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Research Article

Stereoselective separation and determination of triadimefon and triadimenol in wheat, straw, and soil by liquid chromatography–tandem mass spectrometry

A sensitive and rapid analytical method was developed for simultaneous determination of triadimefon (TF) and triadimenol (TN) stereoisomers in wheat, straw, and soil by liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS). The direct enantioseparation of TF and TN was performed on a Lux cellulose-1 column packed with cellulose-*tris*-(3,5-dimethylphenylcarbamate). The effects of mobile-phase composition on the separation were investigated and stereoisomeric elution orders were confirmed with a polarimeter detector. The pesticides were extracted from samples with acetonitrile and cleaned up by solid-phase extraction or activated carbon. Based on the developed stereoselective LC-MS/MS method, for TF and TN stereoisomers, good linearities were obtained over the concentration range of 0.003–4 mg/L; recoveries were 84.2–102.7% in wheat, 84.0–104.0% in straw, and 85.2–106.8% in soil at spiked concentrations of 0.007–2.0 mg/kg; intra-day and inter-day assay precisions were below 12.2%. Limits of detection (LODs) and limits of quantification (LOQs) in wheat, straw, and soil were 0.001–0.005 mg/kg and 0.007–0.02 mg/kg, respectively. Finally, the method was successfully applied to detect TF and TN stereoisomers in wheat, straw, and soil samples from residual trials in farm.

Keywords: Enantioseparation / Environment / LC-MS/MS / Triadimefon / Triadimenol
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1 Introduction

Chiral compounds account for 25% of all agrochemical compounds used commercially and for 26% of the total value of the world agrochemical market [1]. Over the past years, chiral separation has been particularly concerned especially on pesticides and pharmaceuticals. The enantiomers of the chiral pollutants possess similar physicochemical properties in achiral environment but show different activities in biological systems due to stereoselective interactions with enzymes, receptors, and other enantiomeric biological entities [2]. However, most chiral pesticides are almost always manufactured and released into the environment as racemate although the desired biological activity may be derived from only one isomer [3]. Therefore, in order to evaluate the respective properties of individual enantiomer, e.g. physiological, toxicological, and metabolic behaviors, it is essential and urgent to develop enantiomeric separation and analysis methods of chiral pesticides.

Triadimefon (TF) and triadimenol (TN) belonged to the triazole family of chemicals and are two of the most important fungicides in the present use [4]. TF was a systemic fungicide that acts by inhibiting steroid demethylation, and used to control powdery mildews and fungi on fruits, vegetables, wheat, and other agricultural crops. It has a single chiral center and correspondingly present two enantiomers, and can be enzymatically reduced to TN with four stereoisomers in plants, soil, and fungi [5]. The (1S, 2R)-isomer has the highest fungicidal activity (up to 1000-fold more active than the other three) in four stereoisomers of TN [3]. It is known that the biotransformation of TF into TN is stereoselective [6] and the biological response of each TN stereoisomer is different; thus, stereoselective analytical methods are necessary to determine these compounds and study their stereoselective distribution or environmental fate

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Abbreviations: **CDMPC**, cellulose-*tris*-(3,5-dimethylphenylcarbamate); **CSP**, chiral stationary phase; **EW**, emulsion in water; **PD**, polarimeter detector; **TF**, triadimefon; **TN**, triadimenol

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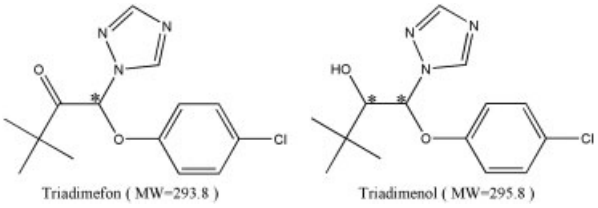

Figure 1. Structures and molecular weights of TN and TF.

[7]. The chemical structures and molecular weights of TF and TN are shown in Fig. 1.

In the past, various chromatographic methods were used for chiral separation of TF or TN. However, most of these methods used ultraviolet detector to separate and detect pesticide stereoisomers with high concentrations [5, 7–14]. There are relatively few publications focusing on trace analyses of chiral pesticides in environmental samples. From the number of papers published over the past years, high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) were two of the most commonly utilized techniques in chiral separation, of which HPLC is the dominant method [15]. To support drug metabolism and pharmacokinetic studies of chiral pharmaceuticals, it is necessary to combine the resolving power of HPLC with the sensitivity of MS detection [16]. The application of LC-MS/MS in pesticide residue analysis has become increasingly popular due to its versatility, specicity, and selectivity, which enables the detection of target compounds in the low ng/L range [17], and also be free of interferences from matrix components present in complex samples such as plant, tissue, and soil. However, as much as we know, scarce work has been done on stereoselective analysis of TF and TN based on LC-MS/MS.

