Science of the Total Environment 551–552 (2016) 484–488

Contents lists available at ScienceDirect

Science of the Total Environment

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

Rapid screening of flonicamid residues in environmental and agricultural samples by a sensitive enzyme immunoassay

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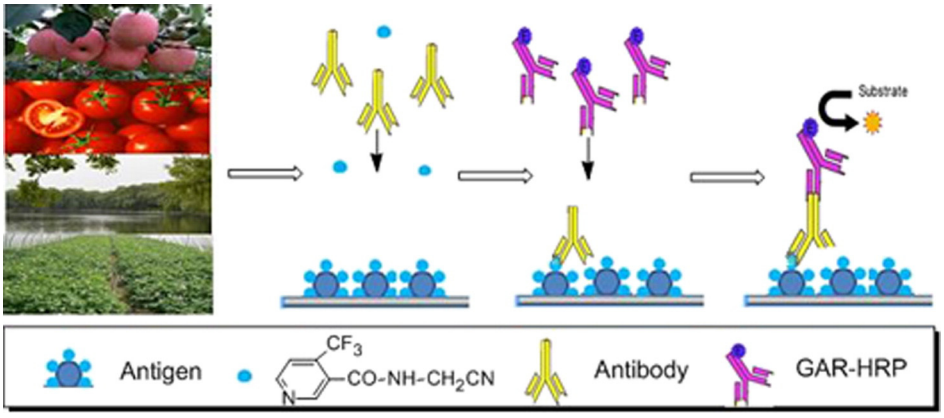
HIGHLIGHTS

- The antibody of flonicamid was obtained.
- A high-throughput, selective and simple ELISA for flonicamid was developed.
- The results of ELISA for the spiked samples were largely consistent with the gas chromatography method.
- This methodology appeared to be useful as a screening method prior to flonicamid analysis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form 2 February 2016
Accepted 2 February 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Flonicamid
Hapten
Polyclonal antibody
Enzyme-linked immunosorbent assay
Environmental and agricultural

ABSTRACT

A fast and sensitive polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) was developed for the analysis of flonicamid in environmental and agricultural samples. Two haptens of flonicamid differing in spacer arm length were synthesized and conjugated to proteins to be used as immunogens for the production of polyclonal antibodies. To obtain most sensitive combination of antibody/coating antigen, two antibodies were separately screened by homologous and heterologous assays. After optimization, the flonicamid ELISA showed that the 50% inhibitory concentration (IC_{50} value) was 3.86 mg L^{-1}, and the limit of detection (IC_{20} value) was 0.032 mg L^{-1}. There was no cross-reactivity to similar tested compounds. The recoveries obtained after the addition of standard flonicamid to the samples, including water, soil, carrot, apple and tomato, ranged from 79.3% to 116.4%. Moreover, the results of the ELISA for the spiked samples were largely consistent with the gas chromatography ($R^2 = 0.9891$). The data showed that the proposed ELISA is an alternative tool for rapid, sensitive and accurate monitoring of flonicamid in environmental and agricultural samples.

1. Introduction

Flonicamid (*N*-cyanomethyl-4-trifluoromethylnicotinamide) is a novel selective systemic pesticide (Tomlin, 2003), which has been widely applied to rice, leafy vegetables, tomato and tea to control

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http://dx.doi.org/10.1016/j.scitotenv.2016.02.017
0048-9697/

noxious insects, with high effectiveness against aphids and other sucking insects (Morita et al., 2000; Hengel and Miller, 2007). As a result, flonicamid is present in river water, soil and agricultural products. To protect consumers from risks related to flonicamid residue, maximum residue limits (MRLs) of flonicamid in agricultural samples have been established by the USA (0.45 mg kg^{-1} for carrot) and Japan (0.4 mg kg^{-1} for tomato and 1 mg kg^{-1} for apple) (Xie et al., 2011). There are no suggested MRLs for flonicamid in China.

