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Determination of Four Chiral Pesticides in Soil by QuEChERS-Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry

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Abstract: The enantiomer residues of metalaxyl, napropamide, triticonazole and metconazole were detected in soil by QuEChERS-ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The soil sample was extracted with acetonitrile containing 1% acetic acid and purified using QuEChERS method. Quantitative analysis for the enantiomers of these four chiral pesticides was performed in MS in multi-response monitoring (MRM) mode by external standard method. All enantiomers showed good linearity in the range of 0.5 to 50 μg/L. The average recoveries of enantiomers in soil were 81.74% to 105.79% with relative standard deviations (RSDs) from 3.69% to 7.86%. The method quantitative limit (MQL) was 5.3-30.3 ng/kg. The method can be used for rapid screening and determination of chiral pesticides in soil.

Key words: ultra performance liquid chromatography-tandem mass spectrometry; QuEChERS; soil; chiral pesticide; enantiomers residues

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0 Introduction

It is reported that more than 25% of pesticides currently used in the world are chiral[1]. The ratio is over 40% in China[2]. Chiral pesticides usually have two or more enantiomers with the same physicochemical properties[3]. However, in many cases, the activity, metabolism, toxicity and persistence of enantiomers show great differences[4,5]. Therefore, it is necessary to study the enantiomer-specific residues and toxicity to accurately assess the risks of chiral pesticides and provide a reliable scientific basis for their environmental safety.

Metalaxyl is a chiral acylanilide fungicide and widely used to control diseases caused by pathogens of oomycota on many plants including crops[6]. Metalaxyl contains two enantiomers. Napropamide is a selective systemic herbicide and used to control the growth of grasses and weeds in many agricultural cultivations[7]. Napropamide has one symmetric center including two enantiomers. Triticonazole and metconazole are broad-spectrum systemic fungicides and widely used in treatment of soybeans[8]. Triticonazole includes two enantiomers and metconazole has two symmetric centers containing four enantiomers. Both of them can interfere with ergosterol biosynthesis and inhibit steroid demethylation against phytopathogen[9]. All of the chiral pesticides mentioned above are widely used in agriculture. These pesticides are expected to remain in soil during application. Therefore, determination of residues of the pesticides is necessary to assess their ecological safety.

However, there is lack of methods to simultaneously analyze their enantiomers in environmental samples.

Many methods have been developed to pretreat samples in environmental analysis including Soxhlet extraction, liquid-liquid extraction, solid-phase extraction, accelerated solvent extraction[10], supercritical fluid extraction[11], liquid-phase microextraction[12], solid phase microextraction[13], gel permeation chromatography[14] and QuEChERS (quick, easy, cheap, effective, rugged, and safe) [15]. The QuEChERS, developed by Prof. Anastassiades of the Department of Agriculture in USA in 2003, is quick, simple, cheap and suitable for pesticide residue analysis in soils, fruits and vegetables[16-18]. Therefore, the method is increasing of application in environmental analysis. Chromatography is the most widely used method to detect organic compounds. Compared to other chromatography, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been widely used in the multi-residues detection of pesticides due to its higher sensitivity, selectivity, resolution and analysis speed. Besides, LC-MS/MS can be easy to fulfil simultaneous separation and identification of various enantiomers coupling with chiral chromatographic column.

The aim of the study is to develop a method to determine enantiomer-specific residues of metalaxyl, napropamide, triticonazole and metconazole in soil by QuEChERS-ultra performance liquid chromatography-tandem mass spectrometry. The method is simple, rapid and can be widely applied to determine pesticides in soil.