The aim of the present study was the development and validation of a simple and rapid LC-MS/MS method for simultaneous quantitative determination of TF and TN stereoisomers in wheat, straw, and soil. Chiral column packed with chiral stationary phase (CSP) of cellulose-*tris*-(3,5-dimethylphenylcarbamate) (CDMPC) was used in the method. The stereoisomeric elution orders were determined by online polarimeter detector (PD). Finally, the optimized method was applied to determine the analytes in real samples. This method can be applied for studying stereoselective metabolism and environmental behaviors of TF and TN, and is helpful to assess the risks posed by the fungicide to environment and public health [18].

2 Materials and methods

2.1 Materials, reagents, and apparatus

Reference standards of racemic TF (99.55%) and TN (99.1%) were purchased from the Institute for Control of Agrochemicals, Ministry of Agriculture of China (Beijing, China). The analytical standards of TN-A (racemate of RS- and SR-enantiomer, 99.9% purity) and TN-B (racemate of RR- and SS-enantiomer, 99.9% purity) were kindly provided by Pesticide Residues and Environmental Toxicology Group, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Beijing, China). Stock solutions of TF and TN were prepared in methanol and stored at −20°C. Working standard solutions were obtained by dilutions of the stock solution with methanol.

Acetone, petroleum ether (60–90°C), and sodium chloride were of analytical grade and purchased from Beijing Chemical Reagent (Beijing, China); the petroleum ether was distilled before use. Activated carbon (particle size <100 μm, water-soluble matter $\leq 0.5\%$) for sample cleanup was obtained from Merck (Darmstadt, Germany). Acetonitrile and methanol were of HPLC grade and purchased from J. T. Baker (Phillipsburg, NJ, USA). Water was Wahaha pure water and purchased from Wahaha Group Co. Ltd. (Hangzhou, China).

Florisil solid-phase extraction (SPE) cartridges (1000 mg, 6 mL) were purchased from Agela Technologies (Beijing, China) and used to sample cleanup. An Anke TDL-40B centrifuge with 50 mL fluorinated ethylene propylene centrifuge tubes was purchased from Aanting (Shanghai, China) and used to separate sample extracts. An SE2020 (OHAUS USA) top-loading balance was used to weigh samples. KQ-600 ultrasonic cleaner was produced by Kunshan Ultrasonic Instrument (Kunshan, China). The shaker (SZX-1370) was obtained from Haerbin Dongfang Electric Control Switch Factory (Haerbin, China).

2.2 Sample preparation

Soil sample was air-dried at room temperature for 48 h and the dried material was sieved through a 2.0-mm sieve. Straw samples were cut into 1–2 cm length using scissors. Wheat grains were grinded to 60–80 mesh with a cereal grinder. All samples were stored at −20°C until further analysis according to the following method. All blank samples were collected from uncontaminated field in Beijing.

2.2.1 Soil sample

Ten grams of soil sample were weighed into a 50-mL centrifuge tube; TF and TN were extracted with 20 mL of acetonitrile by ultrasonic for 20 min. Sodium chloride (5 g) was subsequently added, and the tube was shaken vigorously by hand for 1 min and centrifuged at 3000 rpm for 5 min. A 10-mL aliquot from the upper layer was evaporated to near dryness with a vacuum rotary evaporator at 35°C, and drying was completed under a nitrogen stream. The residue was redissolved in 2 mL of methanol and filtered through a 0.22-μm filter into a sample vial for LC-MS/MS analysis.

2.2.2 Straw sample

Ten grams of straw sample were transferred into a 250-mL flask and extracted by adding 50 mL of acetonitrile, and the

mixture was shaken on a reciprocating shaker for 100 min. Then 0.1 g of activated carbon was added and shaken for 20 min. The samples were filtered through a filter paper into a 50-mL centrifuge tube. Sodium chloride (5 g) was subsequently added, and the tube was shaken vigorously by hand for 1 min and centrifuged at 3000 rpm for 5 min. A 25-mL aliquot was transferred from the upper layer, and following processes were same for the soil sample.

2.2.3 Wheat sample

Ten grams of wheat sample were weighed into a 50-mL centrifuge tube, and extracted with 30 mL of acetonitrile by vortexing for 1 min and ultrasonic for 20 min. Sodium chloride (3 g) was subsequently added, and the tube was shaken vigorously by hand for 1 min and centrifuged at 3000 rpm for 5 min. A 15-mL aliquot from the upper layer was evaporated to near dryness with a vacuum rotary evaporator at 35°C, and drying was completed under a nitrogen stream. The extract was redissolved in 5 mL of acetone/petroleum ether (2:8, v/v) before cleanup.

