Anal Bioanal Chem (2015) 407:8849–8859
DOI 10.1007/s00216-015-9046-y

RESEARCH PAPER

Simultaneous enantioselective determination of triadimefon and its metabolite triadimenol in edible vegetable oil by gel permeation chromatography and ultraperformance convergence chromatography/tandem mass spectrometry

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Received: 8 June 2015 / Revised: 11 August 2015 / Accepted: 11 September 2015 / Published online: 28 September 2015

Abstract A novel, sensitive, and efficient enantioselective method for the determination of triadimefon and its metabolite triadimenol in edible vegetable oil, was developed by gel permeation chromatography and ultraperformance convergence chromatography/tandem triple quadrupole mass spectrometry. After the vegetable oil samples were prepared using gel permeation chromatography, the eluent was collected, evaporated, and dried with nitrogen gas. The residue was redissolved by adding methanol up to a final volume of 1 mL. The analytes of six enantiomers were analyzed on Chiralpak IA-3 column (150×4.6 mm) using compressed liquid CO_2-mixed 14 % co-solvents, comprising methanol/acetonitrile/isopropanol=20/20/60 (*v*/*v*/*v*) in the mobile phase at 30 °C, and the total separation time was less than 4 min at a flow rate of 2 mL/min. Quantification was achieved using matrix-matched standard calibration curves. The overall mean recoveries for six enantiomers from vegetable oil were 90.1–97.3 %, with relative standard deviations of 0.8–5.4 % intra-day and 2.3–5.0 % inter-day at 0.5, 5, and 50 μg/kg levels. The limits of quantification were 0.5 μg/kg for all enantiomers based on five replicate extractions at the lowest fortified level in vegetable oil. Moreover, the absolute configuration of six enantiomers had been determined based on comparisons of the vibrational circular dichroism experimental spectra with the theoretical curve obtained by density functional theory calculations. Application of the proposed method to the 40 authentic vegetable oil samples from local markets suggests its potential use in enantioselective determination of triadimefon and triadimenol enantiomers.

Keywords UPC^2-MS/MS · Absolute configuration · Stereoisomeric separation · Triadimenol · Triadimefon · Gel permeation chromatography

Electronic supplementary material The online version of this article (doi:10.1007/s00216-015-9046-y) contains supplementary material, which is available to authorized users.

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Abbreviations

AC	Absolute configuration
CSP	Chiral stationary phase
DFT	Density functional theory
GPC	Gel permeation chromatography
MRL	Maximum residue limit
RSD	Relative standard deviation
SFC	Supercritical fluid chromatography
SSE	Slope matrix-matched calibration/slope standard calibration in solvent
VCD	Vibrational circular dichroism

Introduction

The edible vegetable oil is rich in saturated and unsaturated fatty acids, triglycerides, antioxidants such as tocopherols, and other fat-soluble vitamins [1], which is one of the important ingredients used in the preparation of our food in daily diet. Pesticides are widely used to control pest damage to crops and raise production. For oil crops, they may accumulate into the oil seeds easily and consequently exist in the oils with high stability because of their lipophilicity. Triadimefon and triadimenol contamination in edible oils [2] showed that they have clear teratogenic effects on the bronchial arches and cranial nerves of rat embryos [3] and also harmful to mammalian central nervous systems and are neurotoxins in rats, mice, and rabbits [4, 5].

Triadimefon (TF) [(*RS*)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-one] and its metabolite triadimenol (TN) [(1*RS*,2*RS*,1*RS*,2*SR*)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol] which has greater fungicidal activity than triadimefon [6] are two registered broad-spectrum systemic fungicide belonging to the family of the triazole pesticides [7]. Triadimefon which has a single chiral center and correspondingly present an equimolecular mixture of the two enantiomers [8] could be enzymatically reduced to triadimenol with four stereoisomers including two pairs of diastereomers [9]. Figure 1 shows their chemical structures. Each stereoisomer of triadimenol could be produced from triadimefon in different relative amounts of soil [8, 10], plants [11], rainbow trout [9, 12], and fungi [13]. Chiral enantiomers have identical physical and chemical properties [14], but they may differ dramatically from their biological efficacy, toxicity, and environmental fate when they interact with other chiral molecules, such as enzymes and biological receptors [15]. In most cases, only one of the pesticide isomers is active, and the other isomer may have less or no activity or may exert toxic effects on nontarget organisms [16]. For example, (1*S*,2*R*)-isomer shows the highest fungicidal activity (up to 1000-fold more active than the other three) in four stereoisomers of triadimenol [17]. Since triadimefon and its metabolite triadimenol used as racemic mixture, it is important to develop a rapid and efficient method to simultaneous separate and determine the enantiomers in edible vegetable oils, in order to obtain a better understanding of the potential human health hazard and the assessment of dietary safety.

In recent years, a few achiral methods have been developed for the detection of triadimefon and triadimenol in olive oil by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [2, 18, 19], with the clean up based on modified QuEChERS and the Florisil SPE cartridge [18]. Meanwhile, some enantiomeric separation methods have been accomplished using different chromatographic techniques, including GC [20], HPLC [21, 22] in water [23] and in cucumber plants [11], supercritical fluid chromatography (SFC) [24, 25], liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) in wheat, straw, and soil [6, 26], HPLC-MS/MS in Chinese lizards [27], and ultraperformance convergence chromatography/tandem mass spectrometry (UPLC-MS/MS) in *Daphnia magna* [28]. However, to the best of our knowledge, there is not any report on simultaneous enantioselective determination of triadimefon and triadimenol in edible vegetable oil.

