



Analytical Methods

Dual-labeled time-resolved fluoroimmunoassay for simultaneous detection of clothianidin and diniconazole in agricultural samples

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ABSTRACT

Europium (Eu³⁺) and samarium (Sm³⁺) were used as fluorescent labels to develop a highly sensitive dual-labeled time-resolved fluoroimmunoassay (TRFIA) for detect clothianidin and diniconazole in food samples. Under the optimized assay conditions, 50% inhibition concentration (IC₅₀) and the limit of detection (LOD, IC₁₀) of clothianidin were 5.08 and 0.021 µg/L, and 13.14 and 0.029 µg/L for diniconazole. The cross-reactivities (CRs) were negligible except dinotefuran (9.4%) and uniconazole (4.28%). The recoveries of clothianidin and diniconazole ranged from 79.3% to 108.7% in food samples. The results of TRFIA for the authentic samples were validated by gas chromatography (GC) analyses, and a satisfactory correlations were obtained. These results indicated that the method was an alternative tool for simultaneous detection of clothianidin and diniconazole in food samples.

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1. Introduction

In the comprehensive management of biological disasters, chemical pesticides are still the most convenient, stable, effective, reliable and inexpensive means for protection of crops (Wang, Jiang, & Cheng, 2003). However, pesticides are considered hazardous substances due to their high toxicity to non-target species and their persistence in the environment (Roex, Keijzers, & Van, 2003). Additionally, there is increasing concern of food and environmental contamination caused by the irrational use of pesticides (Kim, Cho, et al., 2003). Thus, the development of a rapid, sensitive, and economical method for detecting pesticide residues in food and environment is desirable.

Clothianidin is a member of the neonicotinoid insecticides, which shows a highly active, with systemic, tag and stomach poison efficacies (Chen, Huang, Wong, & Li, 2005). Diniconazole belong to the triazole fungicides. It has the strong absorption, efficient, broad spectrum bactericidal characteristics (Stehmann & Waard, 1996). In recent years, clothianidin and diniconazole are commonly used as insecticides and fungicides in field, and they are more and more use as mixed agents. However, several aspects of them, such as contaminating to agricultural products, and toxic

to beneficial organisms has aroused people's attention. Thus, the detection simultaneously of clothianidin and diniconazole in food samples is of great importance.

Immunoassays have been demonstrated to be simple, highly sensitive, cost-effective, and suitable for high-throughput screening analyses in pesticide monitoring programs (Hennion & Barcelo, 1998; Lee et al., 2005; Wanatabe et al., 2001). Generally, immunoassays are developed to recognize a single analyte with high specificity (Hua et al., 2010; Kim, Lee, Chung, & Lee, 2003; Liu et al., 2007). They can also be applied to detect various related compounds in a single test (Puchades, Pastor, Gallego, & Maquieira, 2007; Reder, Dieterle, Jansen, Alcock, & Gauglitz, 2003; Xu et al., 2009). These latter immunoassays have been termed multi-analyte assays. However, the vast majority of multi-analyte assays cannot realize simultaneous, sensitive and quantitative detection of multi analytes. Therefore, this fact becomes the greatest question for working out the advantage of multi-analyte assay.

Time-resolved fluoroimmunoassay (TRFIA) is a new detection technique based on the high specificity of immune reactions and high sensitivity of the combination of labels and tracers (Gui, Jin, Sun, Guo, & Zhu, 2009; Zhang et al., 2013). The labels used in this assay are ions of trivalent rare earth metals such as Eu³⁺, Tb³⁺ and Sm³⁺, which can generate strong fluorescence and have a long decay time (Huang et al., 2012). Because the measurement time is extended and the specific fluorescence of rare earth ions is tested after short-life background fluorescence in the sample has decayed,

