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Rapid Residue Determination of Cyenopyrafen in Citrus Peel, Pulp, and Whole Fruit Using Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry

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Abstract
A sample pretreatment method was established to analyze the residues of cyenopyrafen in citrus peel, pulp, and whole fruit using ultra-performance liquid chromatography coupled with tandem mass spectrometry. The target compound was extracted from all matrices with acetonitrile and then cleaned by dispersive solid phase extraction using 10 mg GCB + 150 mg $MgSO_4$ for citrus peel; 50 mg PSA + 150 mg $MgSO_4$ for citrus pulp, and 50 mg C18 + 50 mg PSA + 150 mg $MgSO_4$ for whole fruits. Determination of the target compound was achieved in less than 5.0 min using an electrospray ionization source in positive mode. Average recoveries in citrus peel, pulp, and whole fruit spiked at 0.01, 0.2, and 2 mg kg^{-1} ranged from 84.9 to 105.1%, with relative standard deviations (RSD_r) of 0.7–7.9%. The reproducibility (RSD_R) ranged from 2.6 to 6.8%. The limits of detection and quantification ranged from 0.00032 to 0.0012 mg kg^{-1} and from 0.0009 to 0.0036 mg kg^{-1}, respectively. This method was used to determine cyenopyrafen residues in citrus fruits to study its dissipation under field conditions. The trial results showed that the half-lives of cyenopyrafen in whole fruits were 10.2 and 6.2 days in Hunan and Guangxi provinces, respectively. The developed analytical method provides a basis to establish maximum residue limits and monitor cyenopyrafen residue in citrus.

Keywords Cyenopyrafen · Citrus · Residue · UPLC-MS/MS

Introduction

Citrus fruits are among the most popular fruits worldwide and are consumed for their dietary and nutritional value (Mehl et al. 2014; Zhao et al. 2015; Zou et al. 2016). Citrus fruits are reportedly rich in various phytochemicals, including limonoids, coumarins, and flavonoids, which are able to prevent diabetes, inflammation, cardiovascular diseases, cancer, and other diseases (Fernandez-Lafuente et al. 2015; Sun et al. 2016). However, citrus suffers from numerous pests and diseases, with a large number of pesticides widely used for pest control in citrus at various stages of cultivation. As a consequence, pesticide residues in citrus are of concern because they are potentially hazardous to health and many determination methods were established (Ucles et al. 2014; Kakimoto et al. 2016; Besil et al. 2017).

Cyenopyrafen (structure shown in Fig. 1) is a relatively novel acaricide developed by Otsuka AgriTechno Co, Ltd. in 2007 (Riga et al. 2015) that is used against spider mites and phytophagous mites at all development stages. Cyenopyrafen is a mitochondrial electron transport inhibitor acaricide with a novel mode of action at complex II that has been recently developed for the control of resistant spider mite populations (Yu et al. 2012). This pesticide offers farmers significantly improved control of spider mites and phytophagous mites in citrus and other crops. Accordingly, cyenopyrafen is in the process of being registered globally for use against pests in fruit and vegetable crops. To establish food safety, detailed investigations of cyenopyrafen residues and detection are important. Unfortunately, to our knowledge, there are only methods reported to determine the residues of cyenopyrafen in fruits and

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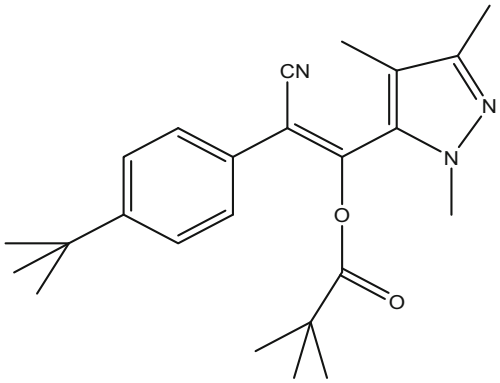

Fig. 1 Chemical structure of a cyenopyrafen

vegetables such as Asian pears, strawberries, and red peppers (Kim et al. 2017; Kabir et al. 2017; Park et al. 2017), but no residue analytical method for cyenopyrafen in citrus has been reported and no maximum residue limit (MRL) has been set for cyenopyrafen in the European Union, China, or the USA. Furthermore, the behavior and fate of cyenopyrafen in citrus fruits has not been reported. Few studies about cyenopyrafen have been reported to date, with most concerning the mechanism of action, insecticidal activity, or biological characterization. Therefore, to manage the impact of cyenopyrafen on environmental and human health safety, it is important to work toward a reliable analytical method for determining cyenopyrafen residues in food.