Several instrument-based detection methods for flonicamid have been developed, such as gas chromatography (GC) (Xie et al., 2011; Shi et al., 2015), high-performance liquid chromatography (HPLC) (Ma et al., 2015) and high-performance liquid chromatography–mass spectrometry (HPLC–MS) (Chen, 2002; Zywitz et al., 2003; Hengel and Miller, 2007; Chen et al., 2012; Ko et al., 2014). Although these methods are characterized by low limits of detection and high precision and sensitivity, they cannot meet the needs of the high-throughput, rapid, screening of large numbers of environmental and agricultural samples. Enzyme-linked immunosorbent assay (ELISA) fulfills these requirements and has become a reliable analytical tool for rapid screening analysis (Liu et al., 2013). ELISA has been successfully used to detect contaminates, including toxins (Sheng et al., 2012; Kawatsu et al., 2014), antibiotics (Adrian et al., 2012; Peng et al., 2012; Sheng et al., 2013; Wang et al., 2015) and pesticides (Gurbuz et al., 2009; Liu et al., 2011; Watanabe et al., 2011; Liu et al., 2013; Navarro et al., 2013; Abad-Fuentes et al., 2014; Hua et al., 2015). However, ELISA of flonicamid has not been reported. In this paper, a rapid and sensitive ELISA was developed for the detection of flonicamid residues in environmental and agricultural samples based on polyclonal antibodies. Furthermore, the ELISA performance was evaluated with conventional GC-NPD in terms of precision and accuracy using spiked samples.

2. Materials and methods

2.1. Reagents

Pesticide-grade flonicamid with a purity of 98.5% was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 4-Trifluoromethylnicotinic acid (TFNA), 4-trifluoromethylnicotinamide (TFNA-AM) and *N*-(4-trifluoromethylnicotinoyl) glycine (TFNG) were purchased from Hayashi Pure Chemical Ind., Ltd (Osaka, Japan). Bovine serum albumin (BSA), ovalbumin (OVA), Freund's complete and incomplete adjuvants, goat *anti*-rabbit IgG-horseradish peroxidase (GAR-HRP), polyoxyethylene sorbitan monolaurate (Tween-20), 3,3′,5,5′-tetramethylbenzidine.

(TMB), *N*-hydroxysuccinimide (NHS), N,N′-dicyclohexylcarbodiimide (DCC) and isobutyl chloroformate were purchased from Sigma Chemical Co. (Shanghai, China). Aminoacetic acid and 4-aminobutyric acid were purchased from Aldrich (Milwaukee, USA). Acetone, acetonitrile, toluene, ethyl acetate, petroleum ether, $SOCl_2$, $NaHCO_3$, NaCl, Na_2CO_3 and so on were purchased from Beijing Chemical Reagent Co., Ltd (Beijing, China). All reagents and solvents were analytical grade.

2.2. Instruments

Nuclear magnetic resonance (NMR) spectra were recorded on a DRX 500 spectrometer (Bruker, Germany). Mass spectral (MS) data were obtained with a LC-MS^{QDECA} (Finnigan, USA). Ultraviolet spectra were recorded on a DU 800 spectrophotometer (Beckman, USA). The 96-well polystyrene microplates (Maxisorp) were purchased from Nunc (Roskilde, Denmark). Absorbance was read with an Infinite M200 microtiter plate reader (Tecan, Switzerland) at 450 nm, and the ELISA plates were washed with a Wellwash Plus (Thermo, USA). The flonicamid ELISA was confirmed with an Agilent 7890A gas chromatograph (Agilent, USA).

2.3. Buffers and solutions

The following buffers were used: (A) coating buffer, 0.05 mol L^{-1} carbonate-buffered saline (CBS), pH 9.6; (B) blocking buffer, 5 g skim milk in 100 mL of phosphate-buffered saline (PBS, 0.01 mol L^{-1}, pH 7.4); (C) washing buffer, PBS containing 0.05% Tween-20 (PBST); (D) the TMB solution contained 0.4 mmol L^{-1} TMB and 3 mmol L^{-1} H_2O_2 in citrate buffer (pH 5.0).

