loaded onto the cartridge and passed through at a flow rate of one to two drops per second. The cartridge was washed with acetonitrile–water (3 mL, 50 : 50, v/v) and then dried with air. The column was eluted with acetonitrile (3 mL), and the eluate was dried under a gentle stream of nitrogen. The residue was redissolved in acetonitrile (1 mL) and filtered through a 0.45 μm filter before HPLC–UV determination.

2.3.3 Recovery assay. The method described for sample preparation was validated by a recovery investigation. Untreated apple and soil samples were fortified with known amounts of pyraflufen-ethyl (10, 100, and 1000 μg kg^{-1}), and the samples were shaken for 1 min by hand, thoroughly mixed, and allowed to settle for 1 h. The extraction and purification were carried out according to the above procedure. Every recovery was done on four replicates.

2.4 *Field trial*

The field trial was carried out in an apple orchard, located in the township of Nankou, Changping district, Beijing, China. The field was divided into 30 m^2-sized blocks for the control as well as the dissipation rate study. The control plots were separated by guard rows to avoid contamination by drift. The trial was conducted from May to August 2005, using a pressurized hand-gun sprayer at high volume to run off.

The weeds in the apple orchard were sprayed, in three replications, with NNH-950 SC (Japan, 30% glyphosate + 0.15% pyraflufen-ethyl) diluted with water at a dosage of 8.81 g of active ingredient of pyraflufen-ethyl per hectare (1.5 times the recommended dosage). Soil samples were collected from directly beneath the treated weed canopy at the surface and from different depths ranging from 0 to 15 cm. The soil samples, which were collected at 0 (1 h after spraying), 2, 5, 7, 14, 21, 35, 42, and 60 days, were put into polyethylene bags and transported to the laboratory. All the sub-samples were kept

deep-frozen (−20°C) until analysis. Control samples were obtained from the soil in the control plot. When they were analysed, all the field samples were based on dry weight.

3. Results and discussion

3.1 *Analytical method*

3.1.1 Calibration curve for pyraflufen-ethyl. Quantification was accomplished by using the standard curve prepared by diluting the stock solution in acetonitrile. The standard calibration curve of pyraflufen-ethyl was constructed by plotting the analyte concentration against peak areas. At 207 nm, the calibration curve was linear in the range of 0.1–20 $\mu g\,mL^{-1}$. The standard curve equation was $y = 104.03x - 1.9021$, $R^2 = 0.9998$.

3.1.2 LOD and LOQ. The limit of detection (LOD) and the limit of quantification (LOQ) were determined as the sample concentration of pyraflufen-ethyl at peak heights of three and 10 times the baseline noise, respectively. The LOD was found to be 4 $\mu g\,kg^{-1}$, and the LOQ was calculated to be 10 $\mu g\,kg^{-1}$, satisfying the Japanese [35], Korean [36], and European [37] MRLs of apple. Considering this result, the method is adequate to determine pyraflufen-ethyl residues in apples and soil.

3.1.3 Precision and accuracy. To assess the precision and accuracy of the chromatographic method, apples and soil containing pyraflufen-ethyl at different concentrations (10, 100, and 1000 $\mu g\,kg^{-1}$) were analysed repeatedly. The recoveries obtained were in the acceptable range of 90.1–102.1%, as shown in table 1. The relative standard deviations of the method (RSD%) were calculated to check the precision of the method, and the RSD% for repeatability ranged from 0.5% to 5.8%. All the batches met the criteria for acceptable quality control [38].

3.1.4 Solid-phase extraction procedure. Figure 2 shows the chromatogram of the standard, blank sample, fortified sample, and field-treated sample extracts with SPE. It was found that cleanup on PSA and C_{18} cartridges eliminated most interference peaks and allowed good recoveries at low fortification levels. To illustrate the importance of the SPE step in the sample preparation, figure 3 shows the chromatograms of fortified

Table 1. Recoveries of pyraflufen-ethyl in apple and soil samples with SPE ($n = 4$).

