

RESEARCH ARTICLE

Separation and determination of triadimefon and its metabolites triadimenol enantiomers in fruit puree by supercritical fluid chromatography

Wen-hua Zhang¹ | Dun-ming Xu² | Jian-bo Hou¹ | Jian-min He¹ | Fei Zhao¹ | Ying-zhu Shi¹ | Yan Qian¹

¹Zhejiang Academy of Science and Technology for Inspection and Quarantine, Hangzhou, P. R. China

²Technical Center of Xiamen Customs, Xiamen, P. R. China

Correspondence

Wen-hua Zhang, Zhejiang Academy of Science and Technology for Inspection and Quarantine, 126 Fuchun Road, Hangzhou 310016, P. R. China.
Email: zwh@zaiq.org.cn

Funding information

National Program on Key Research Project of China, Grant/Award Number: 2019YFE0103900

A method was established for the separation and determination of triadimefon and its metabolite triadimenol enantiomer residues in major complementary fruit puree for infants and young children (banana puree, pineapple puree, and grape puree) by supercritical fluid chromatography. After the samples were extracted with acetonitrile and purified with a solid phase extraction cartridge, Acquity Trefoil CEL2 chiral chromatographic column was adopted for separation, and gradient elution was conducted at the flow rate of 1.0 ml/min under the mobile phase of supercritical carbon dioxide - 0.5% ammonia methanol, the detection wavelength was 220 nm and quantification was conducted with the external standard method. The limits of quantitation of triadimefon and triadimenol enantiomers were both 0.05 mg/kg, the linear ranges were 0.5–50 mg/L, and the linear correlation coefficients were greater than 0.9993. The recoveries in the spiked samples at 0.05, 0.2, and 3.0 mg/kg were from 80.1 to 106%, and the relative standard deviation reached 3.3–7.6%. The method is efficient, rapid, reproducible, and environmentally friendly, enabling accurate analysis of pesticide enantiomers, which can detect the enantiomer residues of triadimefon and its metabolite triadimenol in major complementary fruit puree for infants and young children.

KEYWORDS

chiral separation, enantiomers, puree, supercritical fluid chromatography, triadimefon, triadimenol

1 | INTRODUCTION

Chirality is a common phenomenon in the natural world. With the development of science, there is an increasing demand for optically pure materials, and the separation of chiral enantiomers has attracted people's attention gradually. Traditional studies on chiral pesticides don't distinguish the enantiomers and treat all enantiomers as

the same compound [1]. GB 2763—2021 National food safety standard—Maximum residue limits for pesticides in food just stipulate the safety limit for the use of chiral pesticides raceme, rather than the accurate residue limit of their enantiomers. However, the enantiomers of chiral pesticides vary greatly in bioactivity, environmental behavior, and ecotoxicology [2–4]. For example, fenpropathrin has one chiral center, and (+)-fenpropathrin often presents higher insecticidal activity and faster degradation rate than (–)-fenpropathrin [5]. Therefore, the

Article Related Abbreviation: HLB, hydrophile lipophile balance.

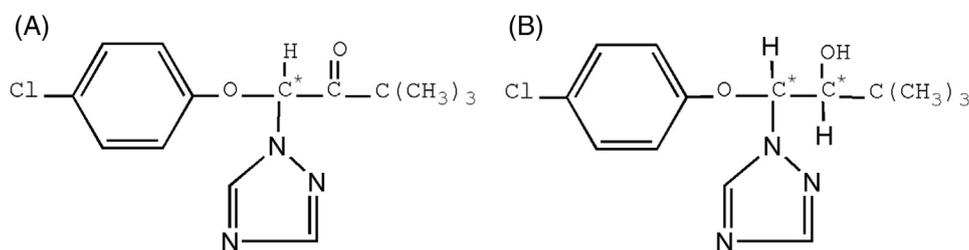


FIGURE 1 The chemical structures of triadimefon (A) and its metabolite triadimenol (B) racemes.

establishment of the separation and analysis method for enantiomers of chiral pesticides has very important significance for the safety evaluation study on chiral pesticides in the toxicity, activity and ecological environment of enantiomers as well as the development of more optical pure enantiomers.

As an efficient bactericide, triazole pesticides can effectively protect crops like fruit, soybean, and grain from the invasion of germs. Therefore, it is extensively applied to agricultural production [6]. The detection methods of triazole pesticides mainly include HPLC [7–9], LC-MS/MS [10, 11], and so on, which mainly detect the raceme of triazole fungicides. Triazole fungicides contain one or two chiral centers, and two or four enantiomers [12]. In the four enantiomers of triadimenol (See Figure 1), the enantiomers with the structure of 1R & 2S and 1S & 2R have relatively higher bactericidal activity [13]. An important direction of the research on chiral pesticides is to study and use high-activity and high-optical pure pesticide enantiomers [13]. The major chiral separation analysis methods for triazole pesticides at present are HPLC [14–17] and LC-MS/MS [18–22]. HPLC features good resolution, but the analysis time is long (>20 min), and consumes a large amount of organic reagents. LC-MS/MS enjoys high accuracy, but the instruments used are expensive. Therefore, it has an extensive application prospect to develop a fast and efficient detection method. SFC has received widespread attention as a high-efficiency chromatographic separation technology. The major mobile phase of this technique is supercritical carbon dioxide (CO₂), which shows a higher mass transfer rate and separation efficiency than the traditional organic mobile phase [23]. Research shows that SFC technology is more suitable for the analysis of isomers and structural analogs which can hardly be separated by traditional LC, and has been successfully applied to separate and determine chiral compounds [1, 24–29].