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the interference of background fluorescence can be eliminated (Yu et al., 2010; Zhou et al., 2012). Besides, the labels have the large stock shift between the excitation and emission wavelength, which effectively avoids environmental interference. During detection, the addition of a fluorescence intensifier can enhance the original fluorescence by 1 million times (Rui et al., 2007; Yan, Zhou, Lin, Ju, & Hu, 2005). Thus, TRFIA has a considerably higher detection limit (10^{-18} mol/L) than ELISA (10^{-10} mol/L). With different emission wavelengths of Eu^{3+} , Tb^{3+} and Sm^{3+} , the detection of the same sample with different wavelengths can simultaneously obtain several indicators simultaneously (Huang, Zhang, Ma, & Yi, 2009; Mitrunen et al., 1995). Based on a new cofluorescence enhancement principle, quadruple-label TRFIA has also been constructed to detect four analytes simultaneously (Yong, 1992). Therefore, multi-labeled TRFIA is feasible for simultaneous, sensitive and quantitative detection of multiply analytes.

In this paper, the trivalent ions of europium and samarium were used as labels, and a dual-label TRFIA for detection of clothianidin and diniconazole was reported for the first time. The dual-label TRFIA can simultaneously, sensitively and quantitatively detect clothianidin and diniconazole. Comparison of enzyme linked immunosorbent assay (ELISA), the sensitivity of dual-label TRFIA was higher, and the accuracy was validated by comparison with gas chromatography (GC) in the authentic samples detection. These results indicate that the dual-labeled TRFIA is a feasible strategy for simultaneous detection of two pesticides in environmental and agricultural samples.

2. Materials and methods

2.1. Reagents and buffer solutions

The standards of clothianidin (97.6% purity) and its analogues were supplied by the Jiangsu Pesticide Research Institute (Jiangsu, China). The standards of diniconazole (92% purity) and its analogues were supplied by the Jiangsu Sevencontinent Green Chemical Co., Ltd. (Jiangsu, China). Bovine serum albumin (BSA), ovalbumin (OVA), and affinity-purified goat anti-rabbit IgG (GAR-IgG) and affinity-purified goat anti-mouse IgG (GAM-IgG) were purchased from Sigma Chemical Co. (St. Louis, USA). N' -[p-Isothiocyanato-benzyl]-diethylene-triamine- N_1 , N_2 , N_3 , N_4 -tetraacetate- Eu^{3+} (DTTA- Eu^{3+}) and DTTA- Sm^{3+} were obtained from Tianjin Radio-Medical Institute (Tianjin, China). The coating antigen, anti-clothianidin monoclonal antibodies (Li, Sheng, Cong, & Wang, 2013) and anti-diniconazole polyclonal antibody (Jiang, Shi, Wu, & Wang, 2011) were prepared and stored in our laboratory. Polyoxyethylene sorbitan monolaurate (Tween-20), tris (hydroxymethyl) aminoethane (Tris) and other chemical reagents were purchased from Aladdin (Shanghai, China). The sepharose CL-6B was supplied by Qisong Biotechnology Co., Ltd. (Beijing, China). The PD-10 column was purchased from Rullwell Co., Ltd. (Shanghai, China). Microtiter plates were obtained from Jiangsu Nuclear-Medicine Institute (Wuxi, China). Carbonate-buffered saline (CBS, 0.05 mol/L, pH 9.6), carbonate buffer saline (CBS, 0.05 mol/L, pH 8.5, containing 0.155 mol/L NaCl), Tris-HCl buffer (TBS, 0.05 mol/L; pH 7.5; containing 0.5 mol/L NaCl), Tris-HCl buffer (TBS; 0.08 mol/L; pH 7.8), and 0.05 mol/L Tris-HCl buffer containing 0.05% Tween-20 (TBST) were prepared and stored in our laboratory. The enhancement solution was obtained from Jiangsu Nuclear-Medicine Institute (Wuxi, China).

2.2. Instruments and equipments

The fluorescence was detected using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA). The washing steps were

carried out using a Wellwash Plus (Thermo, MA, USA). The ultraviolet absorbance was obtained on a Nanodrop-1000 spectrophotometer (Thermo, MA, USA). The TRFIA was confirmed with an Agilent 7890A gas chromatograph (Agilent, Wilmington, DE, USA).