The development of methods to extract fat-soluble compounds from the whole fatty matrix (such as edible vegetable oils) is a challenging issue, because it is difficult to avoid the co-extraction of fatty material, which is harmful for columns and detectors, even in small amounts. This study developed a method of the detection of triadimefon and triadimenol in edible vegetable oil using gel permeation chromatography (GPC); GPC was applied in order to try to separate target components on the basis of molecular size or molecular weight, and it has been used to purify edible oil samples of the detection of other residues previously [29, 30]. The molecular weight of triadimefon and triadimenol is 293.8–295.8 Da, which is much smaller than main matrix in oil such as triacylglycerides and diacyl glycerides. In 2013, a new SFC apparatus as Waters ultraperformance convergence chromatography UPC^2, opened a new possible dimension of analytical instrumentation. UPC^2 is an excellent complement to MS spectrometry because it combines the advantages of SFC and ultraperformance liquid chromatography (UPLC) technology [31]. The use of supercritical CO_2 as the mobile phase could achieve a higher diffusivity and solubility, which makes a short analysis time, good selectivity, and high degree of separation. In addition, the reduction of the amount of organic solvent could reduce the cost and toxicity, which conforms to the idea of green chemistry [32–35]. Chen [36] reported the separation of chiral neonicotinoid sulfoxaflor in vegetables and soil by UPC^2-MS/MS at less than 6.5 min.

In this study, we first report the efficient simultaneous enantioselective analysis method of triadimefon and its metabolite triadimenol in edible vegetable oil by ultraperformance convergence chromatography/tandem mass spectrometry, combined with a simple GPC extraction technique. A systematic discussion on how to improve the stereoselectivity of the stereoisomers by varying the chiral stationary phases (CSPs) and co-solvents provided. As the determination of the absolute configuration (AC) of chiral molecules is an important aspect of chiral analysis and the rapid development of computerized density functional theory (DFT), the AC of the triadimefon and triadimenol enantiomers was determined according to a comparison of experimental and predicted vibrational circular dichroism (VCD). The method was evaluated in terms of linearity, matrix effect, recovery, precision, and limit of detection (LOD)

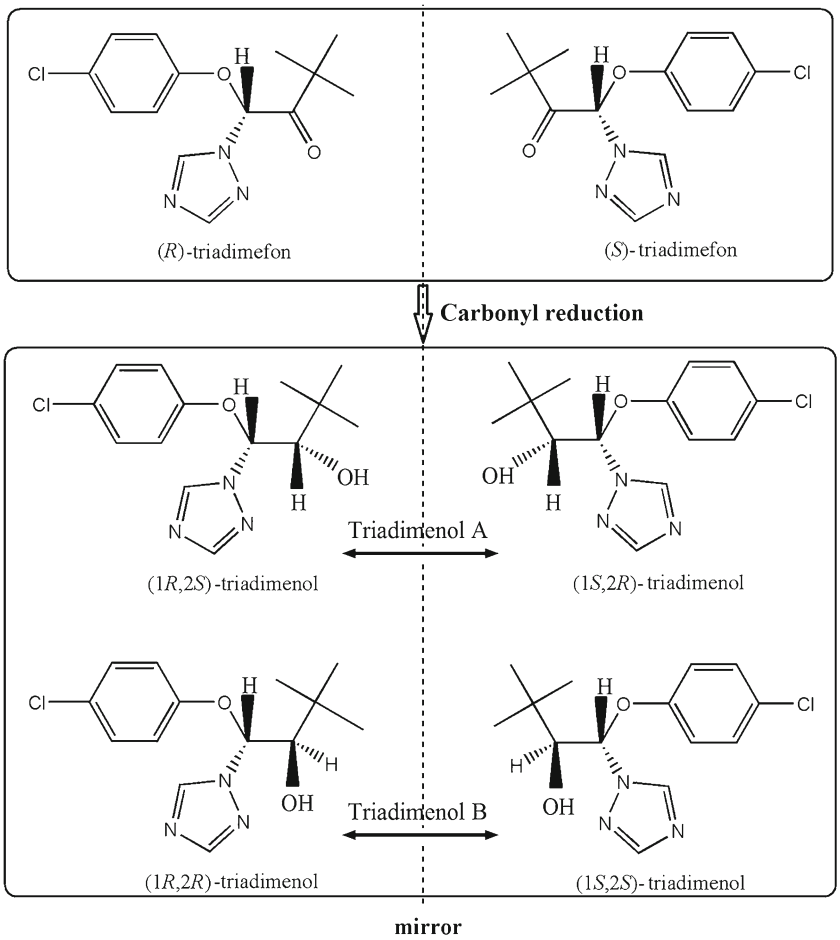

Fig. 1 Chemical structures of triadimefon and its metabolite triadimenol enantiomers

and limit of quantification (LOQ). This developed method of triadimefon and triadimenol enantiomers applied to 40 vegetable oil samples from local markets.

Materials and methods

Chemicals and reagents

Reference standards of racemic triadimefon (99.8 % purity), triadimenol (99.4 % purity), triadimenol isomer A (racemate of RS enantiomer and SR enantiomer, 99.9 % purity), and triadimenol isomer B (racemate of RR enantiomer and SS enantiomer, 100 mg/mL in acetonitrile) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock standard (approximately 100 mg/L) was prepared by dissolving the reference compound in acetonitrile. Working standard solutions were prepared by serial dilution of the stock standard. Stock standards (approximately 100 mg/L) of each enantiomer were prepared in acetonitrile and were used to obtain VCD spectra. All solutions kept in the dark and stored at −20 °C. The working standard solutions underwent no degradation for 3 months.