2.4. Hapten synthesis

The structure and synthesis route of 4-(trifluoromethyl)nicotinoyl chloride (**A**), Hapten 1 and Hapten 2 are shown in Fig. 1.

Compound **A:** 4-(trifluoromethyl)nicotinic acid [2 g (9.7 mmol)] and toluene (30 mL) were added to a 100-mL round-bottomed flask and were cooled in an ice bath. Then, 5.3 g (44.6 mmol) of $SOCl_2$ was slowly added to the solution. After the addition, the temperature was increased to 45 °C for 2 h and was then gradually increased to 80 °C for 1 h. Finally, unreacted reagents were removed by a rotary evaporator, and brown liquid **A** (1.87 g) was obtained.

Hapten 1: A mixture of 1.3 g (175 mmol) of aminoacetic acid and 17.65 g (175 mmol) of triethylamine in 200 mL of toluene was stirred and cooled in an ice bath. Then, liquid **A** was added to the solution slowly, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was moved into a separating funnel, 30 mL water was added, and the organic phase was separated, dried over anhydrous sodium sulfate and evaporated under reduced pressure. This residue was subjected to column chromatography [silica gel, ethyl acetate:petroleum ether (8:1, v/v)]. Yield: 38%. The product was characterized by ESI-MS and NMR: ESI-MS, *m/z*: 339 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6), δ: 4.3 (s, 2H, CH2), 7.58–7.60 (d, 1H, CH), 8.66–8.68 (m, H, CH), 8.77 (s, H, CH).

Hapten 2: This hapten was synthesized using **A** and 4-aminobutyric acid. Yield: 48%. The product was characterized by ESI-MS and NMR: ESI-MS, *m/z*: 367 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6), δ: 1.73–1.78 (m, 2H, CH2), 2.29–2.51 (m, 2H, CH2), 3.26–3.37 (m, 2H, CH2), 7.81–7.83 (d, 1H, NH), 8.74–8.91 (m, 3H, CH × 3), 12.1 (s, 1H, COOH).

2.5. Synthesis and identification of hapten–protein conjugates

Two flonicamid haptens were coupled with BSA using the active ester method to produce an immunogen and were conjugated with OVA by the mixed anhydride method to produce a coating antigen (Li et al., 2014). The conjugates were dialyzed against PBS for 72 h at 4 °C and were stored at −20 °C. The conjugates were confirmed by UV–Vis spectroscopy. The number of hapten molecules per molecule of protein (hapten density) of the conjugate was estimated directly by the molar absorbance at 280 nm (Liu et al., 2011).

$$\text{Hapten density} = (\varepsilon_{\text{conjugate}} - \varepsilon_{\text{protein}})/\varepsilon_{\text{hapten}}$$

2.6. Immunization and antibody preparation

According to the method described by Shan et al. (1999), four male New Zealand white rabbits (approximately 2 kg per rabbit) were divided into two groups and were immunized for Hapten 1-BSA and Hapten 2-BSA via intraperitoneal injection. The rabbits had free access to drinking water and commercial standard laboratory diet (CZZ, Nanjing, China) and were housed according to the EEC 609/86 Directives regulating the welfare of experimental animals. The immunogen (1 mg kg^{-1}) dissolved in physiological saline was emulsified with an equal volume of Freund's complete adjuvant and was injected intradermally at multiple sites on the back of each rabbit. After the first injection, four boosting injections were given at 2 week intervals with the immunogen (1.5 mg kg^{-1}) in Freund's incomplete adjuvant (1:1 v/v). The rabbits