To solve the problems of the above conventional LC in separating the enantiomers of triazole pesticides, a method was established to separate and determine the enantiomer residues of triadimefon and its metabolite triadimenol in the samples of complementary fruit puree for infants and

young children (banana puree, pineapple puree, and grape puree) by SFC technology in this experiment. This method is easy to operate, has a good resolution, and is green and environmental, providing references for the separation and detection of other chiral pesticides (See Table 1).

2 | MATERIALS AND METHODS

2.1 | Instruments, reagents, and standards

Acquity ultra-performance convergence chromatography (Acquity UPC²) (Waters Company, USA, equipped with photo-diode array detector), desk centrifuge (Thermo Company, USA), N-1210BV rotary evaporator (Tokyo Physicochemical Corporation, Japan), JJ500 electronic scale (G&G Company, USA), AE260 Electronic Scale (METTLER TOLEDO Company, Switzerland), WH-861 Vortex Mixer (Taicang Hualida Instrument Factory, China), Synergy185 Ultra-pure Water Purifier (Millipore Company, USA), Microbore-film Filtering Equipment (0.22 μm, organic phase); Chromatographic column CHIRALPAK AD-3(150 mm×3.0 mm, 3 μm, the coating is amylose-tri (3,5-dimethylphenyl carbamate)) (Daicel Chiral Technologies (China) Company), Chromatographic column Acquity Trefoil AMY1 (150 × 3.0 mm, 2.5 μm, the coating is amylose-tri (3,5-dimethyl phenyl carbamate)), Acquity Trefoil CEL1 (150 × 3.0 mm, 2.5 μm), cellulose-tri (3,5-dimethylphenyl carbamate), and Acquity Trefoil CEL2 (150 × 3.0 mm, 2.5 μm, the coating is cellulose-tri (3-chloro-4-methylphenyl carbamate)) (Waters Company).

Isopropanol, methylbenzene, methanol, ethyl alcohol, heptane, ACN, and formic acid (chromatographically pure, Merck Company, Germany), sodium chloride (analytically pure, Shanghai Shisi Hwei Chemical Engineering Company), hydrophile lipophile balance (HLB) cartridge (Oasis, 200 mg, 6 ml) (excitation with 6 ml methanol and 6 ml water successively before use), NH₂ cartridge (Agela, 500 mg, 6 ml), Florisil cartridge (CNW, 1 g, 6 ml), Alumina-N cartridge (CNW, 1 g, 3 ml), ultrapure water,

TABLE 1 The analytical figures of merit with other sample preparation and chromatographic separation methods.

Serial number	This article	The published literature
1	The analysis time was less than 4.5 min. The price of UPC ² was lower than LC-MS/MS. The degrees of separation of six compounds were greater than 1.5.	The analysis time was more than 25 min in the references of 12, 18 and 21. The price of LC-MS/MS was much higher than UPC ² . The chromatographic peaks of the four triadimenol enantiomers did not achieve baseline separation.
2	The analysis time of two triadimefon enantiomers was less than 2.5 min.	The analysis time of two triadimefon enantiomers was more than 10 min in the references of 14 and 19.
3	The analysis time of four triadimenol was less than 4.5 min.	The analysis time of four triadimenol enantiomers was more than 20 min in the references of 14 and 42.

other reagents used in the experiment are all analytically pure unless otherwise stated.

Standards: Triadimefon and triadimenol racemes (purity \geq 99.0%, Dr Ehrenstorfer, Germany). Triadimefon and triadimenol enantiomers: (–)-(R)-triadimefon, (+)-(S)-triadimefon, (–)-(S,R)-triadimenol, (+)-(R,S)-triadimenol, (+)-(R,R)-triadimenol, and (–)-(S,S)-triadimenol (purity \geq 95%, Shanghai Chiralway Biotech).

2.2 | Stock and preparation of working standard solutions

2.2.1 | Standard stock solution of raceme

0.01 g (accurate to 0.1 mg) standard substances of triadimefon and triadimenol racemes were precisely weighed and accurately put into a volumetric flask of 10 ml with isopropanol to prepare 1.0 g/L standard stock solution of raceme.

The standard intermediate solution of triadimefon and triadimenol racemes was as follows: these stock solutions were further diluted with heptane/isopropanol (8:2, v/v) to 100 mg/L to standard intermediate solution.

2.2.2 | Standard stock solution of enantiomer

0.01 g (accurate to 0.1 mg) standard substances of six kinds of enantiomers were precisely weighed and dissolved into a volumetric flask of 10 ml with isopropanol to prepare a 1.0 g/L standard stock solution of enantiomer.

Calibration mixed standards of six kinds of triadimefon and triadimenol enantiomers: Six kinds of triadimefon and triadimenol enantiomers calibration mixed standards were prepared at concentrations of 0.5, 1.0, 2.0, 4.0, 10.0, and 50.0 mg/L with heptane/isopropanol (8:2, v/v) before use.

2.3 | Sample preparation

Note that, a 10 g (accurate to 0.1 mg) sample was weighed and placed in a 50-ml plastic centrifuge tube with a plug, and 20 ml ACN was added for homogeneous extraction. 3.0 g sodium chloride was added, for vortex mixing, and 4,000 r/min centrifugation for 5 min. The supernatant was transferred into a concentration bottle. The remaining residues were reextracted once with 20 ml of ACN, and the extraction was repeated once, and the supernatant was combined twice, and concentrated with a rotary evaporator till it was nearly dry. The dried organic extract was reconstituted in 5 ml of ACN/methylbenzene (3:1, v/v), for purification.