HPLC-grade methanol (CH_3OH), acetonitrile (CH_3CN), and isopropanol ($(CH_3)_2CHOH$) were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared by using Milli-Q water purification system (Millipore Corporation, Billerica, USA). HPLC-grade ethyl acetate and cyclohexane were obtained from Tedia (Fairfield, USA). All other chemicals were of analytical reagent grade and obtained from commercial sources.

Instrumentation and UPC^2-MS/MS conditions

Thar SD-ASFC-2 SFC system from Thar Technologies (Pittsburgh, PA, USA) equipped with a Gilson UV/VIS-151 detector (Middleton, WI, USA) and a Rheodyne 7410 injector with a 20-μL loop volume (Cotati, CA, USA) was used for optimizing the condition of chiral separation and preparing the six enantiomers. The system was controlled by the Thar Instruments Superchrom software. The method of simultaneous enantioselective determination of triadimefon and triadimenol was performed on a Waters ACQUITY UPC^2 system (Milford, MA, USA) with binary solvent manager, sample manager, column manager, and convergence chromatography manager. All data collected in centroid mode was acquired

and processed using MassLynx™ NT 4.1 software with QuanLynx™ program (Waters Corp., Milford, MA, USA).

Chromatographic separation and preparation of two triadimefon enantiomers and four triadimenol stereoisomers were performed on three chiral columns purchased from Daicel Chemical Industries (Tokyo, Japan), including Chiralpak IA-3 column (150×4.6 mm), packed with amylose *tris*-(3,5-dimethylphenylcarbamate) and immobilized on a 3-μm silica-gel support, Chiralpak AD-H column (250×4.6 mm), packed with amylose *tris*-(3,5-dimethylphenylcarbamate) and coated on a 3 μm silica-gel support, and Chiralcel OD-H column (250×4.6 mm), packed with cellulose *tris*-(3,5-dimethylphenylcarbamate), coated on a 3-μm silica-gel support. Chiralpak AD-H and Chiralcel OD-H were used to optimize the condition of separation and preparation of the stereoisomers of triadimefon and triadimenol.

Compressed liquid CO_2 was used as the primary mobile phase throughout the UPC^2-MS/MS procedure. Three co-solvents (methanol, acetonitrile, and isopropanol) were evaluated individually in terms of their ability to achieve optimum separation of the triadimefon and triadimenol stereoisomers. The flow rate of the CO_2-based mobile phase containing mixed co-solvents was 2 mL/min, while methanol was used as the compensation solvent at a flow rate of 0.18 mL/min. The backpressure of the system was 2200 psi and column temperature was 30 °C. The auto-sampler was conditioned at 4 °C, and the sample volume injected was 2.0 μL in each run.

A triple quadrupole Xevo®-TQ-S mass spectrometer (Waters Inc.) equipped with an electrospray ionization source (ESI) was used to quantify the triadimefon and triadimenol stereoisomers. ESI^+ was selected for subsequent experiments because this mode yields higher signal to noise ratios (S/N) than ESI^-. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 294.1→69.3 for triadimefon and *m/z* 296.0→70.0 for triadimenol, respectively. The optimal MS parameters were as follows: source and desolvation temperatures of 150 and 500 °C, respectively; the nebulizer gas was 99.999 % N_2, and the collision gas was 99.999 % Ar (pressure, 2×10^{-3} mbar) in the T-wave cell; cone and desolvation N_2 flows of 150 and 800 L/h were applied; and capillary and cone voltage were 3.20 kV and 40.00 V, respectively.

VCD and IR measurements of triadimefon and its metabolite triadimenol enantiomers were performed on a BioTools ChiralIR-2X FT-VCD spectrometer, equipped with a single photoelastic modulation and a mercury cadmium tellurium detector. Twelve milligrams of each sample was dissolved in 150 μL $CDCl_3$ and placed in a BaF_2 cell with a pathlength of 75 μm. Data were acquired at a resolution of 4 cm^{-1} for 3 h.

Sample preparation

GPC (LC-Tech GPC Vario, Dorfen, Germany) equipped with an auto-sampler, a solvent delivery module, and a fraction collector was used for the analyses (see Electronic supplementary material (ESM) Fig. S1). The standard sample loop was made of polytetrafluoroethene and set to 5 mL. A common glass column (500×25 mm), packed with 50 g of 200–400 mesh Bio-Breads S-X3 resin (Bio-Rad Laboratories GmbH, München, Germany) was used. The mobile phase was ethyl acetate-cyclohexane solution (1:1, *v*/*v*) at a flow rate of 5 mL/min.

Two grams of blank vegetable oil samples were weighed into a 50-mL polypropylene centrifuge tube and spiked with three different concentrations of triadimefon and triadimenol standard solutions. Allowed to stand for 2 h at room temperature to distribute the pesticide evenly and to ensure complete interaction with the sample matrix, then diluted to 10 mL with an ethyl acetate-cyclohexane solution (1:1, *v*/*v*), shaken vigorously and vortex mixed at 2000 rpm for 1 min. Aliquots (5 mL) diluted oil samples injected into the GPC column, and the GPC mobile phase was ethyl acetate-cyclohexane solution (1:1, *v*/*v*). The effective eluent was collected in a 110-mL glass tube and evaporated to near dryness with a vacuum rotary evaporator at 40 °C, and drying was completed under a nitrogen stream. The residue was redissolved by adding methanol up to a final volume of 1 mL. Finally, the solution was vortex mixed for 1 min and filtered through 0.22 μm Teflon filter for UPC^2-MS/MS analysis.

Method validation

Validation of the method for determination of triadimefon and triadimenol enantiomers in samples was composed of the following parameters: testing linearity, linear range, LOD and LOQ, matrix effect, accuracy, and precision.