2.3. Preparation of the Eu^{3+} -labeled GAR-IgG

Europium-labeled GAR-IgG was prepared as previously described with some modifications (Knopf & Papoian, 1991; Wu, Han, Xu, & He, 2003; Xu et al., 2012). 1 mL anti-rabbit IgG (8.1 mg/ml) was loaded on a PD-10 column and eluted by CBS (pH 8.5). Next, the fractions were collected and concentrated to 2 g/L. Subsequently, 500 μL purified anti-rabbit IgG was mixed with 0.2 mg lyophilized Eu^{3+} -DTTA, and the mixture was vigorously stirred at 25 °C for 20 h. The resulting mixture was fractionated on a Sepharose CL-6B column (1×40 cm) and eluted with 0.08 mol/L TBS. The absorbance values of the eluate were measured at 280 nm to obtain the protein concentrations. After purification, equal amount of glycerin and 2% BSA was added before sub-packaged and stored at -20 °C.

2.4. Preparation of the Sm^{3+} -labeled GAM-IgG

The anti-mouse IgG conversion buffer striped with Section 2.4. One mg purified anti-mouse IgG was mixed with 0.2 mg lyophilized Sm^{3+} -DTTA, and vigorously stirred at 25 °C for 20 h. Subsequent steps referred to preparation of the Eu^{3+} -labeled GAR-IgG.

2.5. Preparation of the mixed standard solutions

The standard diniconazole solution (10,000 $\mu\text{g/L}$) was mixed 1:1 (v/v) with a standard clothianidin solution (20,000 $\mu\text{g/L}$) to obtain the mixed standard solution. The series concentrations of the mixtures were prepared using a 10-fold dilution of the mixed standard solution with 10% methanol-TBS.

2.6. Dual-labeling TRFIAs

The mixed coating antigens of clothianidin and diniconazole were diluted to final concentration of 1 mg/L with CBS (pH 9.6), and 100 μL of the mixed coating antigens were added to the plates by incubation at 4 °C overnight. The plates were washed five times with TBST and blocked by incubating with 1% OVA in 0.05 mol/L TBS (200 $\mu\text{L/well}$) for 0.5 h at 37 °C. After another washing step, either the sample or standard in the TBS containing methanol (50 $\mu\text{L/well}$) together with 25 μL of PcAb and 25 μL of McAb in TBS. The plate was incubated for 1 h at 37 °C. After another wash, the diluted Eu^{3+} -labeled GAR-IgG (50 $\mu\text{L/well}$, in TBS) and Sm^{3+} -labeled GAM-IgG (50 $\mu\text{L/well}$, in TBS) was dispensed into each well and incubated for 1 h at 37 °C. Then the plates were washed again and enhancement solution (200 $\mu\text{L/well}$) was added to the plates. The fluorescence intensity (F) was measured after 8 min of mechanical shaking. The measuring conditions of Eu^{3+} were excitation wavelength 340 nm, emission wavelength 615 nm, delay time 0.4 ms, windows time 0.4 ms, and cycle period 1.0 ms. The excitation wavelength and cycling time were not changed for Sm^{3+} , the delay time, emission wavelength, and window time were set at 0.050 ms, 644 nm, and 0.10 ms, respectively.

Determinations were performed in triplicate, and the mean values of F/F_0 (F : fluorescence signal with clothianidin or diniconazole; F_0 : signal without the absence of clothianidin or diniconazole) were plotted against the logarithm of the concentration of analyte obtain the competitive curves. The half-maximal inhibition concentration (IC_{50}) and limit of detection (LOD, IC_{10})

were obtained from a four-parameter logistic equation of the sigmoidal curves using Origin Pro 7.0 software.