The above solution to be purified was moved into the activated NH₂ cartridge, and all effluents were collected immediately after the sample loading. The elution was conducted twice with 5 ml ACN/methylbenzene (3:1, v/v). The eluate was collected and dried by nitrogen in a water bath at 40°C until nearly dry. Constant volume was prepared with 1.0 ml heptane/isopropanol (8:2, v/v), and the constant volume solution was filtered through 0.22 μ m filter membrane.

2.4 | Chromatographic conditions

The SFC analysis was performed using the Acquity Trefoil CEL2 column. The elution gradient (eluent A: CO₂, eluent B: 0.5% ammonium hydroxide-methanol) involved 5%B (initial), 5%–8%B (0.8–1.0 min), and 8%B (1.0–1.4 min), and 8%–5%B (1.4–1.8 min), and 5%B (1.8–2.1 min), and 5%–15%B (2.1–2.3 min), and 15%B (2.3–2.4 min), and 15%–5%B (2.4–2.5 min), and 5%B (2.5–2.6 min), and 5%–15%B (2.6–2.8 min), and 15%B (2.8–3.5 min), and 15%–5%B (3.5–4.0 min), and 5%B (4.0–5.0 min). The backpressure was set to 13.8 MPa. The detection wavelength was 220 nm. The

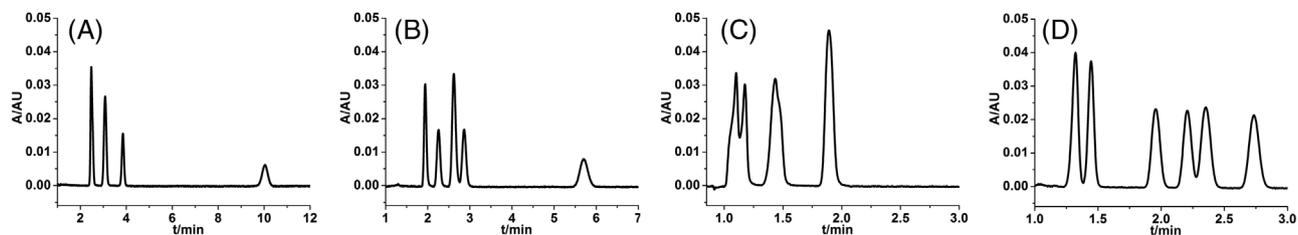


FIGURE 2 Effect of different chromatographic columns on the separation of triadimefon and triadimenol enantiomers. (A) AD-3; (B) AMY1; (C) CEL1; and (D) CEL2.

flow rate was 1.0 ml/min. The column temperature was maintained at 35°C. The injection volume was 5 μ l.

3 | RESULTS AND DISCUSSION

3.1 | Influence of chromatographic column

Chiral stationary phases based on amylose-tri (3,5-dimethylphenyl carbamate) and cellulose-tri (3,5-dimethylphenyl carbamate) are the two most widely used chromatographic stationary phases with good chiral recognition and separation ability, complementing each other in terms of chiral recognition ability [30]. In this experiment, four chiral separation chromatographic columns, namely CHIRALPAK AD-3, Acquity Trefoil AMY1, Acquity Trefoil CEL1, and Acquity Trefoil CEL2 were selected to investigate the resolution of the six triadimefon and triadimenol enantiomers. The isocratic elution condition was carbon dioxide (CO₂)-0.5% ammonium hydroxide-methanol (9:1, v/v). The results showed that the chromatographic peaks of the six triadimefon and triadimenol enantiomers partially overlapped when separated by AD-3, AMY1, and CEL1 chiral columns. In contrast, the separation factor was better when the CEL2 chiral column was used for separation (See Figure 2). Therefore, the CEL2 chiral column was chosen for the separation of the target objects in this experiment.

3.2 | Influence of cosolvent

When a small amount of cosolvents like methanol and isopropanol are added to the main mobile phase of supercritical CO₂ in the SFC system, the elution and solubilization capacity of the mobile phase can be effectively adjusted, and then the resolution and analysis speed of the target object can be precisely adjusted. Isopropanol has a relatively high viscosity, and overpressure alarms often happened to the instrument easily in the experiment process. Therefore, the influence of different cosolvents

like methanol solution containing 0.5% formic acid or 0.5% ammonium hydroxide on the resolution of the target was investigated in the experiment. The results showed that when a methanol solution containing 0.5% formic acid was used as the cosolvent, the chromatogram baseline was unstable, and no obvious chromatographic peak was observed. Comparatively speaking, when a methanol solution containing 0.5% ammonium hydroxide was used, the target compounds were completely separated, and the resolution of the last four chromatographic peaks was better (See Figure 3). The molecular structures of triazolone and triadimenol contain functional groups such as carbonyl and alcohol hydroxyl groups. A hydrogen bond was very likely to produce between these polar groups and the unbonded silanol hydroxyl groups on the surface of the column packing, thus causing chromatographic peak trailing. When 0.5% ammonia was added to the mobile phase, the analytes were deprotonated, the polarity was weakened, the analytes were eluted faster, the trailing phenomena of chromatographic peaks were inhibited and the separation efficiency was improved [31]. Therefore, a methanol solution containing 0.5% ammonium hydroxide was adopted as the cosolvent in this experiment.