Sample	SPE cartridges	Fortification levels ($mg\,kg^{-1}$)	Average recoveries (%)	RSD (%)
Apple	PSA	0.01	95.9	2.3
		0.1	98.1	1.0
		1	96.0	0.5
Soil	C_{18}	0.01	99.0	5.8
		0.1	102.1	4.1
		1	90.1	2.9

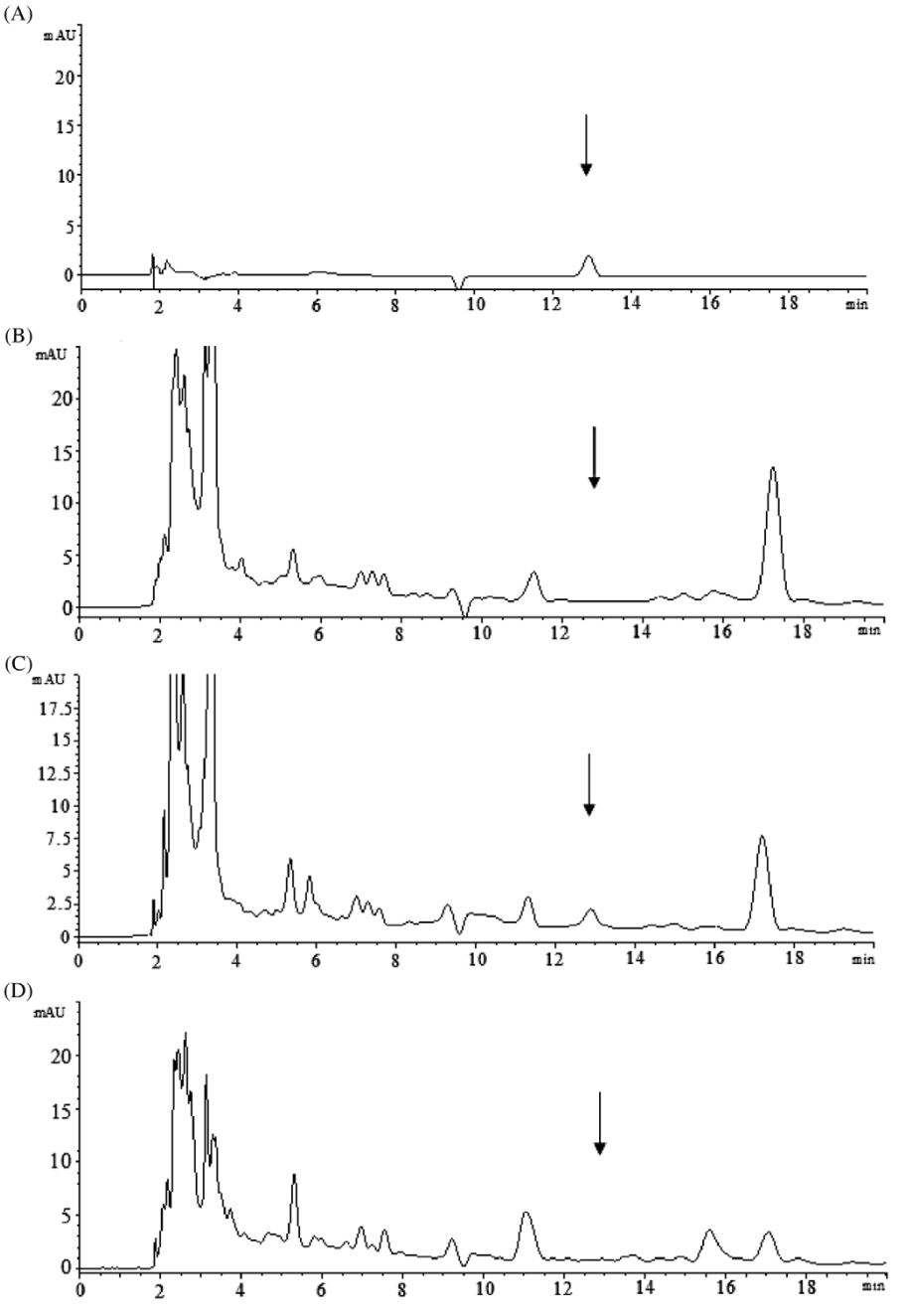

Figure 2. Chromatograms of (A) standard; (B) control apple sample; (C) fortified apple at 0.01 mg kg^{-1}; (D) field-treated apple; (E) control soil sample; (F) fortified soil at 0.01 mg kg^{-1}; (G) field-treated soil at 28 days.