1 Experiment

1.1 Instruments, Reagents and Materials

Ultra performance Liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, Acquity, Xevo TQ-S, Waters, USA); KQ3200B Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co, Ltd. China); LD5-2A Centrifuge (Beijing Medical Centrifuge Factory, China); N-EVAPTM 111 Nitrogen Blower (Organomation, USA); QL901 Automatic Whirlpool Mixer (Haimen City Linbell Instrument Manufacturing Co., Ltd. China)

Standards of metalaxyl (99.5%), napropamide (99.5%) and metconazole (99%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Triticonazole (97.7%) was obtained from Chem Service (West Chester, USA). All enantiomers (＞94%) were provided by Prof. Meirong Zhao, Zhejiang University of Technology.

Acetonitrile (ACN, residue analysis grade) and methanol (LC/MS grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA) and J. T. Baker (Phillipsburg, NJ, USA), respectively. Formic acid and acetic acid (CH_3COOH) were obtained from J. T. Baker (Phillipsburg, NJ, USA). LC-grade water was produced by a MilliQ water purification system (Bedford, MA, USA). Magnesium sulfate anhydrous($MgSO_4$), sodium chloride (NaCl), neutral activated alumina (Al_2O_3) and other chemicals were purchased from Biochem (Shanghai, China). C18 and N-propyl ethylenediamine (PSA) were obtained from Welch Materials (Ellocott City, MD, USA).

Soil samples were air-dried at room temperature, ground and sieved through a 60-mesh screen before pretreatment.

1.2 Standard Solution

Single standard stock solution: Metalaxyl, napropamide, triticonazole, metconazole and their enantiomers standards were individually dissolved in acetonitrile at concentration of 1 mg/L and stored at −20 ℃ in a refrigerator.

Mixed standard working solution: 0.1 mL single standard stock solution of each racemate was mixed and diluted to 1 mL with acetonitrile to prepare mixed stock solution with concentration of 100 μg/L. Working standard solutions were prepared by appropriately diluting the stock solution with acetonitrile.

1.3 Sample Preparation

Appropriately five grams of homogenized soil was placed in a 50 mL centrifuge tube and sonicated for 15 min by adding 10 mL acetonitrile, 100 μL acetic acid and 2 mL ultrapure water. After adding 1 g NaCl and 2 g anhydrous $MgSO_4$, the centrifuge tube was vortexed for 1 min and centrifuged at 6 000 r/min for 10 min. Afterwards, the supernatant was taken into a new centrifuge tube and then cleaned up with 200 mg $MgSO_4$, 25 mg C18, and 40 mg Al_2O_3. The centrifuge tube was vortexed for 1 min and centrifuged at 4 000 r/m for 10 min. The supernatant was moved into a 10 mL tube. The centrifuge tube was washed with 2 mL acetonitrile and centrifuged again. The two supernatants were combined. Finally, the combined supernatant was dried by nitrogen blow, reconstituted in 1 mL methanol and then filtered with 0.22 μm filters to remove particulates. The extracts were analyzed by LC-MS/MS.

1.4 UPLC-MS/MS

UPLC Chiral column: Lux 3u Cellulose-4 (2.0 mm × 150 mm, 1.7 μm); column temperature: 40 ℃; injection volume: 10 μL; mobile phase: acetonitrile/water (60/40); flow rate: 0.3 mL/min; elution mode: isocratic.

MS/MS Ionization source: ESI(electron spray ionization); ionization mode: positive ion mode; desolvation gas: N_2; collision gas: Ar; source temperature: 120 ℃; desolvation gas temperature: 350 ℃; secondary gas flow rate: 10 arb; capillary voltage: 3.5 kV; cone gas flow: 50 L/h; solvent removal gas flow: 800 L/h; detection mode: MRM. The ion monitoring and other mass spectrometry conditions are shown in Table 1.