2.7. Immunoassay optimization

Experimental parameters (concentrations of the coating antigen, antibody and second antigen, organic solvent, ionic strength, and pH) were studied to improve the sensitivity of the dual-label TRFIA. The dual-label TRFIA was run in the TBS solutions which contained different concentrations of methanol (from 5% to 40%, v/v), and Na⁺ (from 0.1 to 0.6 mol/L) and had different pH values (pH 4.5–9.5) to evaluate the effects of the solvent, ionic strength and pH, respectively. The F_0/IC_{50} ratio was used as a primary criterion to evaluate the TRFIA performances, the highest ratio indicates the highest sensitivity.

2.8. Cross-reactivity

Cross-reactivity (CR) was studied using the standards of clothianidin, diniconazole and some of their analogues. The CR values were calculated as follows:

$$CR\% = (IC_{50} \text{ of clothianidin or diniconazole} / IC_{50} \text{ of analogue}) \times 100$$

2.9. Analysis of spiked samples

All samples (wheat, corn, rice, apple, pear, grape, tomato, cucumber, spinach, Chinese cabbage) were obtained from a local supermarket. Before the spiking and recovery studies, each test samples was verified to not contain clothianidin and diniconazole by GC.

All samples (10 g) were spiked with the two pesticides at 10, 100, and 500 µg/kg. The samples were thoroughly mixed, and then allowed to stand at room temperature overnight. The samples were extracted by sonicating for 10 min in 10 mL of methanol and centrifuged at 4000 rpm for 10 min. The supernatants were diluted 5–20 times and analyzed by TRFIA. The experiment of each sample was conducted in triplicate. The recoveries and relative standard deviation (RSD) were calculated.

2.10. Matrix effects on immunoassays

All food samples were used employed to study the matrix effects. The concentrated samples were analyzed using 0-fold, 5-fold, 10-fold and 20-fold dilutions with TBS (containing 10% methanol). The matrix effects were determined by comparing the clothianidin and diniconazole standard curves that were prepared in the matrix extract and those standards curves prepared in the TBS solution of the matrix free.

2.11. Validation of the assay with GC

Authentic samples were from 2.9. The samples were handled according to the aforementioned method and detected with TRFIA. In addition, the samples (20 g) were extracted twice by sonicating with 20 mL of acetonitrile and 3 g sodium chloride for 10 min and subsequently centrifuged at 4000 rpm for 10 min. The supernatants (10 mL) were filtered through anhydrous sodium and evaporated. The remainder was dissolved with 2 mL acetone and determined with GC. Each sample was performed in triplicate.

The clothianidin was detected using a gas chromatograph, which was equipped with ECD (Li et al., 2012). The separation was performed using a DB-17 capillary column with a stationary phase of 50% phenyl-dimethyl siloxane (30 m × 320 µm i.d.,

0.25 µm film thickness). Nitrogen was used as the carrier gas (1.0 ml/min). The column temperature was initially maintained at 180 °C for 1 min and subsequently increased to 220 °C at 15 °C/min and maintained at this value for 3 min.

The diniconazole was detected using a ECD according to (Xiong, Dai, & Huang, 2002). The separation was performed using a DB-17 capillary column with a stationary phase of 50% phenyl-dimethyl siloxane (30 m × 320 µm i.d., 0.25 µm film thickness).

3. Results and discussion

3.1. Optimization of TRFIA conditions

The concentration of immunoreagents (coating antigen, PcAb, McAb, Eu³⁺-labeled antibodies and Sm³⁺-labeled antibodies) were optimized base on the higher F_0/IC_{50} ratio and appropriate F_0 value. Next, 1 µg/mL clothianidin coating antigen, 1 µg/mL anti-clothianidin McAb, 1.2 µg/mL Sm³⁺-labeled GAM-IgG, 1 µg/mL diniconazole coating antigen, 1 µg/mL anti-diniconazole PcAb, 0.6 µg/mL Eu³⁺-labeled GAR-IgG were selected as the optimal concentrations of the dual-labeled TRFIA.