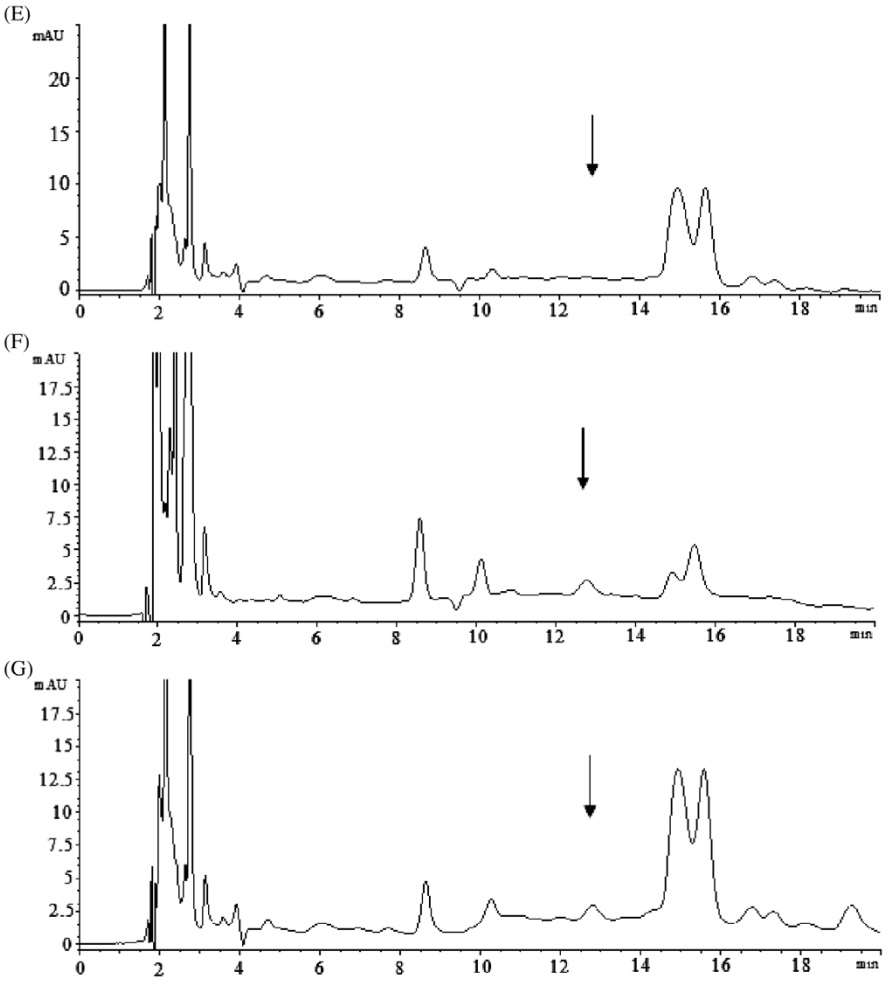

Figure 2. Continued.

apples and soil, prepared following the analytical routine by liquid–liquid extraction (LLE) but without the use of SPE purification steps. According to the data shown in figure 3, the importance and advantages of SPE are apparent for the removal of matrix interferents. It can also be seen that the recoveries of pyraflufen-ethyl extracted by LLE could not be calculated accurately.