The Florisil SPE cartridge was previously conditioned with 5 mL of petroleum ether. The concentrated extract was transferred to the cartridge and eluted twice with 5 mL of acetone/petroleum ether (2:8, v/v). All eluents were collected and evaporated to near dryness with a vacuum rotary evaporator at 30°C and to dryness under a gentle nitrogen stream. The residue was redissolved in 2 mL of methanol and filtered through a 0.22-μm filter into a sample vial for LC-MS/MS analysis.

2.3 Instruments and conditions

2.3.1 LC

The LC system was an Agilent 1200 HPLC system equipped with a G1322A degasser, G1311A quatpump, G1316B column compartment, G1315C diode array detector, G1329A autosampler, and a 20-μL sample loop (Wilmington, DE, USA). The detector was set at 220 nm to evaluate the effect of mobile-phase composition on enantioseparation. Chromatographic separation was carried out on a Phenomenex Lux Cellulose-1 column (250 × 4.6 mm id, 5-μm particles), packed with CSP of CDMPC and obtained from Guangzhou FLM Scientific Instrument (Guangzhou, China). Gradient elution was performed with methanol as mobile phase A and water as mobile phase B at a flow rate of 0.5 mL/min. The gradient elution program was as follows: A was 65% in 0–26 min, linearly increased to 80% in 26–29 min, held at 80% for 29–36 min, returned to 65% in 36.0–36.1 min, finally held at 65% in 36.1–43 min. The initial composition was additionally equilibrated for 1.5 min before the next injection. The injection volume was 10 μL.

The chromatographic parameters including capacity factor (k), separation factor (α) and resolutions (Rs) were calculated from the formulas: $k = (t - t_0)/t_0$, $\alpha = k_2/k_1$, $Rs = 2(t_2 - t_1)/(W_1 + W_2)$, where t was the retention time and t_0 was the void time at given conditions, k was the capacity factor and W was the peak width. Both the separation and resolution values were calculated from the chromatograms recorded under the corresponding chromatographic conditions.

The elution orders of TF and TN stereoisomers were detected with a CHIRALYSER-MP PD (IBZ MESSTECHNIK, Germany) obtained from Beijing Separation Science & Technology Development (Beijing, China). The optical signals were received and processed by an N2000 SP1 chromatographic workstation purchased from Zhejiang University Zhida Information Engineering (Hangzhou, China).

2.3.2 MS

An API 2000 triple quadrupole mass spectrometer equipped with a Turbo Electrospray Ionization (ESI) source was used for LC-MS/MS analysis (Applied Biosystems, USA). Quantification was achieved in positive-ion mode (ESI+). The signals were received and processed with Analyst 1.4.2 software. The optimized major working parameters were as follows: Curtain gas (CUR) 15, collision gas (CAD) 5, ion source temperature (TEM) 400°C, ion source gas 1 (GS1) 25 and ion source gas 2 (GS2) 55, ion spray voltage +5500 V, focusing potential (FP) 400, entrance potential (EP) 10, collision cell exit potential (CXP) 7. Multiple reactions monitoring mode was used; the precursor and product ions of TF and TN with corresponding declustering potentials and collision energies are summarized in Table 1.

2.4 Assay validation

The stock standard solutions of racemic TF and TN were prepared at concentrations of 1000 and 500 mg/L in

Table 1. MRM parameters for determination of TF and TN

Analyte	Q1 mass (*m/z*)	Q3 mass (*m/z*)	Declustering potentials (V)	Collision energies (V)
TF	294.1	197.1	20	22
	294.1	69.1[a)]	25	32
TN	296.1	227.5	17	18
	296.1	70.2[a)]	12	25

a) Multiple reaction monitoring used for quantification. The mass resolution was unit.

methanol. The work standard solutions for calibration curves were prepared by using matrix solutions of blank samples to dilute stock standard solutions of TF and TN. The linear regression equations and the correlation coefficients were obtained from the peak area ratios plotted against their respective concentrations (0.003–4 mg/L).

Recovery evaluation was carried out with six replicates at three fortified concentration levels (0.02, 0.2, and 2 mg/kg for TF enantiomers; 0.013, 0.13, and 1.3 mg/kg for TN-A stereoisomers, 0.007, 0.07, and 0.7 mg/kg for TN-B stereoisomers). The fortified wheat, straw, and soil samples were obtained by adding appropriate amount of the mixed standard solutions in 10 g of samples, and then processed by the above method after 30 min. The recoveries were calculated by the ratio of the peak area of each stereoisomer in extracts from wheat, straw, and soil to that in standard solutions with equivalent concentrations. The standard deviation (SD) and the relative standard deviation (RSD) (RSD = SD/mean × 100%) were calculated in the recovery study. The limit of detection (LOD) of each stereoisomer was considered to be the concentration that produced a signal-to-noise (*S/N*) ratio of 3, and the limit of quantification (LOQ) was defined as the lowest spiking level of each stereoisomer on acceptable recovery. To assess intra-day precision, replicate analysis ($n = 5$) of straw samples spiked at 0.05, 0.5, and 5 mg/kg of rac-TF and rac-TN was performed. The inter-day precision was also tested over 3 days by determinations of each concentration level (as described for intra-day assay) each day. Precision was expressed as RSD [18].