For solvent optimization, methanol was selected to improve the analyte solubility and evaluated its effect on the TRFIAs. With the methanol concentrations being 10%, both clothianidin and diniconazole showed the highest F_0/IC_{50} and the lowest IC_{50} . The F_0 values drastically decrease and the IC_{50} values increase at methanol concentration above 10% (v/v). The change of Na⁺ concentration from 0.1 to 0.6 mol/L also significantly affects the immunoassays. The TRFIA performs better with the increase of Na⁺, the highest F_0/IC_{50} is obtained at 0.5 mol/L Na⁺. The pH value of solutions does not have a notable effect on the assay performance, and the F_0/IC_{50} reached a maximum at pH 7.5. Based on these results, 10% methanol, 0.5 mol/L Na⁺ and pH 7.5 were selected as optimum conditions for the dual-labeled TRFIA (Table 1).

3.2. Sensitivities

The standard curves of clothianidin and diniconazole were obtained under the optimum conditions using the dual-labeled TRFIA (Fig. 1). The dual-labeled TRFIA was shown to have an IC_{50} of 5.08 µg/L, a LOD of 0.0213 µg/L and a linear range (IC_{10} – IC_{90}) of 0.021–973 µg/L for clothianidin. In diniconazole detection, the

Table 1
Effects of organic solvent, ionic strength and pH value on dual-labeled TRFIAs.

Factors		Clothianidin		Diniconazole	
		F_0/IC_{50}	IC_{50} (µg/L)	F_0/IC_{50}	IC_{50} (µg/L)
Methanol (v/v,%)	0%	7242.3	3.21	12665.6	2.24
	5%	6525.1	4.72	10863.5	4.32
	10%	10457.4	3.98	21383.3	3.68
	20%	9203.5	13.7	10113.2	6.88
	30%	5455.6	6.98	7745.3	7.65
	40%	9210.2	9.57	8293.2	8.14
Na ⁺ (mol/L)	0.1	8182.2	3.16	26826.4	3.14
	0.2	5908.4	3.46	28945.2	3.94
	0.3	3338.2	3.98	22027.6	4.57
	0.4	4135.9	4.69	30330.1	4.08
	0.5	9689.6	2.95	69222.5	2.88
	0.6	9236.4	3.75	40106.7	3.95
pH value	4.5	9238.1	7.36	21316.2	3.48
	5.5	8048.6	6.67	26612.6	3.35
	6.5	7632.7	5.41	25518.8	4.32
	7.5	11008.1	3.49	29569.4	3.13
	8.5	7236.3	5.87	20788.8	4.97
	9.5	7412.3	6.41	30975.2	4.35

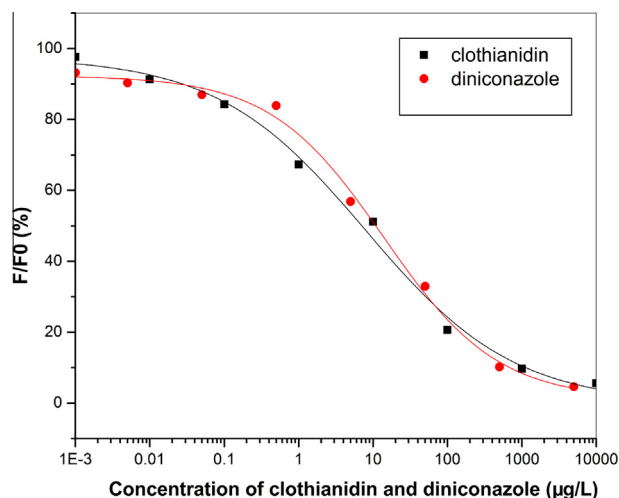


Fig. 1. Standard inhibition curves for clothianidin and diniconazole using the dual-labeled TRFIA.

Table 2
Cross-reactivity of clothianidin to their analogues by dual-labeled TRFIA.