In 2003, Anastassiades et al. developed the outstanding quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for monitoring pesticides in vegetables and fruits cleaned up by dispersive solid phase extraction (Anastassiades et al. 2003). The merits of this method include decreased costs of experimental apparatus, high recovery for pesticides with wide-ranging polarities and volatilities, and the use of smaller amounts of organic solvent. However, compared with other common methods, the main disadvantage of the QuEChERS method is that the final extract concentration (1 g mL^{-1}) is lower than those of concentrated extracts (2–5 g mL^{-1}) in most traditional methods (Dong et al. 2009). Therefore, the QuEChERS method often requires highly sensitive and selective analytical instruments to determine the small extraction samples. Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) has been shown to be a powerful tool for the analysis of pesticides and drugs at trace concentration levels because of its high selectivity, precision, and sensitivity (Pan et al. 2016; Steiner et al. 2016; Xu et al. 2012). Particularly, in MS/MS, the use of multiple reaction monitoring (MRM) mode affords improved limits of detection (LODs) owing to the increased signal-to-noise ratio. Therefore, UPLC in combination with tandem mass spectrometry (MS/MS) is a more robust analytical technique for pesticide residue analysis in different matrices, as shown in numerous recent published reports (Muñoz et al. 2017; Aurayblais et al. 2016; Hu et al. 2016; Rizzetti et al. 2016; Yan et al. 2016).

To our knowledge, the current report is the first to establish an analytical method to detect residues of cyenopyrafen in citrus. A field study was performed to investigate the fate of cyenopyrafen in citrus fruits. Next, a modified QuEChERS method for the determination of cyenopyrafen in citrus using UPLC-MS/MS was developed and validated. Different types of extraction solvents and sorbents were investigated to achieve satisfactory extraction and clean-up of cyenopyrafen in citrus peel, pulp, and whole fruit. This work could help international governments establish the MRL of cyenopyrafen in citrus and provide basic information for the proper use of cyenopyrafen in pest management strategies in citrus farm ecosystems to protect public health.

Experimental Section

Reagents and Materials

Analytical standard cyenopyrafen (purity 99.3%) was obtained from Nissan Chemical Industries, Ltd. (Tokyo, Japan). Chromatography grade acetonitrile (ACN), formic acid, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical grade sodium chloride (NaCl), acetic acid, and anhydrous magnesium sulfate (anhydrous $MgSO_4$) for pesticide residue analysis were purchased from Anhui Tiandi Chemical Regent Co. Ltd. (Hefei, China). Ultra-pure water was prepared using a Milli-Q reagent water system (Bedford, MA, USA). Graphitized carbon black (GCB, 40 μm), primary secondary amine (PSA, 40 μm), and octadecylsilane (C18, 40 μm) sorbents were purchased from Agela Technologies Inc. (Tianjin, China).

Standard stock solutions of cyenopyrafen (1000 mg L^{-1}) were prepared in pure acetonitrile, and the standard solutions required to construct a calibration graph (0.001, 0.005, 0.01, 0.05, 0.1, 0.5, and 2 mg L^{-1}) were prepared from the stock solution by serial dilution with acetonitrile. Correspondingly, matrix-matched standard solutions at the same levels were obtained by adding blank sample extracts to each serially diluted standard solution. Observation for 3 months showed no degradation in the working standard solutions. All solutions were stored in a refrigerator in the dark at below 4 °C.