It has been reported that using a single-bonded normal phase PSA column can provide excellent cleanup of fresh fruit and vegetable extracts for pesticide analysis [39]. Accordingly, we used PSA to analyse pyraflufen-ethyl residues in apple in this work, and we found that the acetonitrile eluate after PSA treatment was colourless. The colour does not necessarily mean interference to the chromatogram, but the pigment might be adsorbed by the LC column. We also evaluated the samples using a PSA column for purification in soil, but we found that the PSA's cleanup efficiency was not better than the C_{18} column for eliminating the matrix interference in soil. So, the study results indicated that the PSA columns were suitable for analysis of

pyraflufen-ethyl residues in apple, while C_{18} columns were more suitable for the analysis of residue in soil. The purification procedures eliminated the need for the solvents that are used in LLE, and allowed increased sample throughput compared with liquid–liquid extraction procedures.

3.2 *Field trial*

The residue analysis in apple and soil matrixes and detection of pyraflufen-ethyl were performed according to the method described above.

3.2.1 Pyraflufen-ethyl residue in apple. No pyraflufen-ethyl residues were detected in apples at harvest time.

3.2.2 Pyraflufen-ethyl residue in soil. The soil under investigation was slightly acidic (pH 5.63), and its texture was sandy loam. The organic matter content was 2.67%, and the cation-exchange capacity (CEC) was 29.7 cmol kg^{-1}. The average residue levels of pyraflufen-ethyl in the top 15 cm degraded from 0.098 to 0.011 mg kg^{-1}, over a period of 35 days (table 2). The dissipation of pyraflufen-ethyl residue with time was described mathematically by a pseudo-first rate equation. The regression line equation for the concentration (C) related to time (t) was $y = 0.08042e^{-0.0583x}$ ($R^2 = 0.9761$) at 1.5 times the recommended pyraflufen-ethyl dosage. The half-life ($t_{1/2}$) of pyraflufen-ethyl in the

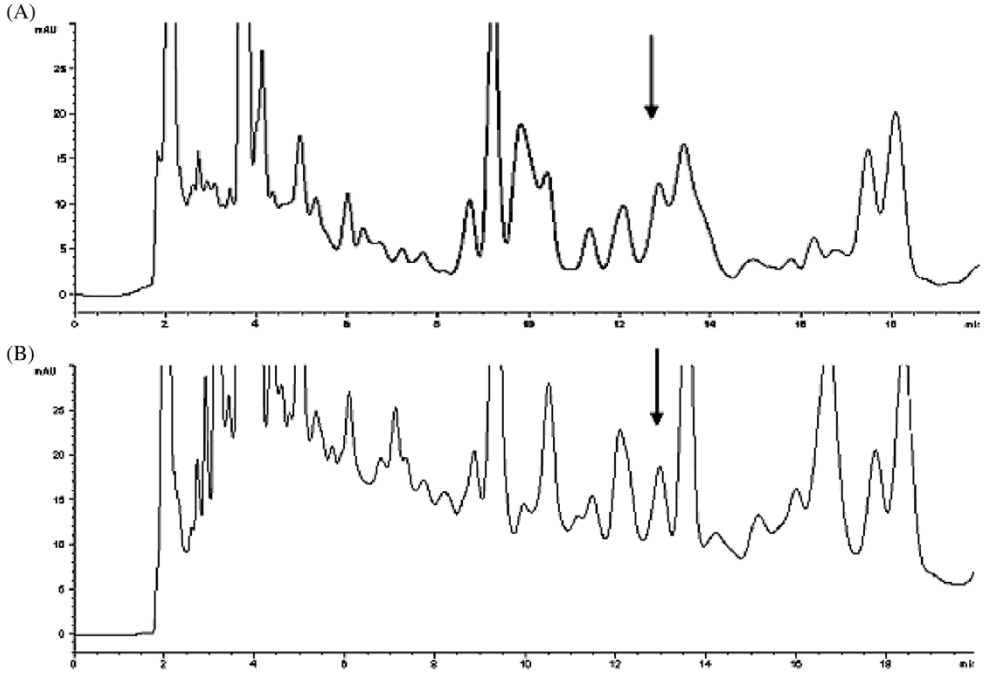

Figure 3. Chromatograms of extracts from apples and soil obtained using LLE and without the SPE step at fortification of 0.1 mg kg^{-1}. (A) Fortified apple sample; (B) fortified soil sample.

soil in the apple orchard was approximately 11.89 days, and on the 42nd day, the residues in treated soil were lower than the LOD. The decrease in residue levels for different days after treatment is presented in table 2, and the corresponding decline curves are shown in figure 4. Our work indicated the less persistent nature of pyraflufen-ethyl under field conditions.