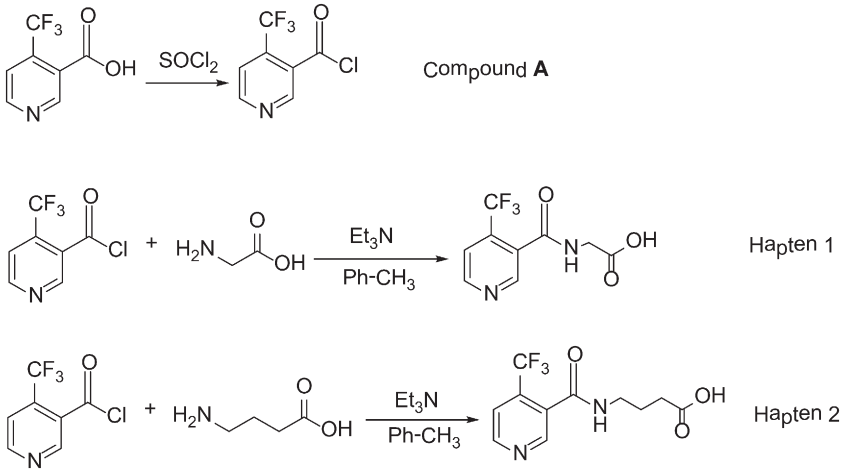


Fig. 1. Synthesis route of flonicamid haptens.

were bled from the ear vein using a 1-mL syringe after each boosting injection. Eight days after the last injection, blood samples were obtained from each rabbit's heart. The antiserum was centrifuged and purified by salting out with caprylic acid–ammonium sulfate (Li et al., 2008) and was stored at −20 °C.

2.7. ELISA protocol

Microplates were coated with coating antigen (100 μL per well, in CBS) overnight at 4 °C. The plates were washed three times with PBST. The binding sites not occupied by coating antigen were blocked with 200 μL of 5% skim milk per well for 1 h at 37 °C. After another washing step with PBST, flonicamid standards or sample extract (50 μL per well) and antibody diluted in assay buffer (50 μL per well) were added to the coated wells and incubated for 1 h. Following a further wash, 100 μL of diluted GAR-HRP was added and incubated for 1 h at 37 °C. After the plates were washed again, 100 μL per well of TMB solution was added and incubated for 15 min. Finally, 2 mol L^{-1} of sulfuric acid (50 μL per well) was added, and the absorbance was measured at 450 nm. The measurement was performed three times in triplicate wells.

The standard curve for flonicamid was obtained using (B/B_0) % versus the concentration of flonicamid and was fitted to a four-parameter logistic equation.

% (B/B_0) was calculated by the following equation:

$$\%(B/B_0) = [(A_x - A_{min})/(A_{max} - A_{min})] \times 100$$

where A_x is the absorbance of the sample, A_{max} is the absorbance in the absence of the analyte, and A_{min} is the absorbance of the background.

2.8. Cross-reactivity

The cross-reactivity (CR) was studied using standard solutions of flonicamid and some analogues. CR was calculated as follows.

$$CR\ (\%) = (IC_{50}\ \text{of flonicamid}/IC_{50}\ \text{of analogues}) \times 100.$$

2.9. Recovery

To evaluate the accuracy and precision of the ELISA, the recoveries of spiked samples (pond water, rice field water, canal water, rice field soil, vegetable field soil, carrot, apple and tomato) were studied. Eight environmental and agricultural samples were collected from the suburbs and local supermarket of Zhenjiang (China). Each analysis was performed in triplicate.

For water samples, simple filtration with filter paper was performed. The filtered water samples were spiked with flonicamid standard solution at 0.05, 0.5 and 5 mg L^{-1}, and were then allowed to stand in the dark at room temperature for 24 h. The spiked water samples were directly analyzed by ELISA.

For soil and agricultural samples, samples (10 g) were spiked with flonicamid at various levels (0.1–10 mg kg^{-1}) and were then allowed to stand in the dark at room temperature for 24 h. The samples were then shaken with 20 mL acetonitrile and 10 mL water. After ultrasonic extraction for 10 min, each suspension was centrifuged for 10 min at 4000 rpm. The supernatant was placed in a separating funnel with 5 g of NaCl and was vigorously shaken. The organic phase (2 mL) was evaporated under vacuum. The residue was dissolved in 4 mL of PBS containing 10% methanol and was analyzed by ELISA. The recoveries and relative standard deviation (RSD) were calculated. The spiked samples were prepared and performed by the same operators.