3 Results and discussion

3.1 Effect of mobile-phase composition on enantioseparation

Mobile phase plays the most important role for enantiomeric separations in terms of efficiency, retention, and resolution of stereoisomers. Therefore, investigation of the composition of the mobile phase should always be included in the optimization of stereoselective separation [18].

3.1.1 Selection of mobile phase

In this study, stereoselective separations of two analytes on the cellulose-based chiral column were performed using methanol/water and acetonitrile/water as the mobile phase at the flow rate of 0.5 mL/min. Methanol and acetonitrile were the most frequently used modifiers for reversed-phase HPLC (RP-HPLC), and the effects of their contents in the mobile phase on the resolution were investigated. The results showed that TN stereoisomers cannot be effectively separated when acetonitrile/water was used as the mobile phase (percent of acetonitrile was from 40 to 90%). Tables 2 and 3 summarize influences of the content of methanol in mobile phase on the separation. Although TF and TN have similar chemical structures, the retention and resolutions were greatly different. The Rs values of TF were all above 2 with different volumes of methanol in mobile phase. The Rs_1 value of TN was 1.45 when the methanol content was 60%. The TF enantiomers showed strong retention on the CSP while the retention of TN stereoisomers was weak.

It can be seen from the results, methanol could provide a more efficient separation compared with acetonitrile. TF and TN stereoisomers could achieve good separations when using methanol as the modifier. The optimal resolution was obtained using 60% of methanol and all Rs values were above 1.45.

3.1.2 Gradient elution

When using isocratic elution of methanol/water (60:40, v/v), the retention times were 70–90 min for TF enantiomers, and 30–50 min for TN stereoisomers. In order to shorten the analysis time and improve the efficiency, the gradient elution procedure using methanol/water as the mobile phase was investigated. The optimized procedure was a linear gradient from 65% of methanol (0–26 min) to 80% in 3 min (26–29 min), holding 7 min (29–36 min) and then returning to 65% in 0.1 min (36–36.1 min), finally

Table 3. The chromatographic separations of TF on Lux Cellulose-1 column

Compound	Methanol/water v/v	k_1	k_2	α	Rs
TF	90:10	0.83	0.98	1.18	2.07
	80:20	1.34	1.78	1.32	2.70
	75:25	2.10	2.65	1.26	4.10
	70:30	3.78	4.86	1.29	4.58
	65:35	6.13	7.88	1.29	4.58
	60:40	11.02	14.24	1.29	4.62

Table 2. The chromatographic separations of TN on Lux Cellulose-1 column

Compound	Methanol/water v/v	k_1	k_2	k_3	k_4	α_1	α_2	α_3	Rs_1	Rs_2	Rs_3
TN	90:10					No separation					
	80:20					No separation					
	75:25	1.45	1.57	1.70	1.92	1.08	1.08	1.13	1.26	1.44	2.41
	70:30	2.22	2.46	2.73	3.18	1.11	1.11	1.16	1.34	1.44	2.22
	65:35	3.39	3.74	4.28	4.83	1.12	1.14	1.13	1.39	2.23	2.23
	60:40	6.06	6.69	7.82	8.76	1.12	1.17	1.12	1.45	2.73	2.16

holding 6.9 min (36.1–43 min). Aiming to get good repeatability, the initial composition was additionally equilibrated for 1.5 min before the next injection. The separation and stabilization time of one injection was 45 min and significantly less than that using an isocratic elution. The best peak shape and separation for six stereoisomers were obtained under this procedure (Fig. 2).

3.2 Elution order

With the development of detection technology, PD has become the new detector for HPLC. It plays an important role in chiral technologies, and HPLC-UV-PD have been used more frequently in qualitative detection of stereoisomers [19]. However, the disadvantage of this detector is lower sensitivity and need for higher concentration of sample solutions to generate the signal. In this work, the HPLC-UV-PD method was used for the determination of the left (−) or right (+) optical rotations of TF and TN stereoisomers by positive or negative optical signals.