Table 1 Mass spectrometry parameters of four chiral pesticide enantiomers

Enantiomer	Retention time /min	Parent ion (M/Z)	Daughter ion(M/Z) 1*/2/3	Cone voltage /V	Collision energy /V
R-metalaxyl	2.25	280.16	192.15*/160.12/220.14	70/70/70	14/20/10
S-metalaxyl	2.49				
R-napropamide	3.59	272.16	171.12*/120.01/199.12	24/24/24	22/14/12
S-napropamide	3.85				
R-triticonazole	3.50	318.27	70.08*/256.34/88.05	22/22/22	14/22/28
S-triticonazole	6.42				
Metconazole-1	3.49	320.20	70.08*/125.03/177.05	44/44/44	20/30/20
Metconazole-2	3.93				
Metconazole-3	4.23				
Metconazole-4	4.40				

Note: ions with * are quantitative ions

2 Results and Discussion

2.1 Optimization of QuEChERS Method

2.1.1 Selection of extraction solvents

Acetonitrile, methanol, acidic methanol and acetonitrile are mostly used as extraction solvents for pesticides in soil. In this study, 1% (*V*/*V*) formic acid-methanol solution, 1% (*V*/*V*) acetic acid-methanol solution, acetonitrile, 1% (*V*/*V*) formic acid-acetonitrile solution and 1% (*V*/*V*) acetic acid-acetonitrile solution were used as extraction solvents to compare the extraction efficiency of the target compounds at spiking levels of 4 and 40 μg/kg in soil. The highest recoveries were achieved with 1% (*V*/*V*) acetic acid-acetonitrile as the extraction solvent, and the recoveries of all four chiral pesticide enantiomers were higher than 80% (Fig.1).

2.1.2 Optimization of purification conditions

PSA, C18, neutral Al_2O_3 and solid phase extraction column packings are usually used as pretreatment materials. PSA is used to eliminate various organic acids, pigments, sugars and fats. C18 works well in removing non-polar impurities. Neutral Al_2O_3 has a specific ability to absorb gases, vapor and liquids.

In this study, the effects of different sorbents such as PSA, C18 and neutral activated alumina were investigated on recoveries of targeted pesticides. The purification conditions were optimized at spiked levels of 4 and 40 μg/kg. The average recoveries of enantiomers at two spiked concentrations with different purification conditions were compared and shown in Fig.2. The results showed that the combination of C18 and neutral Al_2O_3 provided the best recovery of each target compound.

2.2 Optimization of Mass Spectrometry and Chromatographic Conditions

2.2.1 Optimization of mass spectrometry conditions

The continuous injection mode of flow injection

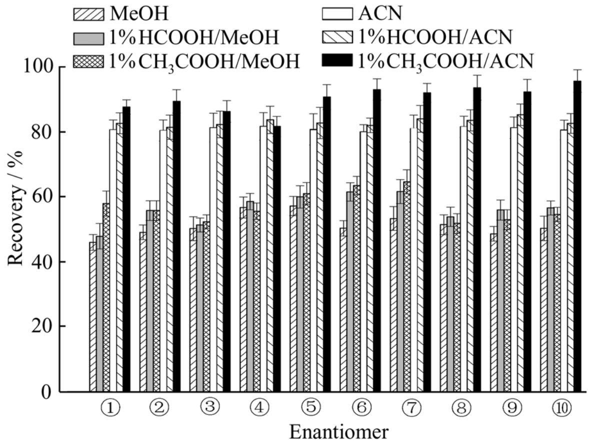


Fig.1 Recoveries of enantiomers in different extraction solvents

① *R*-metalaxyl; ② *S*-metalaxyl; ③ *R*-napropamide; ④ *S*-napropamide; ⑤ *R*-triticonazole; ⑥ *S*-triticonazole; ⑦ Metconazole-1; ⑧ Metconazole-2; ⑨ Metconazole-3; ⑩ Metconazole-4