2.10. Evaluation of the assay by GC

To test the effectiveness of the developed ELISA, the spiked samples were simultaneously analyzed using ELISA and a GC- nitrogen-phosphorus detector (NPD). For GC-NPD, the samples were extracted as described above (ELISA procedure). The extracted residue was purified with a Florisil solid-phase extraction column. Then, the purified extracts concentrated under vacuum were dissolved with acetone and were analyzed by GC-NPD (Shi et al., 2015).

The GC-NPD analysis was performed on a HP-5-fused silica capillary column (30 m × 320 μm × 0.25 μm). The GC conditions were 120 °C for 1 min, a temperature increase to 280 °C at a rate of 25 °C min^{-1} and held for 5 min; a carrier gas (N_2) flow rate of 3.0 mL min^{-1}; an injection temperature of 280 °C using the splitless mode; and a detector temperature of 340 °C (Shi et al., 2015).

Table 1
Homologous and heterologous ELISA for flonicamid.

Antibody	Coating antigen	A_{max}	IC_{50} (mg L^{-1})	A_{max}/IC_{50}
Hapten 1-Ab	Hapten 1-OVA	1.15	5.63	0.20
	Hapten 2-OVA	1.26	7.39	0.17
Hapten 2-Ab	Hapten 1-OVA	1.06	7.32	0.14
	Hapten 2-OVA	1.01	3.87	0.26

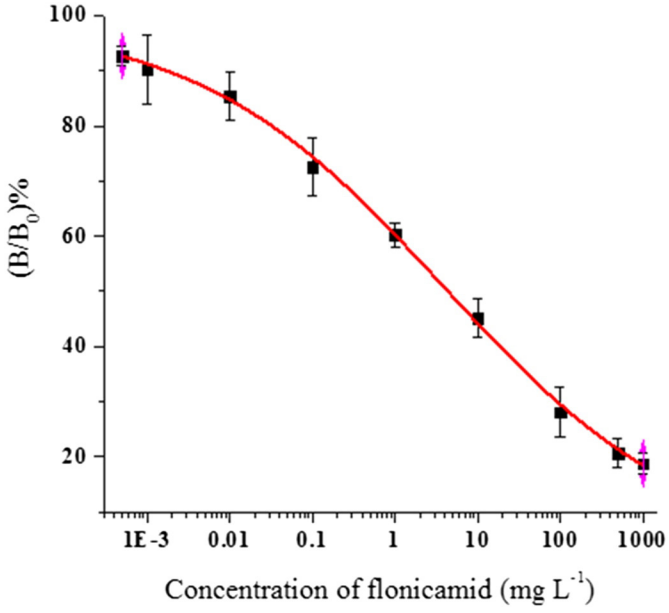


Fig. 2. Standard curve of the optimized ELISA for flonicamid (the combination of Hapten 2-Ab and Hapten 2-OVA was used).

Table 3
Recovery of flonicamid in spiked samples[a] ($n = 3$).

Sample	Spiked concentration (mg L^{-1}, mg kg^{-1})	Dilution times	Mean recovery ± SD (%)	RSD (%)
Rice paddy water	5	0	88.3 ± 4.7	5.3
	0.5		95.8 ± 6.9	7.2
	0.05		79.7 ± 6.4	8.0
Pond water	5	0	89.1 ± 5.3	5.9
	0.5		81.3 ± 7.5	9.2
	0.05		98.1 ± 6.3	6.4
Canal water	5	0	90.6 ± 5.1	5.6
	0.5		85.4 ± 7.8	9.1
	0.05		80.4 ± 6.7	8.3
Rice paddy soil	10	2	112 ± 3.9	3.5
	1		99.8 ± 3.6	3.6
	0.1		111.3 ± 5.4	4.9
Vegetable field soil	10	2	98.7 ± 8.5	8.6
	1		97.5 ± 6.1	6.3
	0.1		99.8 ± 7.2	7.2
Carrot	10	2	116.4 ± 7.9	6.8
	1		100.6 ± 5.9	5.9
	0.1		98.3 ± 6.2	6.3
Apple	10	2	95.3 ± 8.5	8.9
	1		88.7 ± 6.9	7.8
	0.1		89.3 ± 5.6	6.3
Tomato	10	2	86.3 ± 4.7	5.4
	1		79.3 ± 8.4	10.6
	0.1		93.5 ± 5.3	5.7

[a] 100 mg L^{-1} and 50 mg L^{-1} of flonicamid standard solution were used for spiking.