Based on a previous report, (−)-TF was R-configuration and the (+)-TF was S-configuration according to measurements by polarimeter in CCl_4 at 589 nm [20]. However, enantiomeric optical rotation signals can be reversed with change of wavelengths and solvents [21, 22]; so the rotation signals of TF and TN stereoisomers were investigated by PD at 426 nm in different mobile-phase systems. The results showed that the rotation signals of TF enantiomers reversed when changing solvent from CCl_4 to methanol/water (50:50, v/v) or acetonitrile/water (50:50, v/v), but those of TN stereoisomers did not change. Additionally, the signals of all TF and TN stereoisomers did not vary with solvent from

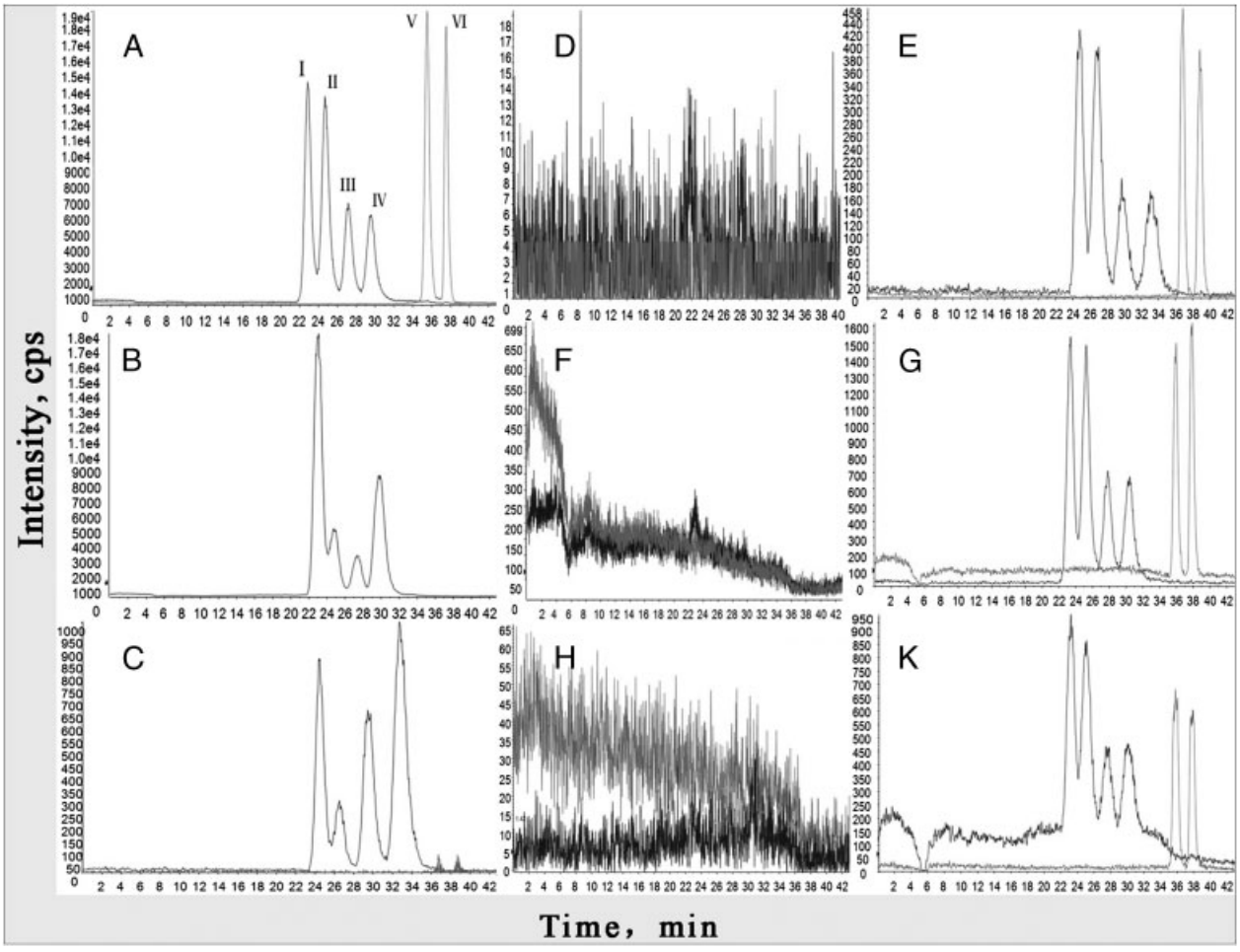

Figure 2. Simultaneous stereoselective separation of TF and TN. Methanol was programmed from 65% (0–26 min) to 80% (26–29 min), held for 7 min (29–36 min), then returned to 65% (36.0–36.1 min), and held for 6.9 min (36.1–43 min); (A) 1 mg/L for each racmate of TF and TN: (I) (−)-TN-A; (II) (+)-TN-A; (III) (−)-TN-B; (IV) (+)-TN-B; (V) (−)-TF; (VI) (+)-TF. Representative chromatograms of (B and C) extracts from real straw and soil samples at 21 days after treatment with *rac*-TF, respectively (the concentrations of (−)-TN-A, (+)-TN-A, (−)-TN-B, (+)-TN-B were 447.7, 88.8, 37.1, 277.3 μg/kg in straw and 33.3, 10.3, 75.7, 110.8 μg/kg in soil, respectively). (D and E) indicate extracts from blank wheat and spiked at LOQ. (F and G) indicate extracts from blank straw and spiked at LOQ. (H and K) indicate extracts from blank soil and spiked at LOQ (all LOQs in three matrices were 0.02, 0.013, and 0.007 mg/kg for each stereoisomer of TF, TN-A, and TN-B, respectively).