Compound	Structure	IC ₅₀ (μg/L)	CR (%)
Clothianidin		5.08	100
Dinotefuran		54.04	9.4
Imidacloprid		403.6	1.2
Imidaclothiz		513.67	0.1
Thiacloprid		>1000	<0.01
Thiamethoxam		>1000	<0.01
Acetamiprid		>1000	<0.01
Nitenpyram		>1000	<0.01

IC₅₀ value, LOD value and linear range were 13.14, 0.029 and 0.029–814 μg/L, respectively.

Compared to the maximum residue limit (MRL) of clothianidin (0.05 mg/kg in USA) and diniconazole (0.05 mg/kg in China), the sensitivity of the dual-labeled TRFIA can satisfy the requirements of clothianidin and diniconazole detection. According to the published articles, the LOD values of ELISA (Li et al., 2013; Mikiko, Eiki, Shigekazu, Seiji, & Shiro, 2012), HPLC (Xiong, Dai, Li, et al., 2002) and GC (Xiong, Dai, & Huang, 2002) was 0.0028, 0.004 and

Table 3
Cross-reactivity of diniconazole to their analogues by dual-labeled TRFIA.

Compound	Structure	IC ₅₀ (μg/L)	CR (%)
Diniconazole		13.14	100
Uniconazole		307.3	4.28
Hexaconazole		356.1	3.69
Triadimefon		>1000	<0.01
Flutriafol		>1000	<0.01
Tebuconazole		>1000	<0.01
Epoxiconazole		>1000	<0.01
Cyproconazole		>1000	<0.01

0.01 mg/L for clothianidin, and 0.02 and 0.01 mg/L for diniconazole, respectively, the dual-labeled TRFIA was more sensitive than above-mentioned detection methods.

3.3. Specificity

The CRs for the analogues were tested using the dual-labeled TRFIA (Tables 2 and 3). The dual-label-TRFIA shows negligible cross-reactivity for the analogues of clothianidin and diniconazole except dinotefuran (9.4%) and uniconazole (4.28%). The results indicated that the developed TRFIA had a high specificity to clothianidin and diniconazole.

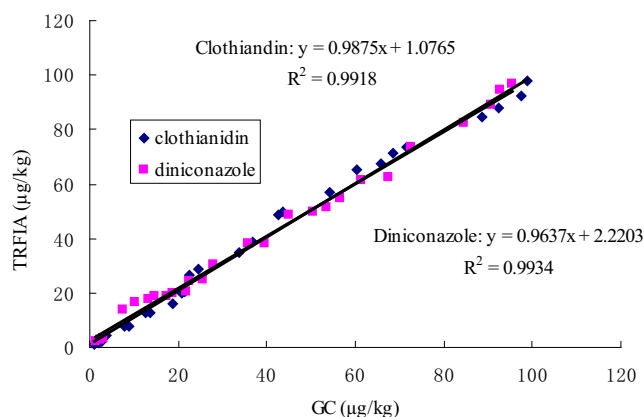
3.4. Matrix interference

Matrix interference is one of the most common challenges in performing immunoassays on complex samples. Sample dilution is the easiest and most immediate method to minimize the matrix effects. The results of matrix effects make different sample matrices have different effects on the sensitivity of the dual-labeled

Table 4

The recoveries of samples spiked with clothianidin and diniconazole by dual-labeled TRFIA.