3. Results and discussion

3.1. Identification of artificial antigens and coupling ratio

The UV–Vis spectra showed qualitative difference between the hapten, carrier proteins and conjugates, especially at 280 nm, and also showed quantitative difference that the coupling ratios of hapten to protein were 31:1, 35:1, 7:1 and 8:1 for Hapten 1-BSA, Hapten 2-BSA, Hapten 1-OVA, and Hapten 2-OVA, respectively. The results indicated that the conjugates were coupled successfully.

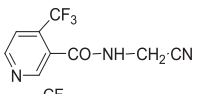
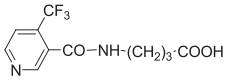
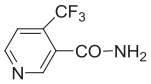
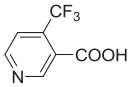
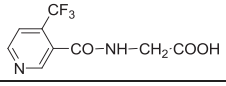
3.2. Development of the ELISA

To develop a sensitive ELISA for flonicamid pesticides, each of the antibodies for analyte recognition was screened by competitive indirect ELISA using each of the two coating antigens. The IC_{50} value and A_{max}/IC_{50} ratio were used as the primary criteria to evaluate the ELISA performance; the lowest IC_{50} value and the highest ratio of A_{max}/IC_{50} indicated the highest sensitivity (Mercader and Montoya, 1999). The results are shown in Table 1. The combination of Hapten 2-Ab and Hapten 2-OVA produced the lowest IC_{50} value, and the highest A_{max}/IC_{50} ratio was used for the ELISA.

The application of ELISA to agricultural and environmental samples requires consideration of several experimental factors, such as the salt concentration, pH and solvents affecting the performance of the immunoassay. In the study, the results indicated that when the PBS buffer contained 10% methanol and 0.3 mol L^{-1} Na^+ at pH 7.5 (Figs. A.1 to A.2, see Supplementary Material), the assay exhibited the highest sensitivity.

When the combination of Hapten 2-Ab and Hapten 2-OVA, 10% methanol, 0.3 mol L^{-1} Na^+ and pH 7.5 were used, a competitive standard curve for flonicamid was obtained by a four-parameter logistic equation. The results are presented in Fig. 2. The limit of detection (LOD, IC_{20} value) and the sensitivity (IC_{50} value) of the ELISA were 0.032 mg L^{-1} and 3.86 mg L^{-1}, respectively. The linear working range determined as the concentrations causing 20–80% inhibition, were 0.032–685.4 mg L^{-1} for the ELISA.

Table 2
Cross-reactivities of related compounds in the flonicamid immunoassay[a].

Compound	Structure	IC_{50} (mg L^{-1})	CR (%)
Flonicamid		3.87	100
Hapten 2		1.26	>100
TFNA-AM		155	0.03
TFNA		>1000	<0.01
TFNG		>1000	<0.01

[a] The combination of Hapten 2-Ab and Hapten 2-OVA was used for the cross-reactivity.

3.3. Specificity

The values of the CR of the antibody with flonicamid and some analogues are shown in Table 2. The ELISA showed negligible CR with its tested metabolites, and the highest CR was TFNA-AM (0.03%). Therefore, the results indicated the antibody had high specificity for flonicamid.

3.4. Analysis of spiked samples

As illustrated in Table 3, the recoveries of flonicamid ranged from 79.3 to 116.4% for the spiked samples (the number of samples was 3), and the RSDs were less than or equal to 10.6%. Therefore, the accuracy and precision for the ELISA were satisfactory for the quantitative detection of flonicamid in agricultural and environmental samples.