A series of standard solutions of triadimefon and triadimenol with 1, 2, 5, 20, 50, 100, and 200 μg/L of each enantiomer were employed as working standards for construction of calibration curves. Blank vegetable oil matrices were made according to the "sample preparation" procedure described, and a series of matrix-matched calibration standards with the same concentrations were also prepared. The calibration curves were obtained by plotting analyte concentrations against peak areas of quantification ion transition with regression analysis of both standard solution and matrix-matched calibration curves. The slope ratios of the linear calibration functions were calculated to differentiate between the extraction efficiency and the matrix-induced signal suppression/enhancement, and the slope matrix-matched calibration/slope standard calibration in solvent (SSE) caused by matrix effects was determined.

The matrix-dependent LOD and LOQ of the method were determined using the blank and calibration standards of the matrices. The LOD was calculated as three times of the signal-to-noise ratio of the quantifier ion transition by the analyses of spiked sample at low concentration levels with five replicate

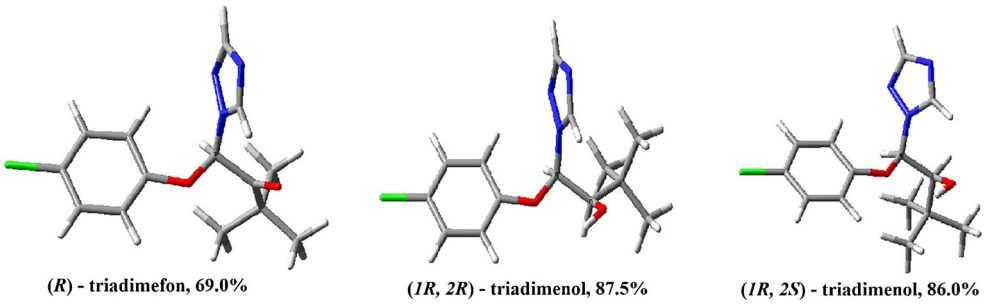

Fig. 2 The most stable conformers for (*R*)-triadimefon, (1*R*,2*R*)-triadimenol, and (1*R*,2*S*)-triadimenol

extractions, whereas the LOQ was defined as the lowest spiking level of each enantiomer on acceptable recovery [37].

The recovery assays were carried out to investigate the accuracy and precision of the method. The recoveries of triadimefon and triadimenol were measured in blank samples that were fortified at three different concentration levels (0.5, 5, and 50 μg/kg for each enantiomer based on five replicates). The samples were left for 1 h to ensure that the spiked pesticides were evenly distributed. The fortified samples were analyzed and the recoveries were calculated by comparing the measured concentration to the fortified concentrations. The precision of the method was determined by the intra-day repeatability and inter-day reproducibility studies and expressed by relative standard deviation (RSD). Inter-day reproducibility was evaluated over 3 days.

Results and discussion

The absolute configuration of triadimefon and triadimenol enantiomer

The assignment of absolute configuration with VCD method is based on comparisons of the experimental spectra with the theoretical curve obtained by DFT calculations. Firstly, molecular models of (*R*)-triadimefon, (1*R*,2*R*)-triadimenol, and (1*R*,2*S*)-triadimenol were built. Monte Carlo conformational searching at the molecular mechanic force field MMFF94 level was then performed using Compute VOA (BioTools Inc., Jupiter, FL, USA). Within a 5-kcal/mol window, five energetically distinct conformers for (*R*)-triadimefon, twenty-one for (1*R*,2*R*)-triadimenol, and eleven for (1*R*,2*S*)-triadimenol were predicted. Geometry optimizations and frequency calculations of these conformers were carried out at the B3LYP/6-31G (d) level of theory using Gaussian 09 (Gaussian Inc., Wallingford, CT, USA). Fractional Boltzmann distributions calculated using relative energies revealed that two conformers for (*R*)-triadimefon, three conformers for both (1*R*,2*R*)-triadimenol, and (1*R*,2*S*)-triadimenol were significantly populated at 298 K. The most stable conformers are shown in Fig. 2. Boltzmann-population-weighted composite VCD and IR spectra were then generated by Compute VOA.

As shown in Figs. S2 and S3 in the ESM, 1-1 and 1-3 are two enantiomers of triadimefon. Their IR curves are exactly the same, and their VCD spectra are mirror images. The excellent agreement between the corresponding spectra of 1-1 and (*R*)-triadimefon leads to an unambiguous assignment of its AC as (*R*). Quantitative evaluation of this assignment was achieved by Compare VOA (BioTools Inc., Jupiter, FL, USA). The related results, including the optimal anharmonicity factor (anH) applied to the calculated frequencies, spectral similarities, and enantiomeric similarity index (the difference between the VCD spectral similarity of the correct and the incorrect enantiomers, ESI) are listed in Table 1. Based on the current Compare VOA database, the confidence level of the (*R*)-triadimefon assignment for 1-1 is 100 %. The AC of 1-3 is therefore assigned as (*S*)-triadimefon. Accordingly, peaks 1′ and 2′ of the chromatograms shown in Fig. 3 were assigned to (*R*)-triadimefon and (*S*)-triadimefon, respectively.