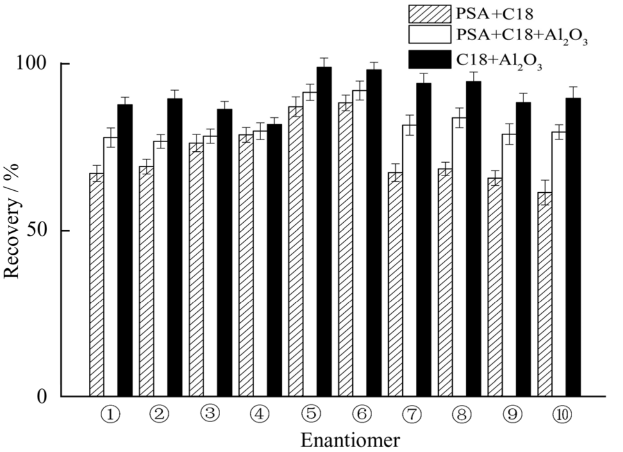


Fig.2 Recoveries of enantiomers in different combinations of sorbents

① *R*-metalaxyl; ② *S*-metalaxyl; ③ *R*-napropamide; ④ *S*-napropamide; ⑤ *R*-triticonazole; ⑥ *S*-triticonazole; ⑦ Metconazole-1; ⑧ Metconazole-2; ⑨ Metconazole-3; ⑩ Metconazole-4

pump was used to optimize the mass spectrometry conditions. The mass spectrometry parameters were optimized including cone voltage, collision voltage, ion source temperature, desolvation gas temperature and flow, collision gas flow, and qualitative and quantitative ions for each compound. The ionization efficiency of each pesticide was also optimized to obtain the best mass spectrometry conditions.

2.2.2 Optimization of chromatography conditions

The composition of mobile phase not only affects the retention time and peak shape of the target compounds, but also is crucial for the ionization efficiency and sensitivity. In this study, the effect of methanol-water, methanol-0.1% (*V*/*V*) formic acid aqueous solution, methanol-10 mmol/L ammonium formate, acetonitrile-water, acetonitrile-0.1% (*V*/*V*) formic acid aqueous solution and acetonitrile-10 mmol/L ammonium formate were compared on the separation, peak shape and sensitivity of the target compounds in gradient elution and isocratic elution modes. The results showed that the best peak shape and highest sensitivity of the target pesticides were observed with methanol-water and acetonitrile-water as the mobile phases. Gradient elution and isocratic elution had little effect on the separation of target pesticides. Considering that acetonitrile was used as the extract solvent in the pretreatment and the acetonitrile-water solution system had a lower column pressure, the acetonitrile-water solution system was used as the mobile phase in isocratic elution mode. The chromatogram is shown in Fig.3.