Sample	Clothianidin		Diniconazole	
	Spiked concentration (µg/kg)	Mean recovery ± SD (% , n = 3)	Spiked concentration (µg/kg)	Mean recovery ± SD (% , n = 3)
Wheat	10	98.7 ± 3.74	10	87.6 ± 7.53
	100	96.5 ± 3.59	100	83.2 ± 6.02
	500	89.7 ± 2.41	500	93.6 ± 2.46
Corn	10	79.3 ± 4.59	10	100.2 ± 5.78
	100	83.4 ± 5.89	100	100.9 ± 2.33
	500	88.7 ± 6.12	500	80.8 ± 8.65
Rice	10	100.5 ± 3.69	10	108.7 ± 8.38
	100	99.3 ± 5.82	100	98.3 ± 5.96
	500	101.7 ± 3.83	500	100.9 ± 2.73
Apple	10	96.1 ± 3.69	10	103.4 ± 2.56
	100	100.2 ± 2.62	100	102.1 ± 8.21
	500	97.5 ± 2.87	500	98.4 ± 3.64
Pear	10	91.6 ± 4.69	10	96.5 ± 5.96
	100	100.3 ± 3.74	100	86.3 ± 5.74
	500	89.7 ± 5.03	500	82.7 ± 3.21
Grape	10	96.4 ± 6.94	10	86.1 ± 7.51
	100	95.2 ± 3.01	100	85.9 ± 5.46
	500	100.3 ± 6.36	500	93.4 ± 3.89
Tomato	10	100.8 ± 4.52	10	97.2 ± 4.82
	100	103.4 ± 4.36	100	100.4 ± 2.33
	500	107.4 ± 8.36	500	100.8 ± 8.41
Cucumber	10	89.4 ± 2.82	10	94.7 ± 5.61
	100	94.3 ± 5.89	100	88.9 ± 4.37
	500	93.5 ± 3.47	500	106.4 ± 5.89
Spinach	10	100.3 ± 9.87	10	105.8 ± 6.42
	100	100.5 ± 5.47	100	102.7 ± 2.14
	500	107.6 ± 6.39	500	91.4 ± 3.57
Chinese cabbage	10	87.6 ± 7.27	10	91.6 ± 9.54
	100	91.4 ± 3.21	100	96.3 ± 2.28
	500	89.7 ± 2.82	500	93.7 ± 4.42

**Fig. 2.** The correlation between the dual-labeled TRFIA and GC for analyses of samples with clothianidin and diniconazole.

TRFIA (see in [Support information Figs. 1S–20S](#)). Matrix interference were adequately removed from the wheat, corn, tomato, cucumber, spinach and cabbage samples with at least a 20-fold dilution, the rice samples with at least a 5-fold dilution, and the apple, pear, grape samples with at least a 10-fold dilution.

3.5. Accuracy and precision

The recoveries of the spiked samples ranged from 79.3% to 108.7%, and the RSDs were lower than 10% ([Table 4](#)). These results indicated that the accuracy and precision of the TRFIA were satisfactory for the quantitative detection of clothianidin and diniconazole residues in food samples.

3.6. Comparison of TRFIA and GC methods

As shown in [Fig. 2](#), good correlations were obtained between the dual-labeled TRFIA (y) and GC (x) for the authentic samples were obtained (clothianidin: $y = 1.0153x + 0.4341$, $R^2 = 0.9905$; diniconazole: $y = 0.9568x + 2.8821$, $R^2 = 0.9877$). These results suggest that clothianidin and diniconazole in the actual samples can be simply, rapidly and accurately detected using the dual-labeled TRFIA.

4. Conclusions

In this study, a simple and reliable dual-labeled TRFIA method to determine clothianidin and diniconazole quantitatively in food matrices (wheat, corn, rice, apple, pear, grape, tomato, cucumber, spinach, Chinese cabbage) was successfully established and validated. Compared with the instrumental analysis, TRFIA can significantly shorten the sample pretreatment time, which often occupies most of the time of the residue analysis. Furthermore, the dual-labeled TRFIA can simultaneously detect clothianidin and diniconazole in the food matrices, whereas the instrumental analysis generally requires two types of instruments of two detectors. Compared with other immunoassay, the dual-labeled TRFIA can shorten the detecting time and decrease the cost. Therefore, this dual-labeled TRFIA is a quick, easy, inexpensive, safe and effective method for the simultaneous, accurate and quantitative determination of clothianidin and diniconazole residues in food samples. This study indicated a new idea to develop dual-labeled TRFIA in pesticides multi-residue immunoassay and provided references to develop dual-labeled TRFIA or multi-labeled TRFIA for other pesticides.

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Notes

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.07.023>.

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