Instrumentation

In this study, the chromatographic separation of cyenopyrafen was conducted using a Waters Acquity UPLC system, which included an Acquity UPLC manager, a Waters Acquity UPLC binary solvent manager, and an Acquity column heater equipped with a Waters Acquity UPLC BEH C18 column

(2.1 × 50 mm 1.7-μm particle size; Milford, MA, USA). The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile. The gradient program was as follows: 0–0.5 min, 90% A; 0.5–1 min, decreased linearly to 5% of A; 1–4 min, 5% A; 4–4.5 min, increased to 90% of A; 4.5–5 min, 90% A. Separation was performed by injecting 3 μL of the sample at a flow rate of 0.30 mL min^{-1} for a total analysis time of 5 min. The sample manager was maintained at 4 °C and the column temperature was set at 35 °C. Under the described conditions, the retention time of cyenopyrafen was approximately 3.35 min.

Cyenopyrafen analysis was conducted on a triple quadrupole (TQD) mass spectrometer (Waters Corp. Milford, MA, USA), MS/MS detection was performed in positive ionization mode, and monitoring conditions were optimized for the target compound. Typical conditions were as follows: capillary voltage, 3.0 kV; source temperature, 120 °C; desolvation temperature, 350 °C; cone gas flow, 50 L h^{-1}; and desolvation gas flow, 650 L h^{-1}. All parameters for MRM transitions, cone voltage, and collision energy were selected to obtain the highest sensitivity and resolution (Table 1).

Sample Preparation Procedure

Samples (10 g, accurate to 0.01 g) of citrus pulp, peel, or whole fruit were weighed into a 100-mL Teflon centrifuge tube and then a suitable volume of working standard solution was added for the recovery experiment. To distribute the pesticide evenly in the sample matrix, the tube was eddied for 30 s and equilibrated for 2 h at room temperature. Acetonitrile (20 mL) and deionized water (5 mL) were added to extract cyenopyrafen from citrus peel. Acetonitrile (20 mL) was added to extract cyenopyrafen from citrus pulp and whole fruit. The tubes were capped and eddied vigorously for 1 min. NaCl (3 g) and $MgSO_4$ (2 g) were then added to the tubes, which were recapped, immediately eddied vigorously for 1 min, and centrifuged at 4000×*g* for 5 min. A 1.5-mL aliquot of the upper layer was transferred into a 2-mL single-use centrifuge tube containing the appropriate sorbent (50 mg PSA + 150 mg $MgSO_4$ for citrus pulp; 50 mg C18 + 50 mg PSA + 150 mg $MgSO_4$ for whole orange; 10 mg GCB + 150 mg $MgSO_4$ for citrus peel). The tubes were well capped, eddied for 1 min, and centrifuged for 3 min at 2810×*g*. The upper layer of the prepared sample was filtered through a 0.22-μm nylon syringe filter before UPLC-MS/MS analysis.

Method Validation

To evaluate the performance of the developed method, it was validated using a conventional validation procedure according to the No. SANTE/11945/2015 guideline (SANTE 2015) that included the following parameters: precision, accuracy, limit of quantification (LOQ), linear range, limit of detection (LOD), stability, specificity, and matrix effect (ME). To investigate the method accuracy and precision, recovery assays were performed. Five replicates of blank samples of citrus peel, pulp, and whole fruit, spiked at 0.01, 0.2, and 2 mg kg^{-1}, were prepared on three different days. Cyenopyrafen was extracted, according to the proposed sample preparation procedure and then determined using the intra-day and inter-day assays. The accuracy was expressed by the recovery (%) of spiked samples and the precision was expressed by the relative standard deviation (RSD). The LOD and LOQ for cyenopyrafen were considered to be the concentrations that produced signal-to-noise (S/N) ratios of 3 and 10, respectively. The linearity of the method was studied by linear regression analysis of both the standard solution and matrix-matched calibration curves in triplicate at seven concentrations ranging from 1 to 2000 μg L^{-1}. ME was estimated by comparing the slopes of matrix-matched calibration curves and solvent (acetonitrile) calibration curve. Results of ME were expressed in percentage and considered significant signal suppression or enhancement effect when the percentage was over than ± 20%.

Field Experiment

Field experiments were conducted in Hunan and Guangxi provinces of China in 2015. Weather conditions were monitored throughout the experimental period. The daily temperatures at the Hunan and Guangxi sites were in the range of 13–38 and 16–35 °C, respectively. The soil organic matter content ranges were 1.5–3.0 and 4.3–8.1%, while the pH ranges were 5.5–6.5 and 4.2–7.5 in Hunan and Guangxi, respectively.