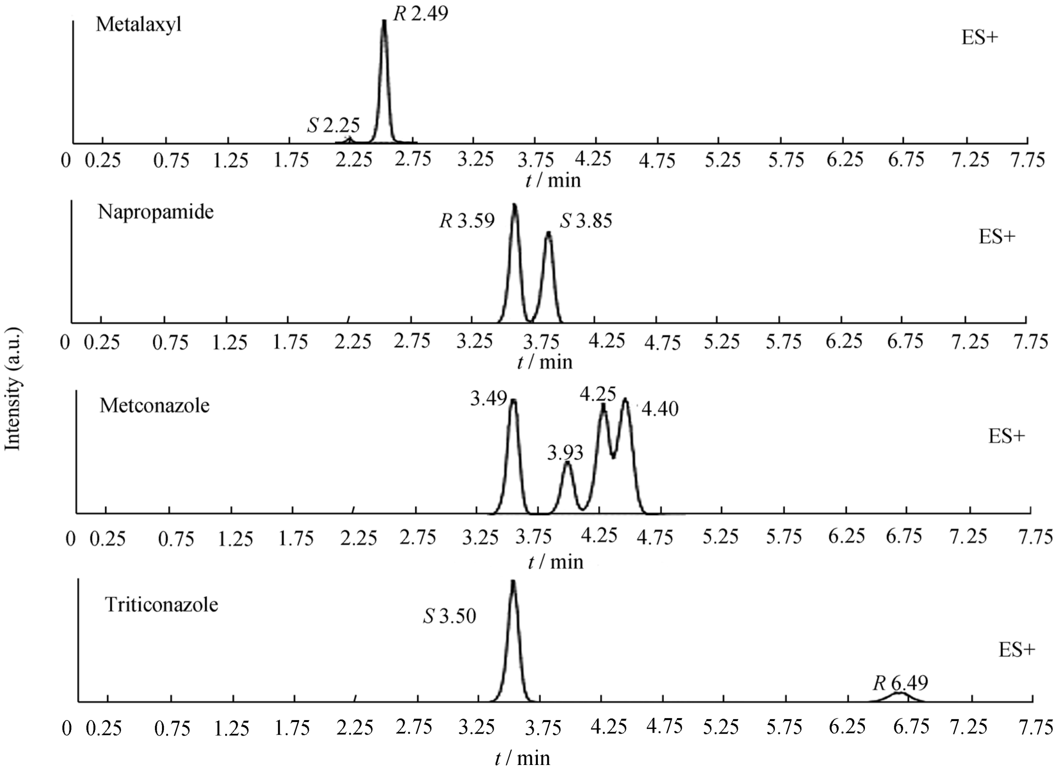
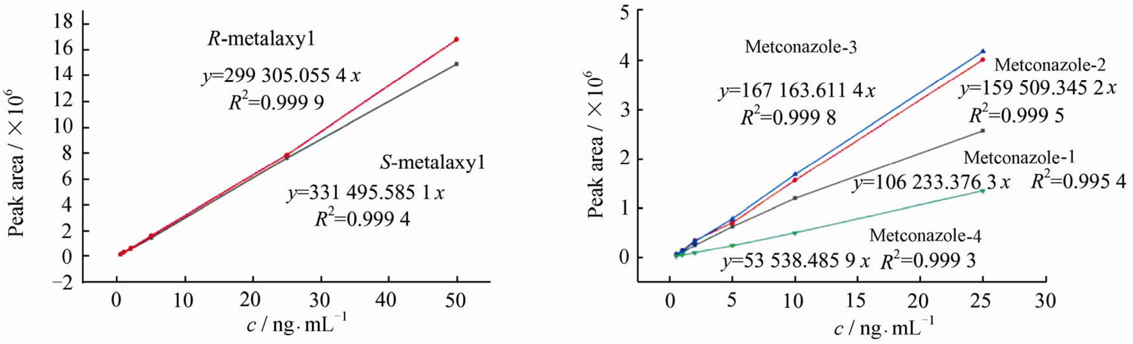


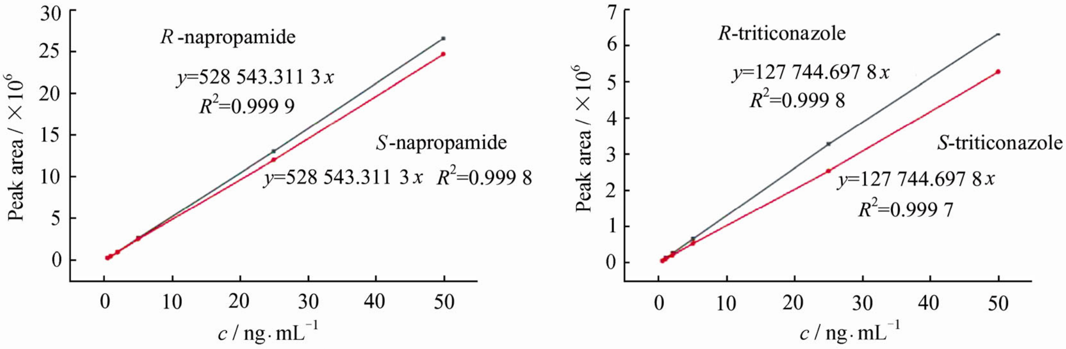
Fig.3 Chromatographic separation of enantiomers of four chiral pesticides

2.3 Linearity and Limit Detection

Eighteen parts of 5.00 g soil sample were weighed and divided into six groups (each group contains three parts as parallel). The six groups were added with 0.01, 0.02, 0.05, 0.1, 0.5 and 1 mL of 100 μg/L mixed standard working solution, respectively, and then diluted to 1, 2, 5, 10, 50, and 100 μg/L (equal to 0.5, 1, 2.5, 5, 25, 50 μg/L of enantiomers) for injection analysis. Linear regression between the concentrations of enantiomers and peak areas revealed good linearity for the four chiral pesticides (Fig.4).



