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Simultaneous determination of oxathiapiprolin and two metabolites in fruits, vegetables and cereal using a modified quick, easy, cheap, effective, rugged, and safe method and liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

An effective and rapid analytical method for the simultaneous determination of a new fungicide oxathiapiprolin and its metabolites (IN-E8S72 and IN-WR791) residues in fruits (grape, watermelon, watermelon peel), vegetables (cucumber, tomato, potato) and cereal (wheat) was developed by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) using the modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction concept. Three target compounds were extracted from all matrices with 1% (V/V) formic acid aqueous solution and acetonitrile then cleaned by dispersive solid phase extraction (dSPE) with octadecylsilane (C_{18}) and graphitized carbon black (GCB). The determination of the target compounds was achieved within a 5.1 min run time by using an UPLC HSS T3 column connected to an electrospray ionization source (ESI, positive ion mode) for oxathiapiprolin and the negative mode for the two metabolites. The method showed excellent linearity ($R^2 > 0.9904$) for target compounds. The limit of detection (LOD) for the three compounds ranged from 0.5 $\mu g\,kg^{-1}$ to 7.5 $\mu g\,kg^{-1}$ and the limits of quantitation (LOQ) were 1 $\mu g\,kg^{-1}$ and 10 $\mu g\,kg^{-1}$ for oxathiapiprolin and the metabolites, respectively. The mean recoveries from seven matrices ranged from 81.5 to 110.7%, with intra-day relative standard deviations (RSD_r) in the range of 0.8–12.0% for all three test compounds. The inter-day RSD_R were less than 14.5% for all of the recovery tests. The method was successfully applied for simultaneous analysis of oxathiapiprolin and its metabolites in actual trial samples, indicating its effectiveness in investigating oxathiapiprolin and its metabolites in the food.

1. Introduction

The oxathiapiprolin, 1-[4-[4-[5-(2,6-difluorophenyl)-4,5-dihydro-3-isoxazolyl]-2-thiazolyl]-1-piperidinyl]-2-[5-methyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]ethanone (Fig. 1), belongs to a new class of fungicides containing oxazole, pyrazole and thiazole rings from DuPont developed under code DPX-QGU42 in July 2012. This fungicide offers a significant improvement for growers in the control of downy mildew and late blight in potatoes, grapes, cucumber and tomato and other crops. IN-E8S72 and IN-WR791 are two metabolites of oxathiapiprolin (Fig. 1). In addition, it is in the process of being registered on a global scale for use against disease of fruits and vegetable crops. Several studies showed that a larger proportion of pesticide metabolites are detected in the environment, representing a potential hazard for non-target organisms including humans [1,2]. For food and environment safety, detailed investigations on its residue and metabolism are very important. However, to the best of our knowledge, analytical methodology for oxathiapiprolin and its metabolite has not been reported. So, it is important to establish reliable analytical method for determining oxathiapiprolin and its metabolites residue in food to ensure food safety.

Pesticide residue analysis methods involve two steps: extraction of target analytes from the matrix and chromatographic separation and determination. Anastassiades et al. [3] developed an approach called "quick, easy, cheap, effective, rugged, and safe" (QuEChERS), which involves extraction with acetonitrile (MeCN) partitioned from the aqueous matrix using anhydrous $MgSO_4$ and NaCl followed by a dispersive-SPE clean up with $MgSO_4$ and primary secondary amine (PSA). This method has many advantages over traditional techniques, such as high recovery for wide polarity and volatility range of pesticides; high sample throughput and the use of smaller amounts of organic solvent [4]. In addition, the use

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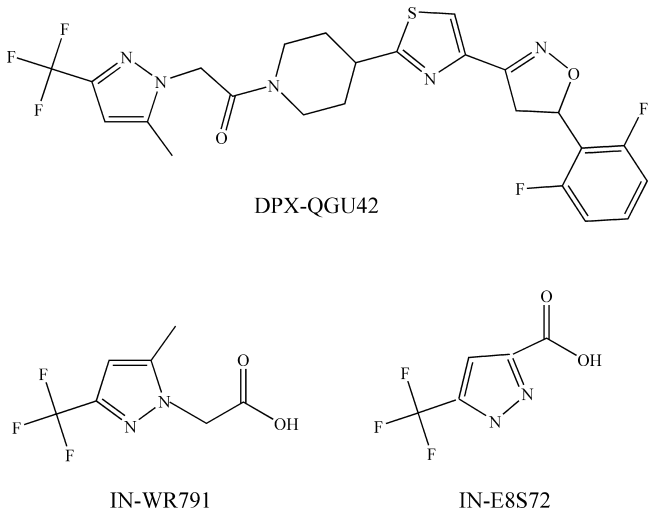

Fig. 1. Chemical structures of oxathiapiprolin and its two metabolites.

of UPLC–ESI-MS and CID-MS/MS offer higher selectivity and sensitivity of detection and shorter analysis time than HPLC–MS/MS, has become a powerful technique for determination of pesticide residue in various matrices including foods at the trace level [5–13].