Figures S4, S5, S6, and S7 in the ESM show the corresponding observed and calculated spectra of four triadimenol stereoisomers. 2-1 and 2-3 (3-1 and 3-3) were deduced to be enantiomers from their IR curve similarity and VCD mirror symmetry. Then, the experimental spectra of 2-1 (or 3-3) was compared with the calculated spectra of both (1*R*,2*R*)-triadimenol and (1*R*,2*S*)-triadimenol. A better agreement is achieved between the VCD spectra of 2-1 and (1*R*,2*S*)-triadimenol, particularly over the range of 1150–1250 cm^{-1}, making the assignment of its AC as (1*R*,2*S*)-triadimenol. 3-3 is determined to have (1*R*,2*R*)-triadimenol configuration. Compare VOA evaluations of the two assignments are given in Table 1, with 99 % confidence levels. Consequently, 3-1 is

Table 1 Evaluations for the AC assignments of 1-1, 2-1, and 2-4

Name	anH	S_{IR}	S_E	S_{-E}	ESI
1-1	0.968	86.8	83.4	2.8	80.6
2-1	0.967	88.3	78.1	6.3	71.8
2-4	0.968	87.4	74.3	5.8	68.5

S_{IR} total neighborhood similarity for IR spectra, S_E VCD spectral neighborhood similarity for the correct enantiomer, S_{-E} VCD spectral neighborhood similarity for the incorrect enantiomer, *ESI* Enantiomeric similarity index

assigned as (1*S,2R*)-triadimenol and 2-3 as (1*S,2S*)-triadimenol. Accordingly, peaks 1, 2, 3, and 4 of the chromatograms shown in Fig. 3 were assigned to (1*R,2S*)-triadimenol, (1*S,2S*)-triadimenol, (1*S,2R*)-triadimenol, and (1*R,2R*)-triadimenol, respectively.

Effect of column and mobile phase

The CSP is a crucial factor for enantiomeric separation which was investigated in our work. Thar SD-ASFC-2 SFC system with a nonpolar mobile phase such as CO_2 was used for optimizing the condition of chiral separation and preparing the triadimefon and triadimenol enantiomers in earlier stage. The wavelength was 230 nm. When separating polar compounds, a little organic solvent can be added to enhance its eluting power, selectivity, and speed of separation. Enantioseparation of triadimefon and triadimenol under CO_2-based mobile phase with methanol, acetonitrile, and isopropanol were studied.

Two different chiral columns (Chiralpak AD-H and Chiralcel OD-H) were selected to systematically explore the influence of the chiral stationary phase on the enantioseparation. The mobile phase is an essential component that is substantially involved in the chiral selector-select and association mechanism at multiple levels, because it defines the properties of the interaction environment [38, 39]. Compressed liquid CO_2 was used as the primary mobile phase throughout the SFC procedure. The initial mobile phase condition was set at 80 % pure CO_2 and 20 % (*v*/*v*) modifier (methanol or acetonitrile or isopropanol) with a flow rate of 2 mL/min and the column temperature at 30 °C. If enantioseparation was unsatisfactory, optimization steps by changing the mobile phase compositions with the percentage of modifier ranged from 20 to 5 % (*v*/*v*). Figure 3 shows the separation results on two chiral columns. Six enantiomers with the mobile phase of modifier at 3 % methanol or 5 % isopropanol on Chiralcel OD-H or 10 % acetonitrile on Chiralpak AD-H could insufficiently be baseline separated, while 7 % methanol or 5 % isopropanol on Chiralpak AD-H or 5 % acetonitrile on Chiralcel OD-H could be entirely separated with a long retention time (more than 30 min).

Considering that single modifiers were incapable of achieving satisfactory resolution of the triadimefon and triadimenol enantiomers, mixed modifiers was introduced into the system. The mixture of methanol, acetonitrile, and isopropanol facilitated the best stereoisomeric resolution of the six stereoisomers. According to the result of single modifiers, co-solvent ratio of modifiers was changed. Mixed 15 % co-solvents comprising methanol/acetonitrile/isopropanol= 20/20/60 (*v*/*v*/*v*) in the mobile phase and a flow rate of 2 mL/min on Chiralpak AD-H column could be entirely separated at less than 11 min, which satisfied the requirement of separation (Fig. 3g).

Chiralpak IA-3 column (150×4.6 mm), packed with amylose *tris*-(3,5-dimethylphenylcarbamate), was the same stuffing as the Chiralpak AD-H column (250×4.6 mm). Chiralpak IA-3 column was used for determination of triadimefon and triadimenol enantiomers on a Waters ACQUITY UPC^2 system according to the separation conditions on Thar SD-ASFC-2 SFC system. After a bit optimization, mixed 14 % co-solvents which comprised methanol/acetonitrile/isopropanol=20/20/60 (*v*/*v*/*v*) in the mobile phase and a flow rate of 2 mL/min on Chiralpak IA-3 by UPC^2-MS/MS could be more suitable for 150 mm column. The time of separation of all six enantiomers was less than 4 min (Fig. 4). Compared with Liang's [26], simultaneous determination of triadimefon and triadimenol stereoisomers in wheat, straw, and soil by liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) and the separation time of one injection was 45 min; UPC^2-MS/MS greatly shortened the detection time. Accordingly, the eluted order of the six enantiomers on Chiralpak IA-3 was (*S*)-triadimefon, (*R*)-triadimefon, (1*R,2S*)-triadimenol, (1*S,2S*)-triadimenol, (1*S,2R*)-triadimenol, and (1*R,2R*)-triadimenol.

Method validation

To obtain better recoveries and improve the purification efficiency, 5 mL of an ethyl acetate-cyclohexane solution (1:1, *v*/*v*) containing 5 μg of each triadimefon and triadimenol enantiomers was injected to the GPC. For the first 10 min, the eluent was discarded, but for the subsequent 20 min, the eluent was collected every 1 min. Each fraction was evaporated at reduced pressure and dried under nitrogen. The fractions from 16 to 22 min were collected to ensure that recovery for the six enantiomers was >95 %. The procedure, where fractions during the first 15 min were discarded and the fractions in the subsequent 7 min were collected, was applied in all the experiments.