A kinetic study was performed at two field plots, each with three citrus trees. Cyenopyrafen (30% suspension) was dissolved in water and sprayed onto citrus fruits at a dosage of 225 mg a.i. kg^{-1} to study the residue behavior. No pesticide

Table 1 Mass-spectrometric conditions to determine cyenopyrafen

Compound	Molecular formula	MW	t_R (min)	CV(V)	Quantification ion transition	CE1 (eV)	Confirmatory ion transition	CE 2 (eV)
Cyenopyrafen	$C_{24}H_{31}N_3O_2$	393.5	3.35	50	394.3 → 310.1	30	394.3 → 294.1	25

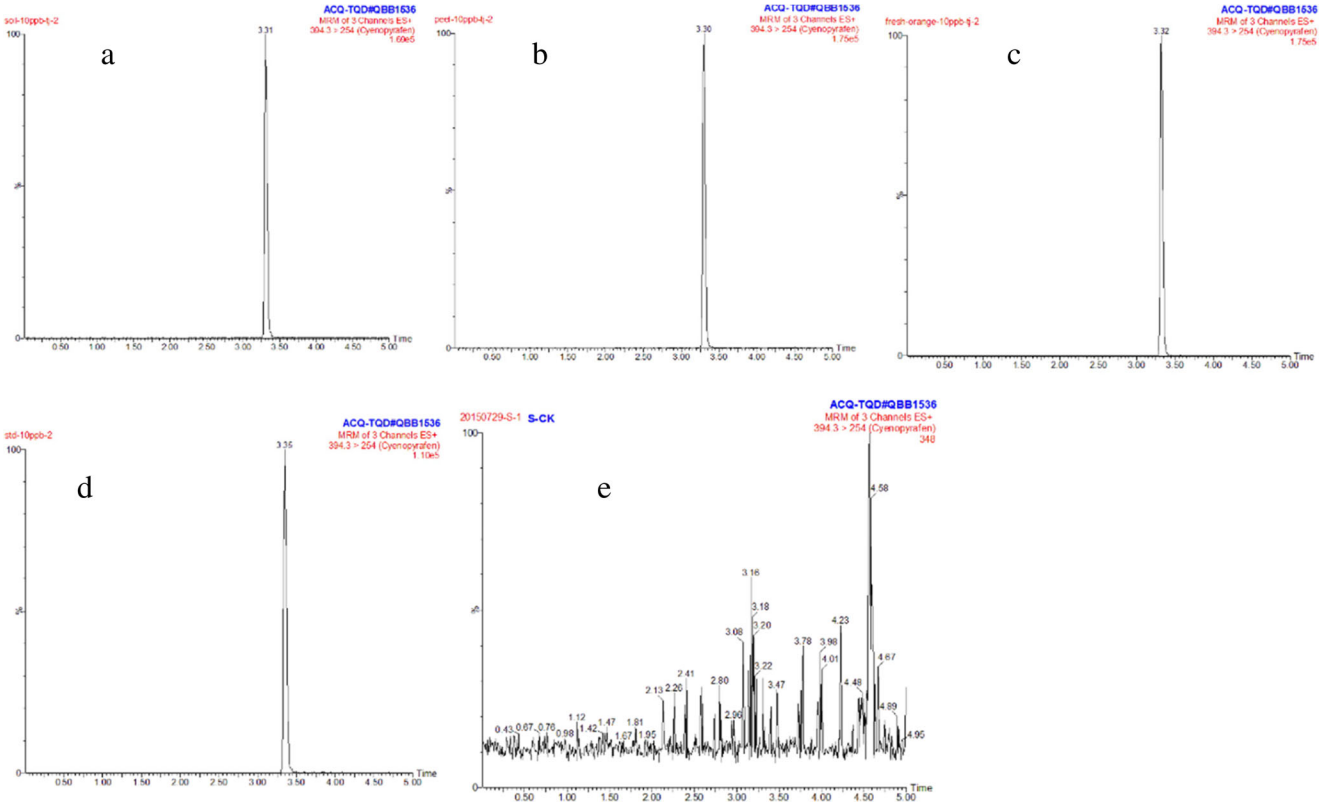

Fig. 2 HPLC-MS/MS chromatograms of the **a** citrus peel spiked at 0.01 mg kg^{-1}, **b** citrus pulp spiked at 0.01 mg kg^{-1}, **c** the standard at 0.01 mg kg^{-1}, **d** citrus whole fruit spiked at 0.01 mg kg^{-1}, and **e** blank sample