3.3 | Influence of chromatographic column temperature

The main mobile phase density of supercritical CO₂ in the SFC system varied with the change in the chromatographic column temperature. When the chromatographic column temperature was increased in the experiment, the mobile phase density decreased, the elution power decreased gradually, and the retention time of the target compounds was extended. Considering that the highest recommended temperature of the Acquity Trefoil CEL2 chiral column is 40°C, the influence of chromatographic column temperatures ranging between 31 and 40°C on the peak shape and separation of the target compounds was investigated in the experiment. Results indicated that the separation factors ranges of the six triadimefon and triadimenol enantiomers were 1.28–6.00, 1.14–7.58, and 0.86–6.05, respectively, when

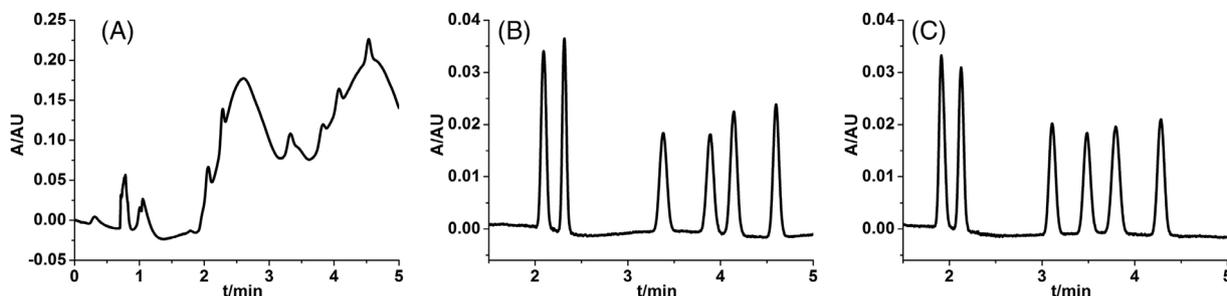


FIGURE 3 Effect of different organic solvent mobile phases on the separation of triadimefon and triadimenol enantiomers. (A) Formic acid-methanol solution(0.5:99.5,v/v); (B) Methanol; (C) Ammonium hydroxide-methanol solution(0.5:99.5,v/v).

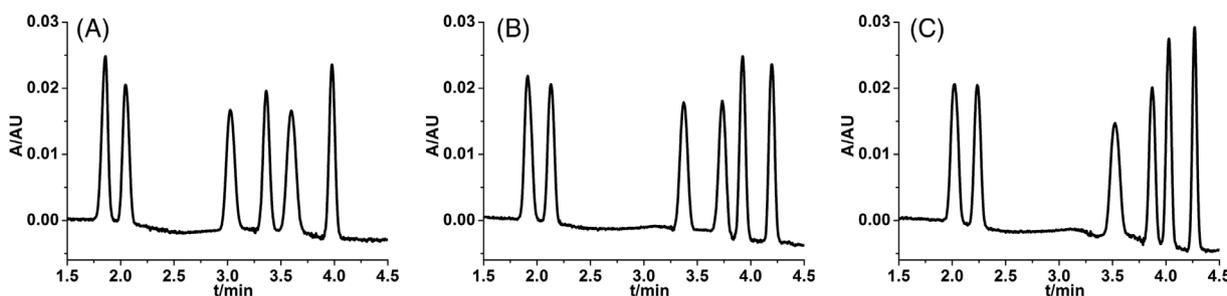


FIGURE 4 Effect of different column temperatures on the separation of triadimefon and triadimenol enantiomers. (A) 31°C; (B) 35°C; (C) 40°C.

the column temperature were 31, 35, and 40°C. The chromatographic peak shapes of the six triadimefon and triadimenol enantiomers were good when the column temperature were 35 and 40°C (See Figure 4). Considering the separation factor and chromatographic peak shape, 35°C was selected as the optimal temperature.

3.4 | Optimization of purification conditions

The experiments were conducted to compare the purification effects of different SPE cartridges like Alumina-N [32], Florisil [33], HLB [34], and NH₂ [35] on the solution extracted from the puree samples. Six kinds of triadimefon and triadimenol enantiomers standard solutions were added to the puree sample without triadimefon and triadimenol, followed by the homogeneous extraction twice with ACN. The extracts were concentrated, dried, and redissolved ACN/methylbenzene (3:1, v/v), and then purified by four different kinds of SPE cartridges, respectively.

The experimental results showed that when the NH₂ cartridge was adopted for purification, the average recovery rate of the target compound was 92.6%. NH₂ cartridge is good for the purification of samples containing some fat and fat-soluble impurities. NH₂ is a weak anion adsorption cartridge, which can separate structural isomers excellently and has a good effect on the purification of

some strong polar impurities, organic acids, pigments, and metal ions in the samples [36, 37]. The recoveries and reproducibility of the spiked samples are good after the purification with the NH₂ cartridge.

When the other three SPE cartridges (Alumina-N, Florisil, and HLB) were adopted, the recovery rate of the target compound was relatively low, and the average recovery rates were 28.6%, 15.9%, and 18.2%, respectively (See Figure 5). The impurities can be purified and removed well with a Florisil cartridge, but some pesticides with strong polarity have also been easily adsorbed at the same time [33]. HLB cartridge can effectively adsorb grease, phospholipid, fat, and pigment due to the bonding of lipophilic groups [38], but it has weak retention of the analytes, thus causing low recovery. Alumina-N cartridge can effectively adsorb pigments and flavonoids, but it has a poor adsorption effect on grease [39]. Therefore, the NH₂ cartridge was applied as the purification cartridge in this experiment.