acetonitrile/water to methanol/water. As shown in Fig. 3A, the PD signals of two enantiomers of TF were approximately opposite to each other; the first and second eluted enantiomers showed (−)- and (+)- optical signals, respectively. Thereby, the enantiomeric elution orders of TF on Lux Cellulose-1 column were (−)-isomer first and (+)-isomer second with methanol/water as the mobile phase by PD at 426 nm. However, the elution orders of fungicide enantiomers may be reversed when changing the mobile phase and CSP. For example, the elute orders of TF enantiomers were opposite on Chiralcel OD-H column packed with CDMPC and Chiralcel OJ-H column with hexane/isopropanol as the mobile phase, the (+)-isomer was first eluted from OD-H column but (−)-isomer first from OJ-H column [23].

Rac-TN has two stereogenic centers and is therefore composed of two pairs of enantiomers herein termed TN-A and TN-B, respectively. Figure 3B–D shows that rac-TN was separated into (−)-TN-A, (+)-TN-A, (−)-TN-B, (+)-TN-B on Lux Cellulose-1 column with methanol/water (70:30, v/v) as the mobile phase at 220 nm. This optical rotation signals were opposite to that on Chiralpak AS-H column with order of (+)-, (−)-, (+)-, (−)-stereoisomers and hexane/ethanol as the mobile phase [24].

3.3 Validation of the method

3.3.1 Linearity

Calibration curves were generated by plotting peak area of each stereoisomer versus its concentration. Linear regression analysis was performed using Microsoft Excel. As observed in Table 5, the method presented typical calibration curve equations and provided a linear range of 0.003–4 mg/L with a good correlation coefficient ($R^2 > 0.99$).

3.3.2 Matrix effect

During the validation of this method, the matrix effect was evaluated by using ratios of the slopes of the calibration curves of each stereoisomer in three different matrices and that in solvent. Data in Table 4 show that most ratios of the slope were higher than 100%, which indicated the presence of matrix effect in this LC-MS/MS method. Especially, the matrix effect of signal enhancement in straw was significantly higher than those in wheat and soil. Therefore, matrix-matched standards were used to eliminate matrix effect with an apparent signal enhancement, and obtained more accurate results according to the above assay validation.

3.3.3 LODs and LOQs

The LODs were 0.002 mg/kg in soil and straw and 0.005 mg/kg in wheat for the two enantiomers of TF. The

Table 4. Matrix effect of TF and TN stereoisomers in wheat, straw and soil

Compound	Matrix effect (the ratio of the slopes of the calibration curve, %)		
	Wheat	Straw	Soil
(−)-TF	104.9	236.1	131.3
(+)-TF	100.2	225.9	98.8
(−)-TN-A	114.9	278.7	114.4
(+)-TN-A	84.3	293.6	98.2
(−)-TN-B	132.1	347.0	136.9
(+)-TN-B	97.2	285.5	108.4