(a) Metalaxyl (b) Metconazole



(c) Napropamide (d) Triticonazole

Fig.4 Matrix matched standard curves of enantiomers

According to the calculation method for limit of detection in mass spectrometry recommended by the EPA [19], the instrument detection limit (IDL) was calculated. The responses of the standard solution at concentrations of 0.05, 0.5, 5 and 50 ng/mL were measured for 5 times. The absolute average ($\overline{X}$) and relative standard deviation (RSD) were calculated for each concentration of individual compound. The calculation formula for IDL is:

$$\mathrm{IDL} = (t_{\mathrm{a}} \times \mathrm{RSD}) / \overline{X} \qquad (1)$$

Among them, t_{a} is the response value at 99% confidence interval. The IDL is the minimum value calculated at different spiked concentrations. The method quantitative limit (MQL) was calculated from the IDL and the relative recovery (Rr) of the method. The formula is:

$$\mathrm{MQL} = 3 \times \mathrm{IDL} \times V / (M \times \mathrm{Rr}) \qquad (2)$$

where V represents final volume of sample extract and M represents sample weight.

The results were shown in Table 2. The MQLs of four chiral pesticides were between 5.3 ng/kg (*R*, *S*-metalaxyl) and 30.3 ng/kg (metconazole-1).

2.4 Matrix Effect

Matrix often produces inhibition or enhancement effect in mass spectrometry. A simple relative response method was used to examine matrix effects. The blank solvent extracts were added with 20 and 200 ng/mL mixed standard solutions. Each concentration was tested for triple and the response was measured as A. Blank matrix extracts were also spiked with the same volume of 20 and 200 ng/mL mixed standard solutions (n=3) and the response was measured as B. Matric effect (ME) can be obtained as:

$$\mathrm{ME}\ (\%) = B/A \times 100 \qquad (3)$$

Table 2 shows that the matrix effects of each enantiomer ranged from 86.37% to 108.22%, which indicated that matrix had no significant inhibition or enhancement effect.

Table 2 Analytical method parameters for enantiomers of four chiral pesticides

Compounds	Rr /%	RSD /%	ME /%	IDL /ng·mL^{-1}	MQL /ng·g^{-1}
S-metalaxyl	87.68	5.61	108.22	0.001 6	0.005 3
R-metalaxyl	89.44	5.45	102.97	0.001 6	0.005 3
R-napropamide	86.33	3.69	90.9	0.002 4	0.008 0
S-napropamide	81.74	4.34	91.4	0.002 1	0.007 0
R-triticonazole	105.79	7.86	94.28	0.003 2	0.010 7
S- triticonazole	103.03	7.21	89.36	0.003 3	0.011 0
Metconazole-1	102.05	7.67	91.38	0.009 1	0.030 3
Metconazole-2	103.59	7.32	86.37	0.005 4	0.018 0
Metconazole-3	92.34	6.89	99.28	0.006 6	0.022 0
Metconazole-4	95.63	6.43	97.46	0.002 2	0.007 3

2.5 Recovery and Precision

For the recovery test, 1 mL of 10 and 100 ng/mL standard working solution were added to blank soil. Triple experiments were performed for each concentration. The average recovery of each enantiomer was calculated at two spiking concentrations and ranged from 81.74% to 105.79% with relative standard deviations ranging from 3.69% to 7.86% (Table 2), which indicated that the method had good accuracy and stability.