3.5 | Method validation

3.5.1 | Linear range and LOQ

Standard working solutions of 0.5, 1.0, 2.0, 4.0, 10.0, and 50.0 mg/L in triadimefon and triadimenol enantiomer series in “2.2.2” were selected, and the chromatographic conditions optimized were adopted for determination. The

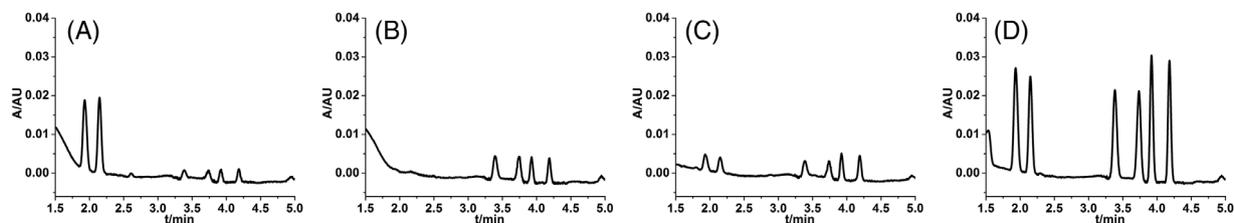


FIGURE 5 Effect of different SPE cartridges on the purification and recoveries of triadimefon and triadimenol enantiomers. (A) Alumina-N; (B) Florisil; (C) HLB; (D) NH_2 .

standard curve was drawn with the peak area as the vertical coordinate (Y) and the corresponding mass concentration of standard solution as the horizontal axis (X). The linear regression and correlation coefficients of the target compounds were calculated. The standard substances were added in the blank puree sample not containing triadimefon and triadimenol, and determination was conducted with the method optimized. The LOQs ($S/N = 10$) of six kinds of triadimefon and triadimenol enantiomers were all 0.05 mg/kg. Good linearity was presented within the linear range of 0.5 and 50 mg/L, and the correlation coefficients were all greater than 0.9993.

3.5.2 | Precision and accuracy

The recovery rate and the precision of the method were determined by adding the standard solution to the blank puree samples without triadimefon and triadimenol. The spiked levels of six enantiomers ((-)-(R)-triadimefon, (+)-(S)-triadimefon, (-)-(S,R)-triadimenol, (+)-(R,S)-triadimenol, (+)-(R,R)-triadimenol, and (-)-(S,S)-triadimenol) were 0.05, 0.2, and 3 mg/kg, respectively. The determination was conducted for six times in parallel, and the recovery rate and RSDs were calculated in the experiment. The results are shown in Table 2. The recovery rate range of the six target compounds was 80.1–106% and the RSDs ($n = 6$) range were 3.3–7.6%. The above recovery rate and precision met the requirements of SANTE/12682/2019 [40], and the analysis requirements for banana puree, pineapple puree, and grape puree samples. Hence, this method can be applied to daily analysis and detection.

3.6 | Application of method

3.6.1 | Separation of standard racemes

The standard substances of the triadimefon and triadimenol racemes purchased were separated and determined with the method established in this paper. As shown in Figure 6, the six kinds of enantiomers presented a

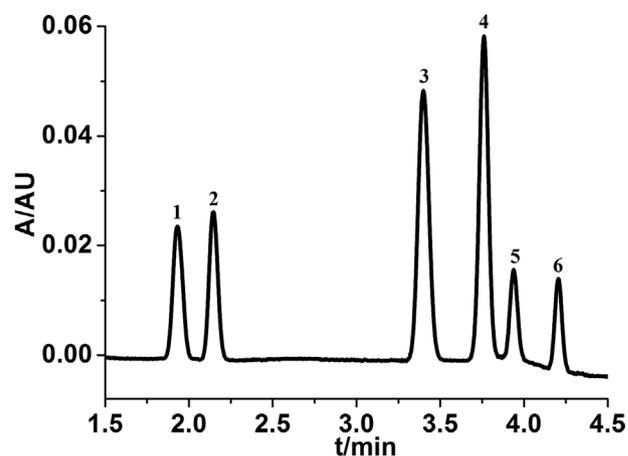


FIGURE 6 Separation of standard triadimefon and triadimenol racemes. Peak 1: (-)-(R)-Triadimefon; Peak 2: (+)-(S)-Triadimefon; Peak 3: (-)-(S,R)-Triadimenol; Peak 4: (+)-(R,S)-Triadimenol; Peak 5: (+)-(R,R)-Triadimenol; Peak 6: (-)-(S,S)-Triadimenol.

good resolution. Effective separation was realized within 4.5 min, and the degrees of separation were 1.6, 7.2, 2.5, 1.5, and 2.4, respectively, which could meet the requirement for complete separation of $R \geq 1.5$ [41]. Ranked according to the retention time of chromatographic peaks, the two triadimefon enantiomers and the four triadimenol enantiomers in order were as follows: (-)-(R)-triadimefon, (+)-(S)-triadimefon, (-)-(S,R)-triadimenol, (+)-(R,S)-triadimenol, (+)-(R,R)-triadimenol, and (-)-(S,S)-triadimenol. Based on the above standard curve, external quantitative method was applied to calculate the contents of two triadimefon enantiomers in 20.0 mg/L standard intermediate solution of triadimefon raceme and the contents of four triadimenol enantiomers in 50.0 mg/L standard intermediate solutions of triadimenol raceme. The content of (-)-(R)-triadimefon was 8.9 mg/L, the content of (+)-(S)-triadimefon was 9.3 mg/L, the content of (-)-(S,R)-triadimenol was 19.5, the content of (+)-(R,S)-triadimenol was 20.8 mg/L, the content of (+)-(R,R)-triadimenol was 4.6 mg/L, and the content of (-)-(S,S)-triadimenol was 4.3 mg/L, respectively. The content ratios of enantiomers in the triadimefon and triadimenol racemes were consistent with the proportion of

TABLE 2 Spiked recoveries and RSDs of six kinds of triadimefon and triadimenol enantiomers in banana puree, pineapple puree, and grape puree matrixes ($n = 6$).