had been applied to the plots beforehand, and the untreated plots were sprayed with water as control. Three replicate plots were tested. A buffer zone without pesticide application was placed between the plots. Citrus samples were collected at 2 h and 1, 2, 3, 5, 7, 10, 14, 21, and 28 days after cyenopyrafen application. At least 2 kg of citrus samples were collected randomly from different parts of treated and controlled trees (Chen et al. 2015).

Citrus samples were put into polyethylene bags and transported to the laboratory where they were mixed, chopped, and divided into sub-samples. The sub-samples were placed in a freezer at − 20 °C until analysis.

Results and Discussion

Optimization of MS/MS and Chromatography

Cyenopyrafen analysis was performed in MRM mode, and full-scan and MS/MS mass spectra were obtained by infusion of the 0.5 mg L^{-1} standard solution at a flow rate of 10 μL min^{-1}, which presented comparable ionization in both negative and positive modes. In this study, positive-mode ESI was selected because it gave a better response signal than negative-mode ESI. In the positive ESI mode, the $[M + H]^+$ ion was selected as the precursor ion for cyenopyrafen. Quantitation was conducted using the more abundant ion transition, while the less abundant ion transition was used for the identification. The precursor ions, molecular weights, collision voltages, and corresponding cone voltages were listed in Table 1.

The mobile phase was modified and different types of column (HSS T3 1.8 μm, 100 mm × 2.1 mm; BEH C18

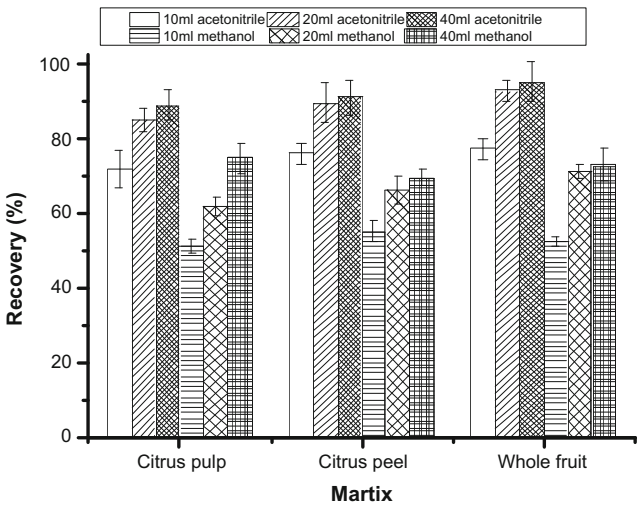

Fig. 3 Comparison of recoveries of cyenopyrafen spiked in citrus pulp, citrus peel, and whole fruit (0.01 mg kg^{-1}) in two types and three volume levels of extraction solvents ($n = 3$)

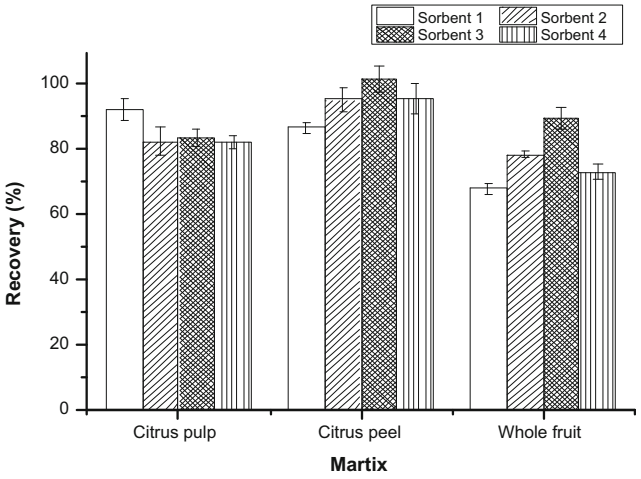

Fig. 4 Effect of different sorbents for the targeted compound in citrus pulp, citrus peel, and whole fruit at the 0.01 mg kg^{-1} level ($n = 3$)