3.5. Validation of the assay by GC

The spiked samples were analyzed by ELISA and GC. The equation of the line obtained from the linear regression of the combined ELISA and GC data (Table A.1, see Supplementary Material) for flonicamid was $y = 0.9609x - 0.1055$ ($R^2 = 0.9891$, $n = 24$). The result of the correlation further demonstrated the reliability of the proposed ELISA method.

4. Conclusions

In summary, a specific and accurate ELISA based on a polyclonal antibody was successfully developed to detect flonicamid in agricultural and environmental samples. The antibody showed high sensitivity and specificity, with an LOD value of 0.032 mg L^{-1}. The cross-reactivity for some analogs was <0.03%. Analysis of spiked samples indicated that the specificity and accuracy of the ELISA were ideal and in good agreement with the GC measurements. Therefore, the proposed ELISA is a feasible quantitative/screening method for flonicamid in agricultural and environmental samples due to its sensitivity and simplicity, rapidity, lower expenses and high sample throughput.

Acknowledgments

This work was supported by the Natural Science Foundation of Jiangsu Province (BK20140543), the China Postdoctoral Science Foundation (2014M561596), the Senior Talent Scientific Research Initial Funding Project of Jiangsu University (14JDG051), the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and the Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.02.017.

References

Abad-Fuentes, A., Agullo, C., Esteve-Turrillas, F.A., Abad-Somovilla, A., Mercader, J.V., 2014. Immunoreagents and competitive assays to fludioxonil. J. Agric. Food Chem. 62, 2742–2744.

Adrian, J., Fernández, F., Sánchez-Baeza, F., Marco, M.P., 2012. Preparation of antibodies and development of an enzyme-linked immunosorbent assay (ELISA) for the determination of doxycycline antibiotic in milk samples. J. Agric. Food Chem. 60, 3837–3846.

Chen, A.W., 2002. Analytical methodology for IKI-220 (F1785) and its major metabolites in/on peach, potato tuber, and wheat straw. Analytical Report (*P*-3561M) for FMC Corporation, Princeton, NJ.

Chen, G., Sun, Y.M., Yang, T., Wu, Y.L., 2012. Simultaneous determination of flonicamid and its metabolites in cucumbers and apples by liquid chromatography–tandem mass spectrometry. Chin. J. Chromatogr. 30, 555–559.

Gurbuz, F., Metcalf, J.S., Karahan, A.G., Codd, G.A., 2009. Analysis of dissolved microcystins in surface water samples from Kovada Lake, Turkey. Sci. Total Environ. 407, 4038–4046.

Hengel, M.J., Miller, M., 2007. Analysis of flonicamid and its metabolites in dried hops by liquid chromatography–tandem mass spectrometry. J. Agric. Food Chem. 55, 8033–8039.

Hua, X.D., Liu, X.F., Yin, W., Xia, Y.Z., Zhou, Q.J., Lu, Y.W., et al., 2015. A sensitive monoclonal antibody-based enzyme-linked immunosorbent assay for the detection of bifenthrin in a chemical soil barrier. Sci. Total Environ. 502, 246–251.

Kawatsu, K., Kanki, M., Harada, T., Kumeda, Y., 2014. A highly rapid and simple competitive enzyme-linked immunosorbent assay for monitoring paralytic shellfish poisoning toxins in shellfish. Food Chem. 162, 94–98.

Ko, A.Y., El-Aty, A.M.A., Rahman, M.M., Jang, J., Kim, S.W., Choi, J.H., Shim, J.H., 2014. A modified QuEChERS method for simultaneous determination of flonicamid and its metabolites in paprika using tandem mass spectrometry. Food Chem. 157, 413–420.

Li, B., Shi, H.Y., Wang, M.H., 2008. An enzyme-linked immunosorbent assay for the detection of bifenthrin. Chin. J. Anal. Chem. 36, 34–38.

Li, M., Hua, X.D., Ma, M., Liu, J.S., Zhou, L.L., Wang, M.H., 2014. Detecting clothianidin residues in environmental and agricultural samples using rapid, sensitive enzyme-linked immunosorbent assay and gold immunochromatographic assay. Sci. Total Environ. 499, 1–6.