The purpose of this study was to develop a rapid and effective residue analytical method for the confirmation and quantitative analysis of oxathiapiprolin and metabolites IN-E8S72 and IN-WR791 in fruits, vegetables and cereal (cucumber, tomato, grape, potato, wheat, watermelon, and watermelon peel). While, the fungicide oxathiapiprolin is a non-polar compound, its two metabolites IN-E8S72 and IN-WR791 are polar compounds. Accordingly, it is very difficult to determine oxathiapiprolin and its metabolites simultaneously. In order to find method that gives satisfactory separation and high recoveries for oxathiapiprolin and its metabolites, different types of UPLC columns and different extraction solvents and sorbents were compared and evaluated their performance for UPLC–MS/MS analysis. To the best of our knowledge, this is the first report for establishing an analytical method for this new pesticide oxathiapiprolin and its metabolites.

2. Experimental

2.1. Reagents and materials

Standard oxathiapiprolin (98.9% purity) and two metabolites IN-E8S72 (99.6% purity), IN-WR791 (99.8% purity) were obtained from DuPont (E. I. du Pont de Nemours and Company, USA). Analytical grade acetonitrile, formic acid, ammonium acetate and sodium chloride (NaCl) for pesticide residue analysis were purchased from Beihua Fine-Chemicals Co (Beijing, China). HPLC grade acetonitrile (MeCN) and methanol was purchased from Sigma–Aldrich (Steinheim, Germany). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA). PSA, C_{18} and GCB (40 μm) sorbents and 0.22-μm nylon syringe filters were purchased from Agela Technologies Inc. (Agela, Tianjin, PRC).

Stock solutions (100 mg L^{-1}) of individual standards were prepared in MeCN. The mixture solutions were prepared by mixing each of the three stock solutions in equal volume to achieve a working mixture of 10 mg L^{-1}. Then standard working solutions of three test compounds at 1, 10, 50, 100, 500 and 1000 μg L^{-1} concentrations were prepared from above working mixture by serial dilution with MeCN. The matrix-matched standard solutions were similarly prepared (1, 10, 50, 100, 500, 1000 μg L^{-1}) by adding the concentrated blank sample extract (cucumber, tomato, grape, potato, wheat, watermelon, and watermelon peel) to each serially diluted standard solution. All solutions were protected against light with aluminum foil and stored in a refrigerator in the dark at −20 °C until use.

2.2. UPLC–MS/MS analysis

UPLC–ESI-MS/MS analysis was conducted using a Waters Acquity UPLC system coupled to a triple-quadrupole mass spectrometer (TQD) equipped with an electrospray ionization source (ESI) (Waters Crop. Milford, MA, USA). The UPLC separation was performed using acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm particle size; Milford, MA, USA). The mobile phases, which were composed of methanol (A) and 2 mM ammonium acetate aqueous solutions (B), were pumped at a flow rate of 0.3 mL min^{-1}. The gradient elution was: 0–0.5 min, 10–40% A; 0.5–2.0 min, 40–70% A; 2.0–3.0 min, 70–90% A; 3.0–4.0 min 90% A; 4.0–4.1 min, 90–10%; then held at 10% A for 1.0 min. Separation and stabilization were achieved in 5.1 min. The column was kept at 40 °C to decrease the viscosity, and the temperature in the sample manager was set at 5 °C. The injection volume was 5 μL oxathiapiprolin and two metabolites were eluted within 5.1 min.

Low-energy collision dissociation tandem mass spectrometric analyses (CID-MS/MS) analysis was performed in the positive and negative ionization switching mode, and the monitoring conditions were optimized for the target compounds. The typical conditions were capillary voltage, 3.0 kV; source temperature, 120 °C; desolvation temperature, 350 °C; desolvation gas (nitrogen, 99.95% purity) flow, 600 L h^{-1}; collision gas (argon, 99.999% purity) pressure 2×10^{-3} mbar in the T-Wave cell. The analytical quantification was performed by CID-MS/MS analyses using the multiple reaction mode (MRM) by measuring the transition of the precursor ion → product ion for the fungicide and its metabolites. Cone voltages and collision energies were optimized for each analyte to obtain the highest sensitivity and resolution (Table 1). The Masslynx NT V.4.1 (Waters, USA) software was used to collect and analyze the data obtained.

2.3. Sample preparation procedure

Approximately, 1000 g samples (cucumber, tomato, grape, potato, watermelon, watermelon peel and wheat) were chopped and homogenized in an Ultra-Turrax homogenizer (IKA-Werke, Staufen, Germany). Amounts of 10 ± 0.1 g of homogenized samples were weighed into 50 mL Teflon centrifuge tubes. Recovery study was performed by spiking each matrix with mixture standard solutions. The tubes were vortexed for 3 min and allowed to stand for 2 h at room temperature to distribute the pesticide evenly. Afterward,

Table 1
Experimental parameters and UPLC–MS/MS conditions of the analytes studied.

Compound	Molecular formula	Molecular weight	t_R (min)	Ion source	CV (V)	Quantification ion transition	CE1 (eV)	Confirmatory ion transition	CE2 (eV)	Ion ratio
Oxathiapiprolin	$C_{24}H_{22}F_5N_5O_2S$	539.52	3.58	ESI^+	60	540 → 500	23	540 → 167	30	1.8
IN-E8S72	$C_5H_3F_3N_2O_2$	180.09	1.68	ESI^-	24	178.7 → 135	10	178.7 → 65	18	2.2
IN-WR791	$C_7H_7F_3N_2O_2$	208.14	1.96	ESI^-	40	206.9 → 163	11	206.9 → 143	18	2.0