Validation of the proposed method included matrix effect, linearity, recovery, and repeatability. It is well known that the presence of matrix components can affect the ionization of the target compounds when ESI is used. Assessment of matrix effects in MS detection is mandatory especially if no isotopically labeled internal standards are available that could, to a great extent, compensate for ionization suppression or enhancement processes [40]. Therefore, in the current study, the matrix effect on the MS/MS (MRM mode) detector was calculated by comparing the slope of matrix-matched standard curve with the slope of the standard calibration curve [41], and Table 2 shows the slope ratios of matrix-matched to solvent-based calibration. In general, there were signal suppressions for the six enantiomers in vegetable oil, as the slope ratios of matrix-matched to solvent-based calibration were in the range of 0.930–0.983. As a result, external matrix-matched

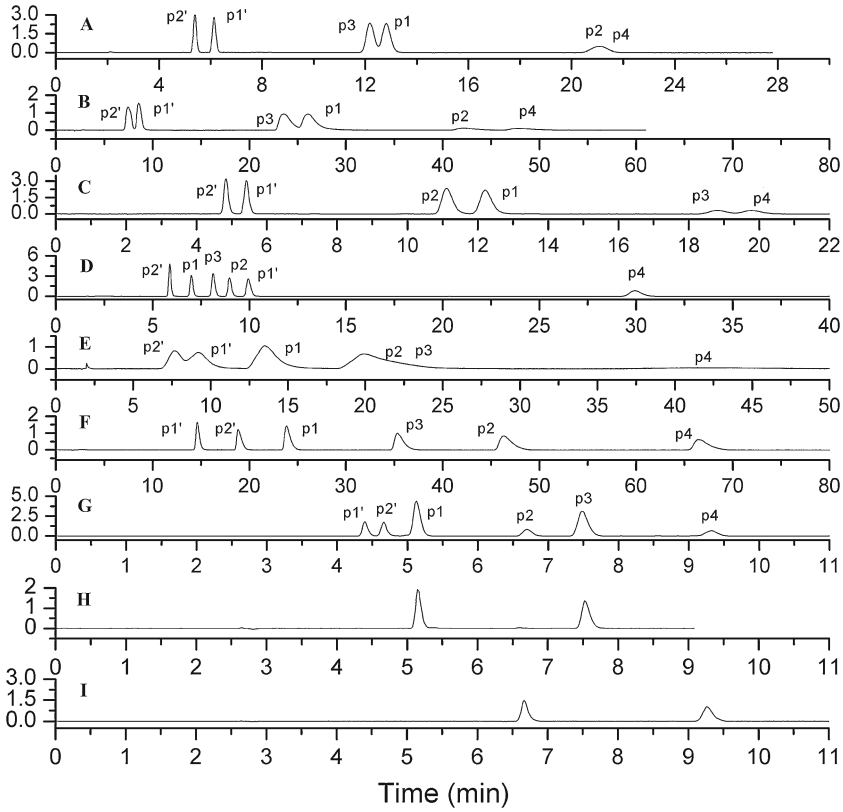

Fig. 3 Typical chromatograms of triadimefon and triadimenol on two chiral columns with a flow rate of 2 mL/min and the column temperature at 30 °C. Separate conditions were **a** 3 % methanol in CO_2 on Chiralcel OD-H, **b** 5 % acetonitril in CO_2 on Chiralcel OD-H, **c** 5 % isopropanol in CO_2 on Chiralcel OD-H, **d** 7 % methanol in CO_2 on Chiralpak AD-H, **e** 10 % acetonitril in CO_2 on Chiralpak AD-H, **f** 5 % isopropanol in CO_2 on Chiralpak AD-H, and **g** mixed 15 % methanol/acetonitrile/isopropanol (2/2/6, *v*/*v*/*v*) as co-solvents in CO_2 on Chiralpak AD-H. **h**, **i** the typical chromatograms of triadimenol isomer A and triadimenol isomer B with the same separate condition of (**g**), respectively

calibration standards were utilized to eliminate the matrix effect and to obtain more accurate determination in oil samples.

Good linear regression results of standard solutions and matrix-matched calibration curves within the range of 1–200 μg/L among the six stereoisomers of triadimefon and triadimenol are summarized in Table 2 and ESM Fig. S8. This indicates the linear equations and coefficients of determination (R^2) of both matrix-matched and standard solutions. Excellent