1.7 μm, 50 mm × 2.1 mm; and HSS PFP 1.8 m, 100 mm × 2.1 mm) were used for each mobile phase composition: (A) water with 0.1% formic acid and (B) acetonitrile. Columns have a significant effect on both the retention and peak shape behavior of the analyte in the LC column, as well as on the MS/MS response. A solvent system consisting of acetonitrile and 0.1% formic acid aqueous solution using a BEH C18 column provided the most efficient chromatographic conditions and was selected for analyses. As shown in Fig. 2, there were no interference peak around the retention time of the analyte, and the retention time of cyenopyrafen was 3.35 min.

Optimization of Extraction and Clean-Up Procedures

The selection of sorbents and solvents has a significant effect on the satisfactory extraction and clean-up of analytes. Different types of sorbents and extraction solvents were tested to achieve good purification and extraction efficiency. A recovery test was performed for optimum purification and extraction of cyenopyrafen from the citrus samples.

Full extraction of the analyte from samples is essential to obtain a satisfactory recovery (Li et al. 2016). Therefore, in the preliminary experiment, a sample (10 g) was weighed into a 100-mL Teflon centrifuge tube, then two types of solvents (methanol, acetonitrile) with three volume levels (10, 20, and 40 mL) were further evaluated at a spiked level of 0.01 mg kg^{-1}. By comparing the extraction results, 20 and 40 mL of acetonitrile obtained almost equivalent recoveries that were slightly better ($P < 0.05$) than those achieved with 10 mL of acetonitrile and the other extraction solvent. This might be attributed to cyenopyrafen being a hydrophobic pesticide (Huang et al. 2016), making it easier to extract cyenopyrafen from samples using acetonitrile. To reduce pollution and waste, minimize the use of water and chemical compounds, and ensure good extraction results, 20 mL of acetonitrile was selected for optimum sample extraction. QuEChERS is an appropriate method for the analysis of matrices with high water contents (more than 80%) (Dong et al. 2012; Lehotay et al. 2005). Therefore, deionized water (5 mL) was added before the extraction procedure to improve the water content and achieve good extraction efficiency for citrus peel. Additionally, NaCl (3 g) was used to initiate separation of the acetonitrile from water phases, and $MgSO_4$ (2 g) was used to absorb traces of water in the organic solvent. Figure 3 showed the mean recoveries of the six extraction tests.

PSA, C18, and GCB are usually employed as sorbents during the clean-up process. PSA is frequently used to clean-up non-polar compounds, such as sugars and fatty acids, from polar samples because it has a weak anion exchange function (Pan et al. 2016). Conversely, C18 is suitable for extracting non-polar to moderately polar compounds from polar samples. Finally, GCB can remove sterols and pigments from green vegetable extracts because it is a weakly polar or non-polar sorbent (Tian et al. 2016). Four sorbents (sorbent 1, 50 mg PSA + 150 mg $MgSO_4$; sorbent 2, 50 mg C18 + 150 mg $MgSO_4$; sorbent 3, 50 mg C18 + 50 mg PSA + 150 mg $MgSO_4$; sorbent 4, 10 mg GCB + 150 mg $MgSO_4$) were respectively used to investigate the influences on recovery rate in samples in our study. As shown in Fig. 4, all four sorbent combinations achieved satisfactory recovery when used to clean-up citrus peel extracts. Nevertheless, it was difficult to achieve satisfactory recovery using only PSA, C18, or GCB for whole fruit, for which the compound recoveries

Table 2 Calibration equations, R^2, LODs, LOQs, and ME

Matrix	Regression equation	R^2	Slope ratio	ME (%)	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
Acetonitrile	$y = 896.08x + 3101.5$	0.9998	–	–	–	–
Citrus peel	$y = 1762.7x + 22{,}792$	0.9969	1.97	96.71	0.00032	0.0009
Citrus pulp	$y = 1052.4x + 9056.3$	0.9986	1.17	17.44	0.0012	0.0036
Whole fruit	$y = 1578.86x + 10{,}255.74$	1	1.76	76.20	0.0011	0.0033