It is noteworthy that pyraflufen-ethyl in the apple and soil samples was identified by its retention time and specific ions. A confirmation assay was performed by HPLC-MS using the conditions described above (figure 5), and the following fragments were obtained: $[M+H]^+$ at m/z 413, $[M+H\text{-}COOC_2H_5]^+$ at m/z 339 and $[M+H\text{-}COOC_2H_5\text{-}CL]^+$ at m/z 304. The LOD and the LOQ obtained using LC-MS were determined as the sample concentration of pyraflufen-ethyl at peak heights of 3 and 10 times the baseline noise, respectively; the LOD was found to be $0.15\,\mu g\,kg^{-1}$; and the LOQ was calculated to be $0.5\,\mu g\,kg^{-1}$. It can be seen that the pyraflufen-ethyl detected by MS detector is more sensitive than that detected by the UV detector.

Table 2. Pyraflufen-ethyl residues in soil at different time intervals ($n=3$).

Days after treatment (days)	Residue concentration of pyraflufen-ethyl ($mg\,kg^{-1}$)	RSD (%)
0	0.098	2.2
2	0.074	1.4
5	0.053	4.7
7	0.047	3.8
14	0.034	6.2
21	0.024	7.9
35	0.011	9.1
42	BDL[a]	–
60	BDL	–

[a] Below limit of detection (LOD).

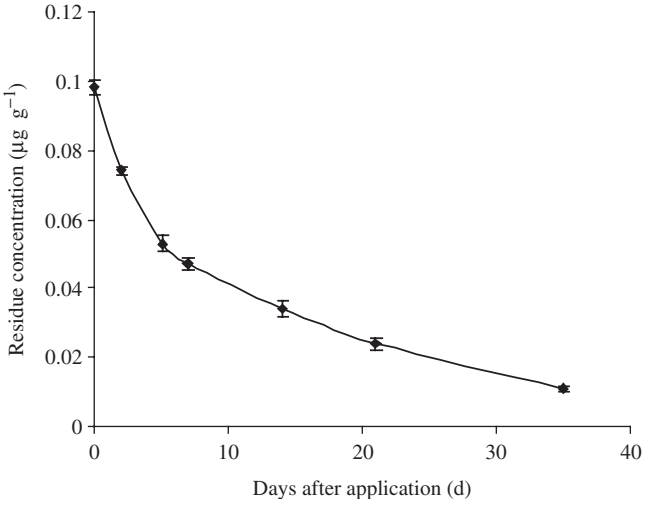

Figure 4. Dissipation rate of pyraflufen-ethyl in soil. Vertical bars represent ± standard of the mean of three replicates.