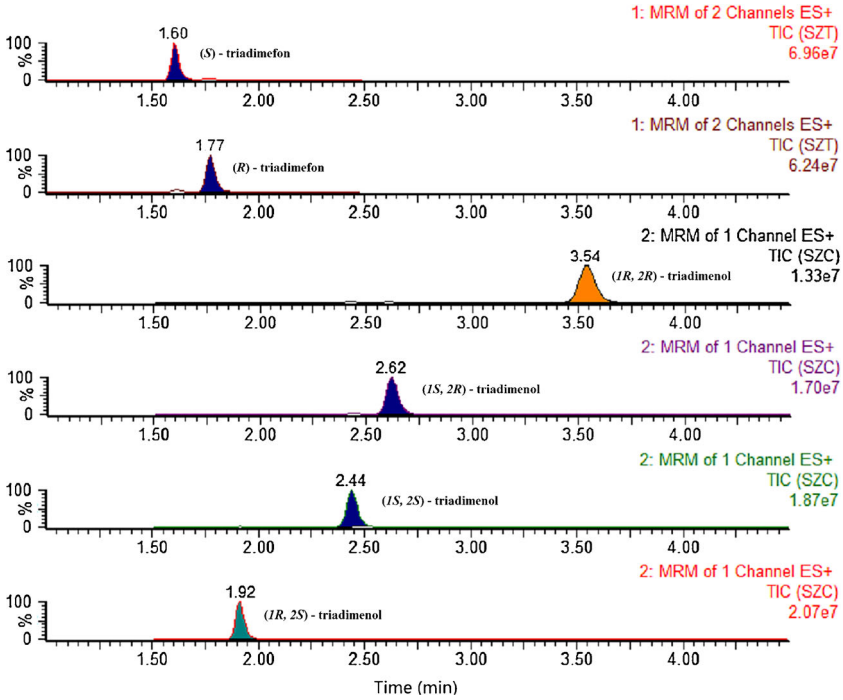

Fig. 4 Typical UPC^2-MS/MS chromatograms of triadimefon and triadimenol, using 14 % methanol/acetonitrile/isopropanol (2/2/6, *v*/*v*/*v*) as co-solvents in CO_2 on Chiralpak IA-3 column

Table 2 Comparison of matrix-matched calibration and solvent calibration (1–200 μg/L)

Enantiomers	Matrix	Standard linear equation	R^2	SSE	LOD (μg/kg)	LOQ (μg/kg)
(*R*)-(−)-triadimefon	Methanol	$y=4213x-145$	0.9999		0.1	0.5
	Vegetable oil	$y=4071x-161$	0.9997	0.966	0.1	0.5
(*S*)-(+)-triadimefon	Methanol	$y=4229x-198$	0.9998		0.1	0.5
	Vegetable oil	$y=4146x-219$	0.9997	0.98	0.1	0.5
(1*R*,2*S*)-(+)-triadimenol	Methanol	$y=4268x-157$	0.9997		0.1	0.5
	Vegetable oil	$y=3985x-336$	0.9992	0.934	0.1	0.5
(1*S*,2*R*)-(−)-triadimenol	Methanol	$y=4293x-238$	0.9996		0.1	0.5
	Vegetable oil	$y=4193x-449$	0.9991	0.977	0.1	0.5
(1*R*,2*R*)-(+)-triadimenol	Methanol	$y=4934x-276$	0.9997		0.1	0.5
	Vegetable oil	$y=4849x-374$	0.9994	0.983	0.1	0.5
(1*S*,2*S*)-(−)-triadimenol	Methanol	$y=4867x-118$	0.9998		0.1	0.5
	Vegetable oil	$y=4524x-109$	0.9993	0.93	0.1	0.5

R^2 coefficients of determination, *SSE* slope matrix-matched calibration/slope standard calibration in solvent

linearities were observed for the enantiomers ($R^2 \geq 0.9991$), which were adequate for enantiomeric-specific quantitative trace analysis.

As shown in Table 2, the LODs for both triadimefon and triadimenol enantiomers were estimated to be 0.1 μg/kg in vegetable oil. The LOQs were established as being 0.5 μg/kg of each enantiomer based on five replicate extractions at the lowest fortified level in vegetable oil. China has established the maximum residue limit (MRL) for triadimefon and triadimenol (GB 2763-2014) which were 200 μg/kg for rapeseed and 100 μg/kg for maize, respectively. This sensitivity is far better than the monitoring requirements.

The analytical precision and accuracy were evaluated by spiking the blank vegetable oil samples with triadimefon and triadimenol enantiomers at three concentration levels (0.5, 5, and 50 μg/kg) in five replications. The process was repeated

Table 3 Accuracy and precision of the proposed method in vegetable oil

Enantiomers	Spiked level (μg/kg)	Intra-day ($n=5$)						Inter-day ($n=15$)
		Day 1		Day 2		Day 3		
		Average recoveries (%)	RSD (%)	Average recoveries (%)	RSD (%)	Average recoveries (%)	RSD (%)	RSD (%)
(*R*)-(−)-triadimefon	0.5	94.3	3.5	93.1	3.1	90.1	3.2	3.6
	5	93.4	3.3	90.7	4.7	92.8	3.8	3.1
	50	91.5	3.3	92.8	3.9	94.8	2.4	2.9
(*S*)-(+)-triadimefon	0.5	93.1	2.8	96.8	2.1	91.5	5.4	3.7
	5	91.2	4.1	91.8	5.3	93.4	4.7	5.0
	50	95.7	2.1	92.9	3.4	91.6	3.2	3.4
(1*R*,2*S*)-(+)-triadimenol	0.5	97.3	3.7	92.3	4.5	94.0	5.2	4.9
	5	91.2	2.7	90.5	4.1	92.8	3.1	3.3
	50	93.7	2.9	94.1	3.6	96.8	3.6	3.1
(1*S*,2*R*)-(−)-triadimenol	0.5	93.2	3.1	94.8	3.2	90.5	2.0	2.9
	5	90.6	3.6	91.5	3.8	91.2	2.8	3.5
	50	92.2	3.7	93.4	2.4	93.1	1.9	3.2
(1*R*,2*R*)-(+)-triadimenol	0.5	92.2	2.9	91.6	2.4	90.7	2.1	2.9
	5	94.5	0.8	94.0	1.7	92.8	2.3	2.3
	50	95.7	4.1	92.4	2.6	93.1	1.9	2.8
(1*S*,2*S*)-(−)-triadimenol	0.5	93.2	1.9	93.4	3.7	91.8	4.3	2.9
	5	93.4	2.2	90.1	3.7	92.9	4.1	3.1
	50	94.3	2.8	92.8	3.6	92.3	3.7	3.2