2.6 Soil Sample Testing

Soil samples were collected from Lin'an, Wenzhou, Taizhou, Jiaxing, Suzhou and Baiyanghu during the experiment and analyzed according the method developed. As shown in Fig.5, all enantiomers of four chiral pesticides were detected in five soils. The residues of enantiomers ranged from 7.5 ng/kg (*R*-metalaxyl) to 8 233.1 ng/kg (*R*-triticonazole).

3 Conclusion

In this study, a method was developed to detect four chiral pesticides (10 enantiomers in total) in soil by QuEChERS-ultra-performance liquid chromatography-tandem mass spectrometry. Soil was extracted with acetonitrile containing 1% (*V*/*V*) acetic acid and purified with magnesium sulfate, neutral activated alumina and

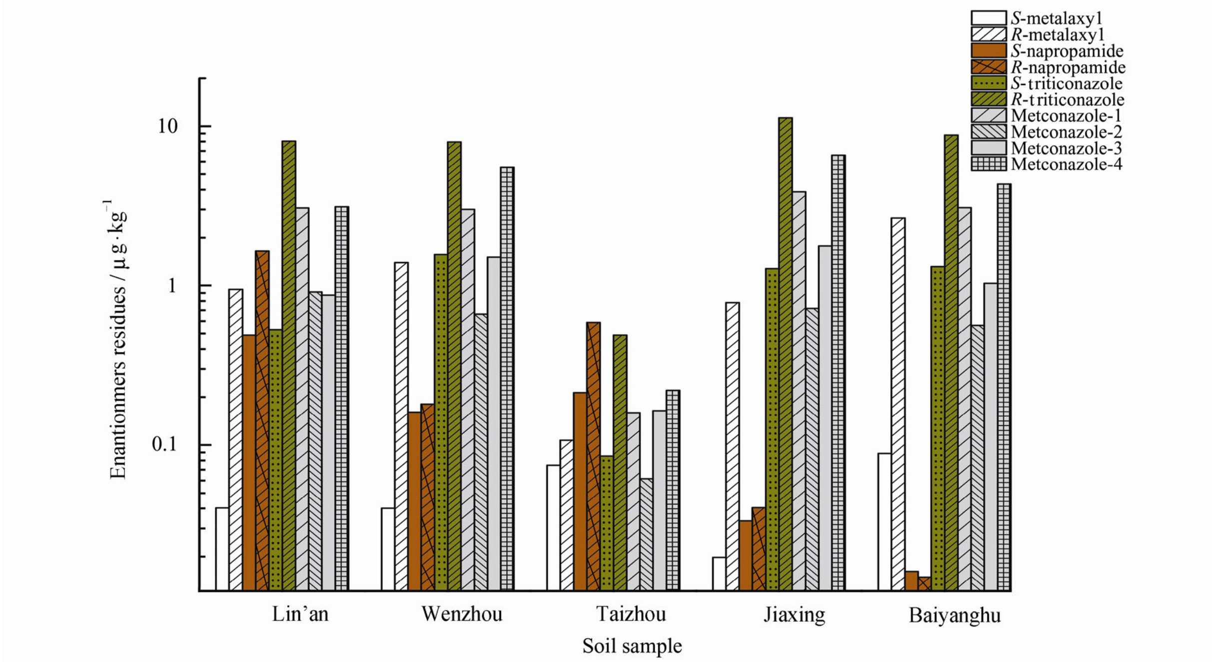


Fig.5 Residues of enantiomers of four chiral pesticides in soils

C18. The recoveries of the enantiomers of four chiral pesticides ranged from 81.74% to 105.79%, the relative standard deviations were 3.69%-7.86%, and the method quantitative limits were 5.3-30.3 ng/kg in soil. The method was successfully applied to determine enantiomer-specific residues of these chiral pesticides in real soil samples.

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