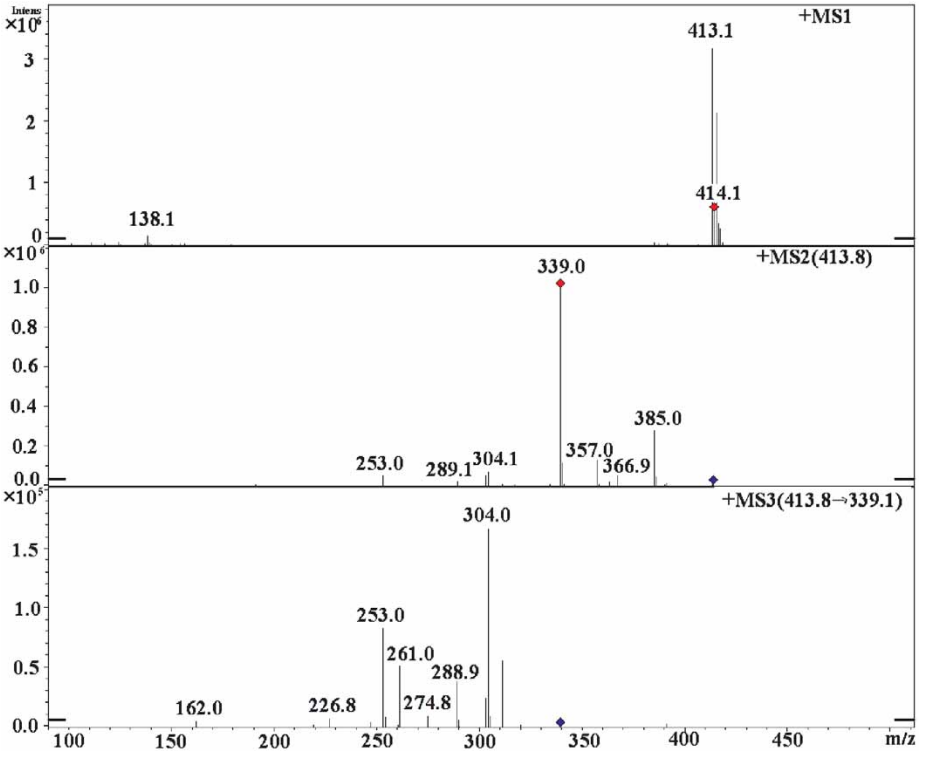

Figure 5. Mass spectra of pyraflufen-ethyl.

4. Conclusion

A simple and efficient method for the determination of pyraflufen-ethyl residues in apples and soil by HPLC with UV detection using SPE has been developed. In this work, we used PSA and C_{18} SPE for the cleanup of pyraflufen-ethyl in apples and soil, respectively. This procedure as an alternative to the classical liquid–liquid extraction method removes the volumes of organic solvents used, and allows increased sample throughput compared with liquid–liquid extraction procedures. It also has a better cleanup performance than LLE.

The proposed method was applied to the determination of pyraflufen-ethyl residues in apples and soil in an apple orchard in Beijing after application of pyraflufen-ethyl. The half-life obtained following the application of pyraflufen-ethyl was 11.89 days in soil, and no pyraflufen-ethyl residues were detected in apples at harvest time.

Acknowledgements

This work was supported by the Ministry of Agriculture of the People's Republic of China. We also would like to thank professor Hanhong Mo and Fengchun An for their guidance and advice.