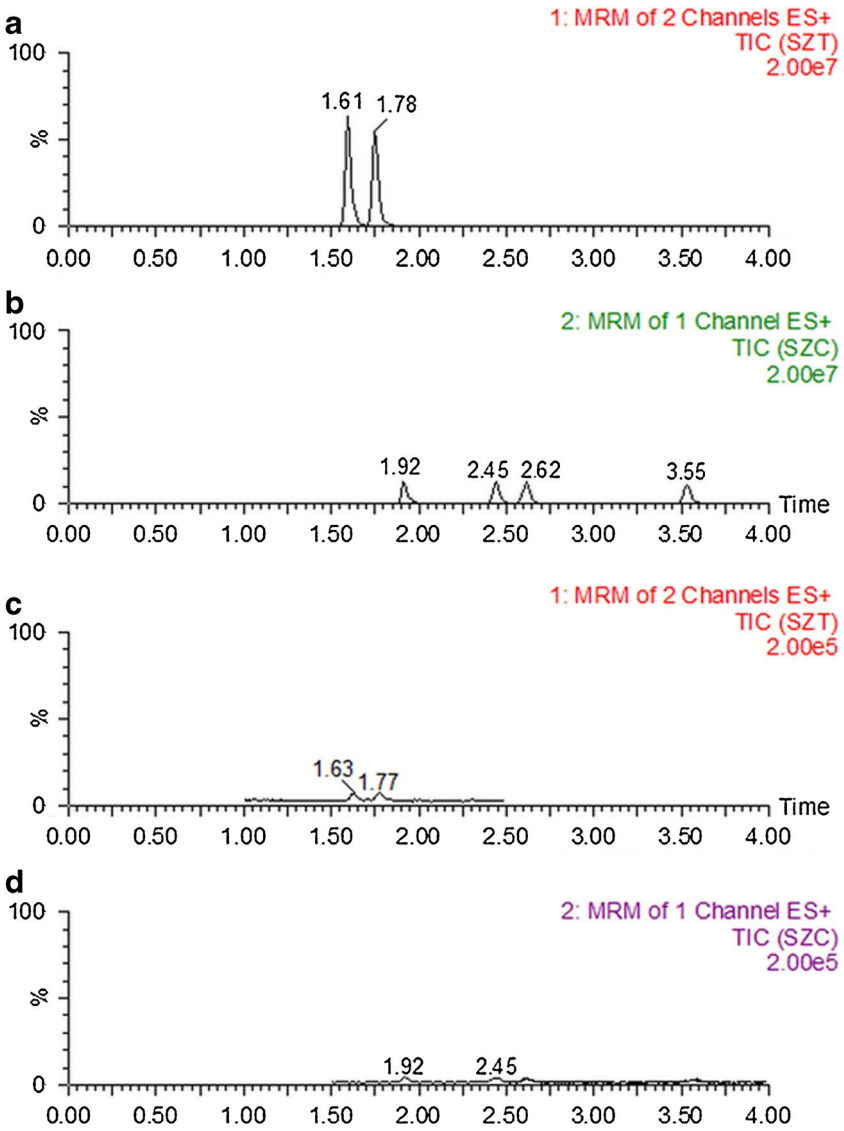

Fig. 5 Total ion chromatogram (*TIC*) of **a** triadimefon standard solution with 10 μg/kg for each enantiomer in the vegetable oil matrix, **b** triadimenol standard solution with 10 μg/kg for each enantiomer in the vegetable oil matrix, **c** triadimefon in authentic vegetable oil sample, and **d** triadimenol in authentic vegetable oil sample

for 3 days using the same instrument but handled by different operators. The results showed recoveries in low-, intermediate-, and high-spiked levels were in the ranges of 90.1–97.3, 90.1–94.5, and 91.5–96.8 %, respectively. Good repeatability, as indicated by RSD of less than 5.4 %, was also obtained. In general, the intra-day (n=5) and inter-day (n=15) RSD for the proposed method ranged from 0.8 to 5.4 % and from 2.3 to 5.0 %, respectively (Table 3). The method presented satisfactory mean recoveries and precision, and no significant difference of inter-day and intra-day assays. The results demonstrated that the method of UPC^2-MS/MS was efficient and reliable.

Application to real samples

To assess the applicability of the proposed method of UPC^2-MS/MS for the detection of triadimefon and its metabolite triadimenol stereoisomers in vegetable oil samples, 40 samples including 16 maize oils, six peanut oils, six rapeseed oils, six soybean oils, and six sunflower oils purchased from local markets, were tested. Each kind of sample was analyzed in quintuplicate. The results showed that the residual contents of the six stereoisomers in all the samples were lower than their corresponding LOQs. A typical chromatogram of vegetable oil is presented in Fig. 5.

Conclusions

In the present study, convenient sample preparation was achieved by using GPC, and UPC^2-MS/MS was applied to the stereoisomeric separation and determination of triadimefon and its metabolite triadimenol stereoisomers in edible vegetable oil. Under the optimum conditions, baseline resolutions of two chiral fungicides were achieved, and the

fast separation was achieved within 4 min using a Chiralpak IA-3 column with 86 % compressed liquid CO_2 (A) and 14 % methanol/acetonitrile/isopropanol (2/2/6, *v*/*v*/*v*) (B) as mixed co-solvents at 30 °C. The absolute configurations of triadimefon and triadimenol stereoisomers were determined by a combination of experimental and predicted VCD spectra.

The developed chiral UPC^2-MS/MS method was useful for determination of triadimefon and its metabolite triadimenol stereoisomers in plant and environmental samples and could be used for enantioselective degradation studies of each stereoisomer.

Acknowledgments This work was financially supported by the National Natural Science Foundation of China (no. 21207118), the Project of Science and Technology Plan of Zhejiang Province, China (no. 2014C37103), and the Natural Science Foundation of Zhejiang Province, China (no. Y15B070017).

Conflict of interest The authors declare that they have no competing interests.

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