Compound	Spiked (mg/kg)	Banana puree		Pineapple puree		Grape puree	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
(–)-(R)-Triadimefon	0.05	81.6–92.5	6.5	86.1–102	6.3	84.6–104	6.5
	0.2	87.5–99.8	6.2	81.5–98.7	5.7	85.4–101	5.8
	3.0	83.4–95.6	3.8	82.6–99.1	4.5	81.5–95.3	4.3
(+)-(S)-Triadimefon	0.05	82.6–95.1	6.6	86.1–102	6.8	85.4–104	6.4
	0.2	82.7–98.4	5.3	81.5–98.6	5.6	86.2–103	4.7
	3.0	85.4–94.5	3.8	84.6–99.1	4.3	81.4–95.3	3.3
(+)-(R,S)-Triadimenol	0.05	81.6–95.2	6.5	86.1–102	6.3	85.4–106	6.5
	0.2	83.7–98.4	5.4	81.4–98.6	5.7	86.3–101	4.9
	3.0	85.4–94.4	3.8	83.5–99.1	4.2	81.5–95.6	3.3
(–)-(S,R)-Triadimenol	0.05	84.6–104	6.2	82.3–97.6	6.8	82.4–104	7.6
	0.2	85.1–90.2	4.5	87.2–102	5.3	84.2–104	6.4
	3.0	86.1–95.6	3.5	85.1–97.3	4.2	82.5–98.3	3.8
(–)-(S,S)-Triadimenol	0.05	81.87–97.4	6.3	81.4–103	7.3	86.5–101	6.8
	0.2	82.1–99.6	5.5	82.6–99.4	6.5	85.1–99.2	5.2
	3.0	85.2–101	4.7	84.3–98.2	4.8	83.1–98.1	4.6
(+)-(R,R)-Triadimenol	0.05	81.4–97.2	6.5	81.4–105	6.4	83.7–105	7.5
	0.2	81.5–93.6	4.8	84.3–103	5.7	80.1–97.2	6.4
	3.0	85.5–95.3	3.5	83.4–98.7	4.5	83.3–95.6	4.8

enantiomers in the triadimefon and triadimenol racemes of industrial products in literature [1, 21, 42].

3.6.2 | Real samples analysis

In order to investigate the effectiveness and practicability of this method, it was applied to detect the enantiomer residues of triadimefon and its metabolite triadimenol in 30 pieces of commercially available banana puree, pineapple puree, and grape puree samples randomly selected (including 10 pieces of banana puree, 14 pieces of pineapple puree and six pieces of grape puree). Results showed that the triadimefon and triadimenol enantiomer components were detected in a banana puree sample (See Figure 7), in which, the content of (–)-(R)-triadimefon was 0.20 mg/kg, the content of (+)-(S)-triadimefon was 0.23 mg/kg, the content of (–)-(S,R)-triadimenol was 0.54 mg/kg, the content of (+)-(R,S)-triadimenol was 0.51 mg/kg, the content of (+)-(R,R)-triadimenol was 0.13 mg/kg, and the content of (–)-(S,S)-triadimenol was 0.12 mg/kg, respectively.

4 | CONCLUDING REMARKS

In this paper, the SFC method was established to separate and determine the enantiomer residues of triadimefon

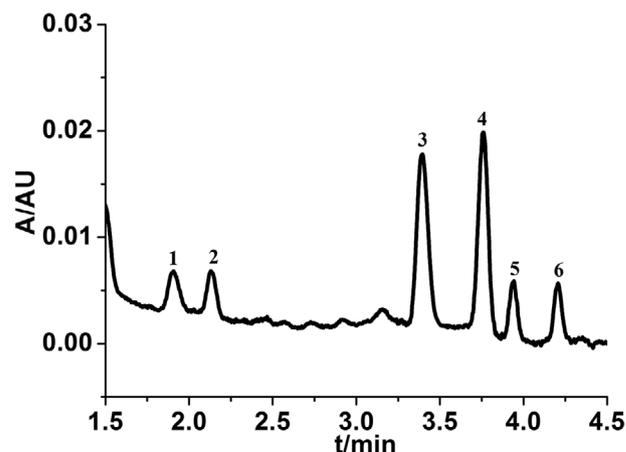


FIGURE 7 Chromatograms of the actual positive banana puree sample. Peak 1: (–)-(R)-Triadimefon; Peak 2: (+)-(S)-Triadimefon; Peak 3: (–)-(S,R)-Triadimenol; Peak 4: (+)-(R,S)-Triadimenol; Peak 5: (+)-(R,R)-Triadimenol; Peak 6: (–)-(S,S)-Triadimenol.

and its metabolite triadimenol in major complementary fruit puree for infants and young children (banana puree, pineapple puree, and grape puree). The samples were extracted with ACN, purified with an NH_2 cartridge, separated by Acquity Trefoil CEL2 chiral column, eluted with supercritical CO_2 -0.5% ammonia methanol as mobile phase gradient, and quantified by external standard

method. The LOQs were both 0.05 mg/kg, the recoveries ranged from 80.1 to 106%, and the RSD reached 3.3–7.6%. This method was used to detect the enantiomer residues of triadimefon and its metabolite triadimenol in 30 pieces of fruit puree samples purchased from the market, and the detected amount ranged from 0.12 to 0.54 mg/kg.