References

[1] T. Mabuchi, Y. Miura, T. Ohtsuka. *J. Pestic. Sci.*, **27**, 39 (2002).
[2] S. Murata, A. Yamashita, Y. Kimura, K. Motoba, T. Mabuchi, Y. Miura. *J. Pestic. Sci.*, **27**, 47 (2002).
[3] Y. Mura, T. Mabuchi, M. Higashimura, T. Amanuma. *J. Pestic. Sci.*, **28**, 219 (2003).
[4] J.A. Ivany. *Crop Prot.*, **24**, 836 (2005).
[5] P. Lopez-Roldan, M.J. Lopez de Alda, D. Barcelo. *Anal. Bioanal. Chem.*, **378**, 599 (2004).
[6] V. Andreu, Y. Picó. *Trends Anal. Chem.*, **23**, 772 (2004).
[7] S.C.N. Queiroz, C.H. Collins, I. Jardim. *Quim. Nova*, **24**, 68 (2001).
[8] M.C. Hennion. *J. Chromatogr. A*, **856**, 3 (1999).
[9] N. Li, H.K. Lee. *Anal. Chem.*, **72**, 3077 (2000).
[10] O. Pozo, E. Pitarch, J.V. Sancho, F. Hernández. *J. Chromatogr. A*, **923**, 75 (2001).
[11] E. Ayano, H. Kanazawa, M. Ando, T. Nishimura. *Anal. Chim. Acta*, **507**, 211 (2004).
[12] S. Moret, J.M. Sánchez, V. Salvadó, M. Hidalgo. *J. Chromatogr. A*, **1099**, 55 (2005).
[13] J. Hernández-Borges, F.J. García-Montelongo, A. Cifuentes, M.Á. Rodríguez-Delgado. *J. Chromatogr. A*, **1100**, 236 (2005).
[14] F. Peña, S. Cárdenas, M. Gallego, M. Valcárcel. *Talanta*, **56**, 727 (2002).
[15] C. Cacho, E. Turiel, A. Martín-Esteban, D. Ayala, C. Pérez-Conde. *J. Chromatogr. A*, **1114**, 255 (2006).
[16] J. Engebretson, G. Hall, M. Hengel, T. Shibamoto. *J. Agric. Food Chem.*, **49**, 2198 (2001).
[17] E. Bichon, M. Dupuis, B. Le Bizec, F. André. *J. Chromatogr. A*, **838**, 96 (2006).
[18] A. Li, M.P. May, J.C. Bigelow. *J. Chromatogr. B*, **836**, 129 (2006).
[19] A.O. Olsson, S.E. Baker, J.V. Nguyen, L.C. Romanoff, S.O. Udunka, R.D. Walker, K.L. Flemmen, D.B. Barr. *Anal. Chem.*, **76**, 2453 (2004).
[20] J. Norrgran, R. Bravo, A.M. Bishop, P. Restrepo, R.D. Whitehead, L.L. Needham, D.B. Barr. *J. Chromatogr. B*, **830**, 185 (2006).
[21] J. Tronczynski, C. Munschy, G. Durand, D. Barceló. *Sci. Total Environ.*, **132**, 327 (1993).
[22] V. Pacáková, K. Stulík, J. Jiskra. *J. Chromatogr. A*, **754**, 17 (1996).
[23] B.S. Clegg, G.R. Stephenson, J.C. Hall. *J. Agric. Food Chem.*, **49**, 2168 (2001).
[24] J.K. Lee, K.C. Ahn, O.S. Park, Y.K. Ko, D.W. Kim. *J. Agric. Food Chem.*, **50**, 1791 (2002).
[25] E. Watanabe, R. Hoshino, Y. Kanzaki, H. Tokumoto, H. Kubo, H. Nakazawa. *J. Agric. Food Chem.*, **50**, 3637 (2002).
[26] M. Khrolenko, P. Dżygiel, P. Wieczorek. *J. Chromatogr. A*, **975**, 219 (2002).
[27] M.Y. Selisker, D.P. Herzog, R.D. Erber, J.R. Fleeker, J.A. Itak. *J. Agric. Food Chem.*, **43**, 544 (1995).
[28] A. Sannino. *J. AOAC Int.*, **81**, 1048 (1998).
[29] J. Fillion, R. Hindle, M. Lacroix, J. Selwyn. *J. AOAC Int.*, **78**, 1252 (1995).
[30] S. Chiron, S. Papilloud, W. Haerdi, D. Barcelo. *Anal. Chem.*, **67**, 1637 (1995).
[31] G. D'Ascenzo, A. Gentili, S. Marchese, A. Marino, D. Perret. *Chromatographia*, **48**, 497 (1998).
[32] C. Aguilar, I. Ferrer, F. Borrull, R.M. Marcé, D. Barceló. *J. Chromatogr. A*, **794**, 147 (1998).
[33] G.M. Kienhuis, R.B. Geerdink. *Trends Anal. Chem.*, **19**, 249 (2000).
[34] G.M. Kienhuis, R.B. Geerdink. *Trends Anal. Chem.*, **19**, 460 (2000).
[35] Ministry of Health, Labour and Welfare. Available online at: http://www.mhlw.go.jp/english/topics/mrls/second/dl/mrls36.pdf (accessed August 2004).
[36] Korean Food & Drug Administration Notice. Available online at: http://www.fas.usda.gov/ffpd/WTO_SPS_TBT_Notifications/Fishery_Products/Kor148text.pdf (accessed October 2003).
[37] The European Commission. Available online at: http://europa.eu.int/comm/food/plant/protection/resources/mrl_crop.pdf (accessed November 2004).
[38] L.V. Podhorniak, J.F. Negron, F.D. Griffith Jr. *J. AOAC Int.*, **84**, 873 (2001).
[39] F.J. Schenck, S.J. Lehotay, V. Vega. *J. Sep. Sci.*, **25**, 883 (2002).