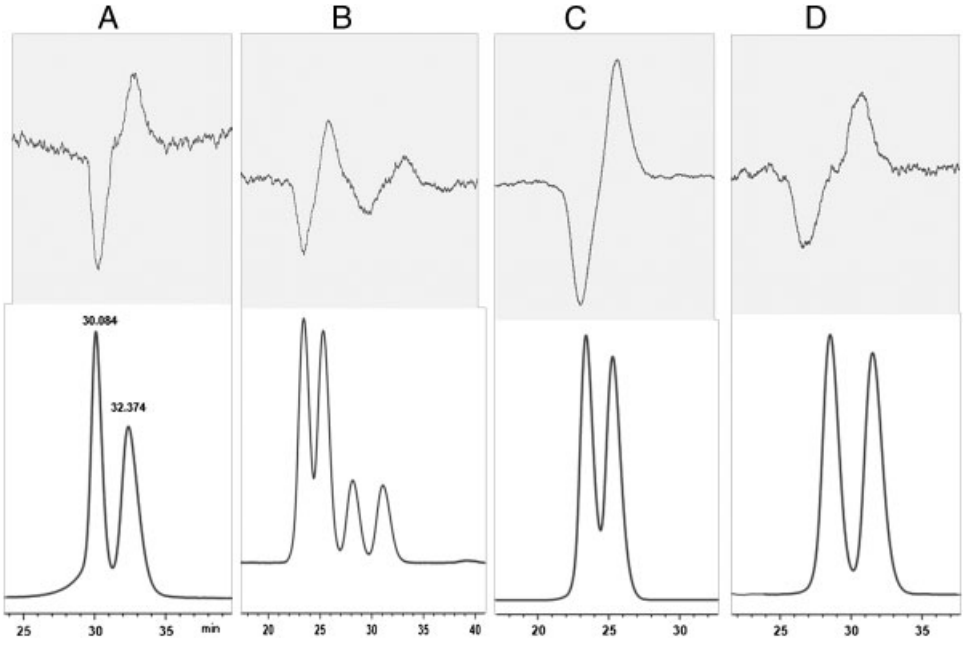

Figure 3. Stereoisomeric elution orders of TF (A), TN (B), TN-A (C), and TN-B (D) on Lux Cellulose-1 column; PD (above) and UV (below) at 220 nm; methanol/water (70:30, v/v) at flow rate of 0.5 mL/min.

Table 5. Summary of recoveries and linearity equations for TF and TN stereoisomers in wheat, straw and soil ($n = 6$)

Compound	Fortified level (mg/kg)	Recovery (average ± SD, %)			Linearity				
		Wheat	Straw	Soil	Concentration range (mg/L)	Matrix	Linear equation	R^2 a)	RSD[a] (%)
(−)-TF	0.02	90.80 ± 3.42	91.78 ± 2.96	93.81 ± 2.91	0.01–4.0	Wheat	$y = 164\,627x + 395.04$	0.9998	9.21
	0.2	89.25 ± 8.13	95.21 ± 3.30	99.38 ± 1.79		Straw	$y = 37\,0631x + 17681$	0.9986	14.45
	2	91.77 ± 4.32	96.32 ± 8.21	100.50 ± 9.09		Soil	$y = 20\,6187x + 7980.2$	0.9984	13.72
(+)-TF	0.02	87.62 ± 3.93	83.97 ± 3.18	85.23 ± 5.41	0.01–4.0	Wheat	$y = 21\,7693x + 7277.8$	0.9979	10.13
	0.2	96.83 ± 8.81	104.02 ± 6.91	100.21 ± 3.68		Straw	$y = 49\,0753x + 22333$	0.9984	11.88
	2	95.60 ± 5.77	99.68 ± 7.33	106.14 ± 8.52		Soil	$y = 21\,4565x + 12681$	0.9985	12.09
(−)-TN-A	0.013	84.18 ± 1.99	94.85 ± 2.29	95.21 ± 2.72	0.007–3.3	Wheat	$y = 97\,097x + 336.53$	0.9999	3.78
	0.13	102.67 ± 2.42	93.02 ± 2.27	99.30 ± 2.77		Straw	$y = 26\,5606x + 10819$	0.9991	2.48
	1.3	89.96 ± 4.44	94.56 ± 4.79	102.58 ± 6.41		Soil	$y = 96\,634x + 5713.4$	0.9990	5.35
(+)-TN-A	0.013	97.88 ± 4.04	91.15 ± 2.98	92.78 ± 8.52	0.007–3.3	Wheat	$y = 74\,949x + 5753.6$	0.9979	2.55
	0.13	99.67 ± 1.78	93.23 ± 2.91	98.83 ± 2.11		Straw	$y = 27\,5767x + 16364$	0.9984	3.29
	1.3	94.54 ± 2.16	94.06 ± 6.25	102.36 ± 2.69		Soil	$y = 92\,222x + 2521.6$	0.9992	2.77
(−)-TN-B	0.007	96.48 ± 3.74	93.65 ± 2.76	95.08 ± 4.53	0.003–1.7	Wheat	$y = 43\,633x - 1842.5$	0.9979	3.42
	0.07	96.65 ± 4.59	90.06 ± 1.08	100.18 ± 2.54		Straw	$y = 114\,601x + 14681$	0.9956	11.65
	0.7	97.78 ± 6.51	90.44 ± 8.48	106.78 ± 7.28		Soil	$y = 45\,200x + 2333$	0.9989	6.52
(+)-TN-B	0.007	89.61 ± 5.42	90.18 ± 5.31	98.98 ± 2.11	0.003–1.7	Wheat	$y = 64\,179x - 3072$	0.9985	4.11
	0.07	98.61 ± 1.19	86.32 ± 2.56	100.05 ± 0.79		Straw	$y = 188\,539x + 17246$	0.9975	9.12
	0.7	93.25 ± 4.94	93.70 ± 7.50	100.40 ± 5.99		Soil	$y = 71\,586x - 2009.3$	0.9997	0.01

a) R^2, Coefficients of determination; RSD, coefficient of variation on the slope of the calibration curve.