ACKNOWLEDGMENTS

This work was supported by the National Program on Key Research Project of China (2019YFE0103900).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- Zhang WH, Xie W, Hou JB, Chen QK, Li SM, Zhu ZL, et al. Chiral separation of six triazole pesticide enantiomers by ultraperformance convergence chromatography and residue determination in cucumber. *Chin J Chromatogr.* 2019;37:1356–62.
- Li RN. Studies on difference in bioactivity, ecotoxicity and enantioselective behavior of fluxametamide and imazalil enantiomers. Beijing: Chinese Academy of Agricultural Sciences; 2020.
- Wang Q, Huang Z, Xu MR, Kou XL, Li XR, Li T. Review of chiral separation and environmental behavior of chiral pesticides. *Gansu Agr Sci Techn.* 2020;1:67–70.
- Chang WX. Study of etoxazole stereoselective environmental behavior in orchard and toxicology effect. Beijing: Chinese Academy of Agricultural Sciences; 2020.
- Zhu MN, Li ZY, Li QL, Cao YH, Chen FN. Study on the enantioselective degradation of fenpropathrin in soil. *Jiangsu Agric Sci.* 2011;39:481–3.
- Cheng BP, Li L S, Zhou RD, Li L., Zhang HF. Enantioseparations of triazole chiral pesticides on two β -cyclodextrin-bonded stationary phases with different linkages by HPLC. *Chem J Chin Univ.* 2015;36:872–80.
- Hermawan D, CacuSuwandri, Fatoni A, Aboul-Enein HY. Application of high performance liquid chromatography method for triadimenol analysis in water sample. *J Phys Conf Ser.* 2020;1494:012032.
- Zhao CJ, Pang J, Gao WH. Analysis of three triazole fungicide residues in food by molecularly imprinted solid phase extraction-HPLC. *J Chin Insti Food Sci Technol.* 2015;3:175–80.
- Gao WH, Wang JJ. Molecularly imprinted solid-phase extraction membrane-high performance liquid chromatography for the determination of triazole fungicide content in grains, *Modern Food Sci Technol.* 2014;30:225–9.
- Shuang YZ, Zhang TC, Zhong H, Li LS. Simultaneous enantiomeric determination of multiple triazole fungicides in fruits and vegetables by chiral liquid chromatography/tandem mass spectrometry on a bridged bis(β -cyclodextrin)-bonded chiral stationary phase. *Food Chem.* 2020;345:128842.
- Zhao YF. How to use high performance liquid chromatography-tandem mass spectrometry to determine triadimefon residues in soil. *China Qual Technol Supervis.* 2019;9:64–5.
- Li YB. Studies on the analysis, environmental behaviors, and toxicity of typical chiral triazole fungicide enantiomers. Beijing: Chinese Academy of Agricultural Sciences; 2013.
- Li CY, Zhan YC, Li QL, Wang WX, Li JY. Chiral separation and enantiomerization of triazole pesticides, *Chin. J. Anal. Chem.* 2010;38:237–40.
- Zhang YC. Study on enantioselective environmental behavior of chiral triazole pesticides. Shijiazhuang: Hebei University of Science and Technology; 2010.
- Sang N, Zhou MM, Ku TT. Advanced on triazole fungicide pesticide residue analysis technology. *J Shanxi Univ.* 2021;44:125–33.
- Lv C, Zhou Z, Chiral HPLC separation and absolute configuration assignment of a series of new triazole compounds. *J Sep Sci.* 2011;34:363–70.
- Lv CG, Jia GF, Zhu WT, Qiu J, Wang XQ, Zhou ZQ. Enantiomeric resolution of new triazole compounds by high-performance liquid chromatography. *J Sep Sci.* 2007;30:344–51.
- Liang HW, Qiu J, Li L, Li W, Zhou ZQ, Liu FM, et al. Stereoselective separation and determination of triadimefon and triadimenol in wheat, straw, and soil by liquid chromatography-tandem mass spectrometry. *J Sep Sci.* 2012;35:166.
- Huang YF, Luo C, Huang JX, Tang XM, Wang F H, Wang K, Simultaneous extraction and determination of three typical chiral pesticides in soil using a QuEChERS-based method and liquid chromatography-tandem mass spectrometry. *Qual Saf Agro-Prod.* 2019;2:29–34.
- Yao ZL, Li XG, Miao YL, Lin M, Xu MF, Wang Q, et al. Simultaneous enantioselective determination of triadimefon and its metabolite triadimenol in edible vegetable oil by gel permeation chromatography and ultra performance convergence chromatography/tandem mass spectrometry. *Anal Bioanal Chem.* 2015;407:8849.
- Liu QJ, Chen W, Han P. Determination of triadimefon and its metabolites in 2 kinds of solanaceous organic vegetables by QuEChERS-ultra performance liquid chromatography-tandem mass spectrometry. *J Food Saf Qual.* 2021;12: 6457–64.
- Li Y, Dong F, Liu X, Xu J, Li J, Kong Z, et al. Enantioselective determination of triazole fungicide tebuconazole in vegetables, fruits, soil and water by chiral liquid chromatography/tandem mass spectrometry. *J Sep Sci.* 2012;35:206–15.
- Lesellier E, West C, The many faces of packed column supercritical fluid chromatography – a critical review. *J Chromatogr A.* 2015;1382:2–46.
- Jiang H, Yang L, Xing XD, Yan ML, Guo XY, Yang BY, et al. Development of an analytical method for separation of phenolic acids by ultra-performance convergence chromatography (UPC²) using a column packed with a sub-2- μ m particle. *J Pharmaceut Biomed.* 2018;153:117–25.
- Yu WS, Liu X, Zhang YZ, Lin YN, Qiu J, Kong FY. Simultaneous determination of pigments in tea by ultra-performance convergence chromatography (UPC²). *Anal Lett.* 2020;53:1654–66.
- Khater S, Canault B, Azzimani T, Bonnet P, West C, Thermodynamic enantioselective behavior of phenylthiohydantoin-amino acid derivatives in supercritical fluid chromatography on