Liu, Z.J., Yu, P.M., Fang, S., Fan, J.Q., Wang, M.H., 2011. Development of an enzyme-linked immunosorbent assay for determination of pretilachlor in water and soil. Ecotoxicol. Environ. Saf. 74, 1595–1599.

Liu, Z.J., Yan, X., Xu, X.Y., Wang, M.H., 2013. Development of a chemiluminescence enzyme-linked immunosorbent assay for the simultaneous detection of imidaclothiz and thiacloprid in agricultural samples. Analyst 138, 3280–3286.

Ma, M., Chen, M., Wang, M.H., 2015. Quantitative analysis of flonicamid by HPLC. World Pestic. 37, 51–53.

Mercader, J.V., Montoya, A., 1999. Development of monoclonal ELISAs for azinphos-methyl. 2. Assay optimization and water sample analysis. J. Agric. Food Chem. 47, 1285–1293.

Morita, M., Ueda, T., Yoneda, T., Koyanagi, T., Murai, S., Matsuo, N., et al., 2000. Ishihara Sangyo Kaisha, Ltd. IKI-220—a novel systemic aphicide. BCPC Conference-Pests & Disease, p. 5965.

Navarro, P., Pérez, A.J., Gabaldón, J.A., Núñez-Delicado, E., Puchades, R., Maquieira, A., et al., 2013. Detection of chemical residues in tangerine juices by a duplex immunoassay. Talanta 116, 33–38.

Peng, D.P., Ye, S.Q., Wang, Y.L., Chen, D.M., Tao, Y.F., Huang, L.L., et al., 2012. Development and validation of an indirect competitive enzyme-linked immunosorbent assay for the screening of tylosin and tilmicosin in muscle, liver, milk, honey and eggs. J. Agric. Food Chem. 60, 44–51.

Shan, G.M., Stoutamire, D.W., Wengatz, I., Gee, L.H., Hammock, B.D., 1999. Development of an immunoassay for the pyrethroid insecticide esfenvalerate. J. Agric. Food Chem. 47, 2145–2155.

Sheng, Y.J., Jiang, W.X., Saeger, S.D., Shen, J.Z., Zhang, S.X., Wang, Z.H., 2012. Development of a sensitive enzyme-linked immunosorbent assay for the detection of fumonisin *B*1 in maize. Toxicon 60, 1245–1250.

Sheng, W., Yang, L., Wang, J.P., Zhang, Y., Wang, S., 2013. Development of an enzyme-linked immunosorbent assay for the detection of gentamycin residues in animal-derived foods. LWT Food Sci. Technol. 50, 204–209.

Shi, K.W., Tang, C.F., Li, W., Li, L., 2015. Residue and dietary risk assessment of flonicamid in apple. Agrochemicals 54, 674–677.

Tomlin, C.D.S., 2003. The pesticide manual. 13th ed. British Crop Protection Council, Surrey, UK.

Wang, Z.H., Mi, T.J., Beier, R.C., Zhang, H.Y., Sheng, Y.J., Shi, W.M., et al., 2015. Hapten synthesis, monoclonal antibody production and development of a competitive indirect enzyme-linked immunosorbent assay for erythromycin in milk. Food Chem. 171, 98–107.

Watanabe, E., Baba, K., Miyake, S., 2011. Analytical evaluation of enzyme-linked immunosorbent assay for neonicotinoid dinotefuran for potential application to quick and simple screening method in rice samples. Talanta 84, 1107–1111.

Xie, J.J., Chen, J., Wang, L., Jiao, H., Ye, H.Y., Lai, Z.F., 2011. Study on the determination of flonicamid residues in plant-based foods by gas chromatography. J. Northwest A F Univ. (Nat. Sci. Ed.) 39, 82–86.

Zywitz, D., Anastassiades, M., Scherbaum, E., 2003. Simultaneous determination of neonicotinoid insecticides in fruits and vegetables by LC–MS and LC–MS–MS: methodology and residue data. Dtsch. Lebensm.-Rundsch. 99, 188–196.