LODs for each stereoisomer of TN were 0.001 mg/kg in soil and straw, and 0.002 mg/kg in wheat. The LOQs were established as being 0.02 mg/kg for TF enantiomers, 0.013 mg/kg for TN-A stereoisomers, 0.007 mg/kg for TN-B stereoisomers, based on the lowest fortication level in wheat, straw, and soil.

3.3.4 Recovery assays

The blank wheat, straw, and soil samples spiked with different concentrations of mixed standard solutions were analyzed by the developed methods. Table 5 shows the analytical results obtained by stereoselective LC-MS/MS method. Average recoveries at three fortified levels for TF and TN stereoisomers were 84.2–103% with RSD of 1.22–9.09% in wheat, 84.0–104% with RSD of 2.44–8.79% in straw, and 85.2–107% with RSD of 0.79–9.18% in soil.

3.3.5 Repeatability and stability

A summary of the results of the precision and accuracy experiments is given in Table 6. RSDs of the intra-day precision in five samples were <9.69% and the inter-day precision in 3 days was <12.2% for TF and TN stereoisomers at the three fortified concentration levels, indicating a good repeatability.

The ratios between each stereoisomer of TF and TN in standard solutions and in matrix samples after extraction and purification process were calculated, respectively. The results showed that stereoisomeric ratios in standard solutions and in matrix samples did not have significant differences, indicating that TF and TN stereoisomers were stable and had no racemization in the sample preparation.

Table 6. Intra- and inter-day precision of the developed method for determination of TF and TN stereoisomers

Compound	Intra-day (RSD%, $n = 5$)			Inter-day (RSD%, $n = 3$)		
	1[a]	2	3	1	2	3
(−)-TF	4.22	3.39	1.00	9.12	2.20	7.12
(+)-TF	8.19	8.57	2.07	5.30	5.80	2.13
(−)-TN-A	4.34	2.84	2.50	9.56	3.83	3.51
(+)-TN-A	6.30	2.11	2.14	9.79	2.27	5.56
(−)-TN-B	3.30	3.71	1.15	12.20	3.04	2.60
(+)-TN-B	9.69	2.57	3.12	12.00	8.36	5.41

a) 1, 2, 3 represent concentrations of 0.025, 0.25, 2.5 mg/kg for TF enantiomers, 0.033, 0.33, 3.3 mg/kg for TN-A stereoisomers, and 0.017, 0.17, 1.7 mg/kg for TN-B stereoisomers, respectively.

3.4 Comparison with previous methods

Some stereoselective methods were reported for the determination of TF or TN. Wu et al. [9, 10] developed a method for simultaneous separation of TF and TN by sulfated β-cyclodextrin-mediated CE, and applied this method to study biotransformation of TF to TN by soil microorganisms. Kenneke et al. [5] reported on the integration of metabolomics and *in vitro* metabolism assays for investigating the stereoselective transformation of TF in

rainbow trout by GC-MS. Dong et al. [24] analyzed stereoselective degradation of TN in cucumber plants by HPLC. Compared with CE and HPLC, the extraction and purification procedures of this LC-MS/MS method were fast and the sensitivity was better. So, the developed method has more advantages on selectivity, sensitivity, and analytical efficiency for TF and TN stereoisomers.

3.5 Application to practical samples

Wheat, straw, and soil samples obtained from a residual trial in Beijing were analyzed using the above optimized method. Field trial was conducted as follows: TF (15% emulsion in water (EW)) was applied to the wheat plots at 180 g/a.i.ha (the recommended dose, means gram of effective chemicals in EW per hectare) two times at interval of 12 days. The wheat, straw, and soil samples were collected at harvest of 21 days after this application. The results showed no detectable residues of TF in real wheat, straw, and soil at harvest. However, different concentrations of TN stereoisomers were detected in straw and soil, and the residue level was higher in straw. The concentrations of (−)-TN-A, (+)-TN-A, (−)-TN-B, (+)-TN-B were 447.7, 88.8, 37.1, 277.3 μg/kg in straw and 33.3, 10.3, 75.7, 110.8 μg/kg in soil, respectively. The typical chromatograms are presented in Fig. 2. These results indicated that TF can stereoselectively degrade to TN in straw and soil.

4 Concluding remarks

In this work, a new stereoselective LC-MS/MS method was developed for simultaneous separation and determination of TF and TN stereoisomers in wheat, straw, and soil. The effective enantiomeric resolutions of two fungicides were achieved on a Lux Cellulose-1 chiral column on RP-HPLC. The investigation on effects of organic modiers on enantioseparation showed that methanol/water with a gradient elution procedure provided the best results. The assay evaluation results showed that this LC-MS/MS method has good linearity, recovery, repeatability, low LODs, and LOQs. The method is simple, rapid, sensitive, and reliable for determination of TF and TN stereoisomers in wheat, straw, and soil, and can be widely applied in analysis and study of stereoselective environmental behaviors of TF and TN.

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