- polysaccharide chiral stationary phases. *J Sep Sci.* 2018;41:1450–9.
27. West C, Lemasson E, Nagai K, Shibata T, Franco P, Bertin S, et al. Characterization of novel polymer-based pyridine stationary phases for supercritical fluid chromatography. *Chromatographia* 2019;82:143–52.
 28. Tang S, Liu S, Frank BP. Feasibility of supercritical fluid extraction-supercritical fluid chromatography mass spectrometry for the determination of polycyclic aromatic hydrocarbons in particulate matter samples. *J Sep Sci.* 2021;44:3717–26
 29. Yamamoto K, Nishimura T, Machida K, Kotani A, Hakamata H. Supercritical fluid chromatography with post-column addition of supporting electrolyte solution for electrochemical determination of tocopherol and tocotrienol isomers. *J Sep Sci.* 2022;45:1797–805.
 30. Okamoto Y, Yashima E. Polysaccharide derivatives for chromatographic separation of enantiomers, *Angew Chem Int Ed.* 1998;37:1020–43.
 31. Hu S, Hua ZD, Huang Y, Cheng FB, Liu Y. Fast separation and detection of fentanyl isomers by ultra performance convergence chromatography-mass spectrometry. *Chin J Anal Chem.* 2022;50:964–72.
 32. Liu J, Li CH, Zhong Y J, Xu QS. Determination of triadimefon, chlorthalonil and vinclozolin residues in vegetables by solid phase extraction-high performance liquid chromatography. *J Food Saf Qual.* 2017;8:4798–803.
 33. XU YJ. Determination of six triazole pesticides residues in vegetables by SPE-GC- μ ECD. *J Anhui Agri Sci.* 2014;42: 5813–15.
 34. Zhao SM, Meng YL, Han HL, Lei GM, Zhang ZC, Fang YL. SPE-GC/MS/MS method for determination 5 triazole pesticide residues in dendrobium officinale. *J Guangdong Pharm Univ.* 2020;36:210–14.
 35. Huan ZB, Xie DF. Simultaneous determination of triadimefon and triadimenol in four matrixes of animal origin. *Chin J Trop Crops.* 2016;37:2434–40.
 36. Ye RH, Su JF. Determination of 61 organophosphorous pesticide residues in fruits vegetables, milk, vegetable oils and animal muscles by dispersive solid-phase extraction and ultra performance liquid chromatography-tandem mass spectrometry. *Chin J Chromatogr.* 2011;29:618–23
 37. Wang J, Ai LF, Ma YS, Zhang HC, Li W, Yu M. Determination of 213 pesticide residues in milk and milk powder by gas chromatography-triple quadrupole tandem mass spectrometry. *Chin J Chromatogr.* 2015;33:1175–85
 38. Yang F, Ji Y, Liu SS, Wang Y, Fan ZY, Deng HM, et al. Determination of multiple pesticide residues in heated cigarettes by liquid chromatography tandem mass spectrometry and gas chromatography tandem mass spectrometry combined with pass-through solid phase extraction. *Acta Tabacaria Sinica.* 2021;27:1–10
 39. Li W, Wang W, Guan RF, Zhang Y, Li X, Shen GX. Determination of squalene in olive oil by solid phase extraction and ultra high performance liquid chromatography. *Food Industry.* 2021;42:303–9
 40. SANTE/12682/2019 Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed. https://www.eurl-pesticides.eu/docs/public/tmpl_article.asp? Accessed October 16, 2022
 41. Kuang LJ, Li CX, Cao SL, Li HR, Wang Z, Wu SS, Fan Y. *Baipharm. Pharmacopoeia of the People's Republic of China. Volume I.* China Medical Science and Technology Press, Beijing, 2020.
 42. Li ZY, Zhang YC, Li QL, Wang WX, Li JY. Chiral separation and enantiomerization of triazole pesticides. *Chi J Anal Chem.* 2010;38:237–42.

How to cite this article: Zhang W-H, Xu D-M, Hou J-B, He J-M, Zhao F, Shi Y-Z, Qian Y. Separation and determination of triadimefon and its metabolites triadimenol enantiomers in fruit puree by supercritical fluid chromatography. *J Sep Sci.* 2023;2200875. <https://doi.org/10.1002/jssc.202200875>