ME (%) = [(slope (matrix match)/slope (solvent curve) − 1] × 100

Slope ratio = matrix/ACN

Table 3 Recoveries ($n = 15$, percent), RSD_r and RSD_R for target compound from different matrices at three spiked levels, RSD_r intra-day, which is the relative standard deviation for repeatability ($n = 5$); RSD_R inter-day, which is the relative standard deviation for reproducibility ($n = 15$)

Matrix	Spiked level (mg kg^{-1})	Intra-day ($n = 5$)						Inter-day ($n = 15$) RSD_R (%)
		Day 1		Day 2		Day 3		
		Average recoveries (%)	RSD_r (%)	Average recoveries (%)	RSD_r (%)	Average recoveries (%)	RSD_r (%)	
Citrus peel	0.01	86.0	4.1	85.0	4.0	84.9	4.6	4.0
	0.2	98.1	3.6	98.6	2.4	96.6	3.9	3.2
	2	96.1	1.8	95.7	1.9	94.0	3.6	2.6
Citrus pulp	0.01	93.0	6.8	94.7	4.3	90.7	7.9	4.8
	0.2	96.2	6.6	99.2	7.9	92.9	3.9	6.8
	2	95.7	4.7	89.3	3.1	93.9	4.8	5.1
Whole fruit	0.01	91.4	5.4	94.1	4.8	91.5	6.4	5.3
	0.2	105.1	2.0	101.2	4.8	97.0	6.0	5.5
	2	100.1	4.9	97.9	0.7	96.6	2.0	3.3

ranged from only 67.7 to 78.1%. This was due to the large amount of sugars and fatty acids in the citrus fruit. For the citrus pulp, recoveries and RSDs were kept in the acceptable range using sorbents 1–3. Considering that PSA is much cheaper than C18, sorbent 1 was selected for purifying the target compound from citrus pulp.

Method Validation

Linearity, LODs, and LOQs

The linearity, LODs, and LOQs were obtained from the peak areas of the product ions obtained by MS/MS, as summarized in Table 2. A linear range of 1–2000 μg L^{-1} with a good determination coefficient ($R^2 > 0.99$) was achieved in all matrices. The limits of detection and quantification (LOD and LOQ) for the analyte were estimated for spiked samples (0.01 mg kg^{-1}) based on signal-to-noise (S/N) ratios of 3:1 and 10:1, respectively. The LODs of cyenopyrafen ranged from 0.00032 to 0.0012 mg kg^{-1}, while the LOQs were 0.0009 to 0.0036 mg kg^{-1}. The LOD and LOQ values did not surpass the established maximum limits of cyenopyrafen (2 mg kg^{-1}) in Japan. Therefore, this sensitivity was sufficient to verify the compliance of foodstuffs with legal tolerances. However, MRLs of cyenopyrafen in citrus fruits were not established in the European Union, the USA, China, and other countries; this method will be helpful for the registration and setting of MRLs for cyenopyrafen.

Precision and Accuracy

To evaluate the performance of the proposed method, a recovery assay was performed. The blank samples (citrus pulp, peel, and whole fruit) were spiked at three concentration levels (0.01, 0.2, and 2 mg kg^1; Table 3) and then analyzed in five replications each on three distinct days. The method precision was determined from repeatability and reproducibility studies and expressed as the relative standard deviation (RSD). The repeatability (RSD_r) was measured by comparing the standard deviations of the recovery percentage spiked samples on the same day. The RSD_R was determined by analyzing spiked samples on three different days. As shown in Table 3, the satisfactory mean recoveries of cyenopyrafen were in acceptable ranges of 91.4–105.1% (RSD_r, 0.7–6.4%), 89.3–99.2% (RSD_r, 3.1–7.9%), and 84.9–98.6% (RSD_r, 1.8–4.6%), for citrus whole fruit, pulp, and peel, respectively. Correspondingly, the inter-day RSD_R ($n = 3$) for the proposed method ranged from 2.6 to 6. 8%, which were in agreement with Document No.SANTE/11945/2015 (mean recovery between 70 and 120% and RSD ≤ 20%, with RSDr ≤ 20%). The results of the recovery studies revealed that this analysis method can achieve satisfactory sensitivity, precision, and recovery for cyenopyrafen analysis in citrus fruits.

Table 4 Half-lives and other parameters for cyenopyrafen dissipated in citrus whole fruit

Matrix	Locality	Regression equation	Coefficient (R^2)	Half-life (day)
Whole fruit	Hunan	$C_t = 0.166e^{-0.068t}$	0.774	10.2
	Guangxi	$C_t = 0.237e^{-0.111t}$	0.801	6.2

Matrix Effect

ME values for cyenopyrafen in citrus whole fruit, pulp, and peel were shown in Table 2. All three matrices showed an effect of signal enhancement, among which citrus pulp did not have significant effect on the recovery of cyenopyrafen with the ME value of only 17.4% (< 20%), while matrix effects of citrus peel and whole fruit were significant with the ME values of 96.7 and 76.2% (> 20%), respectively. As a result, matrix-matched calibration was selected for real sample analysis.

Dissipation of Cyenopyrafen in Citrus Under Field Conditions

It was assumed that pesticide degradation in citrus samples under field conditions followed first-order kinetics, $C_t = C_0 e^{-Kt}$, where C_0 is the initial concentration, C_t is the concentration at time t, and K is the dissipation rate constant. The 50% (DT_{50}) dissipation time of the initial pesticide concentration was calculated from K using the equation, $DT_{50} = \ln 2/K$.

The initial concentrations of cyenopyrafen in citrus fruits were 0.218 and 0.299 mg kg^{-1} in Hunan and Guangxi provinces, respectively. Cyenopyrafen was not detected (< 0.01 mg kg^{-1}) in whole citrus fruit 28 days after treatment in both testing sites. The regression line equations for the concentration (C) of cyenopyrafen in the whole fruit from Hunan and Guangxi with respect to time (t) were $C_t = 0.166e^{-0.068t}$ ($R^2 = 0.774$) and $C_t = 0.237e^{-0.111t}$ ($R^2 = 0.801$), respectively, after applying the pesticide at 225 mg a.i. kg^{-1}. As shown in Table 4, the half-lives of cyenopyrafen were 10.2 and 6.2 days in whole fruit samples from Hunan and Guangxi, respectively. Degradation of cyenopyrafen in whole fruits from Guangxi was faster than that from Hunan, which may be related to volatilization, wash-off, and photodegradation at these sites. The effects of climate conditions, including temperature, rain, and pH, at these two sites might contribute to geographic differences in the pesticide half-life (Heimbach et al. 2016; Sun et al. 2016; Zhang et al. 2016).

Conclusions

In the present study, a simple and reliable method for the trace analysis of a new generation acaricide, cyenopyrafen, in citrus peel, pulp, and whole fruit was developed and validated. The devised UPLC-MS/MS method combined with a modified QuEChERS approach showed satisfactory validation parameters in terms of precision, accuracy, lower limits, and linearity. The mean recoveries in citrus for cyenopyrafen ranged between 84.9 and 105.1%, and the intra-day RSD_r and inter-day RSD_R of the method were in the ranges of 0.7–7.9 and 2.6–6.8%, respectively. The LODs of cyenopyrafen ranged from 0.00032 to 0.0012 mg kg^{-1}, while the LOQs were 0.0009 to 0.0036 mg kg^{-1}. The trial results showed that the half-lives of cyenopyrafen were 10.2 and 6.2 days in whole orange samples from Hunan and Guangxi provinces, respectively.

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Compliance with Ethical Standards

Conflict of Interest Huihua Tan declares that he has no conflict of interest. Yanping Gu declares that she has no conflict of interest. Sihong Liu declares that she has no conflict of interest. Hui Zhang declares that she has no conflict of interest. Xuesheng Li declares that he has no conflict of interest. Dongqiang Zeng declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent All authors named in a manuscript are entitled to the authorship and have approved the final version of the submitted manuscript.

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