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Development and Validation of Analytical Methods for Picoxystrobin Determination in Agricultural Products by GC-ECD and GC-MS

Jung-Ah Do, Min-Hye Lee, Hyejin Park, IL-Hyun Kang, Kisung Kwon, and Jae-Ho Oh*

Food Chemical Residues Division, National Institute of Food and Drug Safety Evaluation, KFDA, Osong, Cheongwon, Chungbuk 363-700, Korea

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ABSTRACT - A simple and sensitive analytical method was developed using gas chromatography with electron capture detector (GC-ECD) and gas chromatography-mass spectrometry (GC-MS) for determination of Picoxystrobin in agricultural products (apple, hulled rice, mushroom, pepper, soybean, and mandarin). Picoxystrobin residues were extracted with acetonitrile, partitioned with saline water, and then they were cleaned up on a florisil solid-phase extraction (SPE) cartridge to obtain an extract suitable for analysis by GC-ECD and GC-MS. The method was validated using 6 agricultural product samples spiked with Picoxystrobin at different concentration levels (0.02, 0.05 and 0.5 mg/L). Average recoveries of Picoxystrobin (using each concentration three replicates) ranged 64.0~98.3% with relative standard deviations less than 10%, calibration solutions concentration in the range 0.1~5 mg/L, and limit of detection (LOD) and limit of quantification (LOQ) were 0.005 and 0.02 mg/L, respectively. The result showed that the developed analytical method is suitable for Picoxystrobin determination in agricultural products.

Key words: Picoxystrobin, acetonitrile extraction, GC-ECD, GC-MS

Introduction

Picoxystrobin, methyl (E)3-methoxy-2-{2-[6-(trifluoromethyl)-2-pyridyloxymethyl]phenyl}acrylate, is the broad spectrum strobilurin fungicide for the control of yellow, brown and crown rusts, powdery mildew, sooty mold, net and leaf blotch, and tan spot on cereal crops, including wheat, barley, and oats[1-3]. It discovered and developed as Acanto by Zeneca Agrochemicals (now Syngenta) in 2002[4]. It blocks the electron transport between cytochrom b and cytochrom c1, which causes inhibition of ATP formation and mitochondrial respiration[4,5].

Picoxystrobin was approved in the European Union (EU) in 2003[6] and it has been registered at the U.S. Environmental Protection Agency (EPA) as a new chemical candidate[7]. The European Commission (EC) has stated a maximum residue limit (MRL) of 0.05 mg/kg for Picoxystrobin residue in oat in 2005[8]. In republic of Korea, Picoxystrobin was approved in 2011[9] and it has been registered for controlling anthracnose, downy mildew, phytophthora blight and phomopsis blight in persimmon, pepper, apple and cucumber[10]. The MRL of Picoxystrobin in republic of Korea was set to pepper (0.5 mg/kg), apple (0.3 mg/kg), persimmon (1.0 mg/kg), and cucumber (1.0 mg/kg)[11,12].

Picoxystrobin has a wide range of applications and is commonly used in agriculture; so it is necessary to determine levels of Picoxystrobin residues in agricultural products. Therefore, very reliable and sensitive analytical method for determining Picoxystrobin residues is required. A number of analytical methods have been reported for the determination of fungicide residues, using solid-phase micro extraction (SPME)[13], supercritical fluid extraction (SFE)[14], and matrix solid-phase dispersion (MSPD)[15]. These methods have the advantages of simplicity and rapidity. However, it is difficult to use those methods in research and official laboratories because of the need of specific and expensive laboratory tools and skilled personnel. Moreover, there is little work to develop the analytical method using high-performance liquid chromatography (HPLC) or gas chromatography (GC). Therefore, the objective of this study was to develop a routine analytical method including liquid-liquid partition, solid-phase extraction, and GC analysis for Picoxystrobin in apple, hulled rice, mushroom, pepper, soybean, and mandarin.

Materials and Methods

Standards, Reagents, and Samples

A certificated analytical standard of Picoxystrobin (99.9%) was obtained from Dupont Products (USA). The physic-

*Correspondence to: Jae-Ho Oh, Food Chemical Residues Division, National Institute of Food and Drug Safety Evaluation, KFDA, Osong, Cheongwon, Chungbuk 363-700, Korea
Tel: 82-43-719-4207, E-mail: chopin68@kfda.go.kr

Table 1. Physiochemical characteristics and toxicities of Picoxystrobin

Property	Content
IUPAC name	methyl(2*E*)-3-methoxy-2-{2-[6-(trifluoromethyl)-2-pyridyloxymethyl]phenyl}acrylate
CAS No.	117428-22-5
Classification	Strobilurin
Melting point	75°C
Boiling point	-
Density	1.4 (20°C)
Log P_{ow}*	3.6
Vapor pressure (25°C, mPa)	5.5×10^{-3}
pKa**	-
Stability	Stable at pH 5 and pH7 ; DT_{50} c. 15 d (pH9, 50°C)(EU Review Report)
Solubility	Water : 3.1 mg/L (20°C) Methanol : 96 g/L (20°C) 1,2-dichloroethane : > 250 g/L (20°C) Acetone : > 250 g/L (20°C) Xylene : > 250 g/L (20°C) Ethyl Acetate : > 250 g/L (20°C)
Toxicity	-

*n-Octanol/water partition coefficient
**Dissociation constant at 25°C.

chemical properties of Picoxystrobin are shown in Table 1. Pesticide grade acetone, acetonitrile, and n-hexane were purchased from Merck (Darmstadt, Germany). Other reagents such as sodium chloride and sodium sulfate anhydrous were purchased from Wako (Japan). Florisil cartridge (SPE, 6CC, 1 g) and 0.45 μm GHP syringe filter were purchased from Waters (USA) and General Electric Company (USA), respectively.

To prepare applicability in a variety of agricultural products, apple and mandarin (fruits), hulled rice (cereal grains), oyster mushroom (mushrooms), pepper (vegetables), and soybean (beans) were selected as the representative crops. Agricultural products used in this experiment were purchased from nation's major retailer.

Stock and working standard solution

Standard stock solution of Picoxystrobin was prepared in acetone at 500 μg/mL and stored in a freezer at −18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from stock solutions by serial dilution using acetone, immediately prior to sample preparation.

Extraction procedure

Each 20 g of the apple, hulled rice, mushroom, pepper, soybean, and mandarin samples were weighed out and homogenized with 100 mL of acetonitrile for 10 min (In case of dried samples like hulled rice and soybean water addition hydration step is required prior to acetonitrile extraction). The homogenized samples were conducted vacuum filtration, and then transferred to 500 mL separatory funnel. 10 g of sodium chloride was added and then vigorously shaken for 30 min. The eluent (acetonitrile layer) which passed through anhydrous sodium sulfate for dehydration was collected into a round bottom flask and evaporated to near dryness by rotary evaporator at 40°C. Finally make up with 5 mL of 20% acetone in n-hexane.

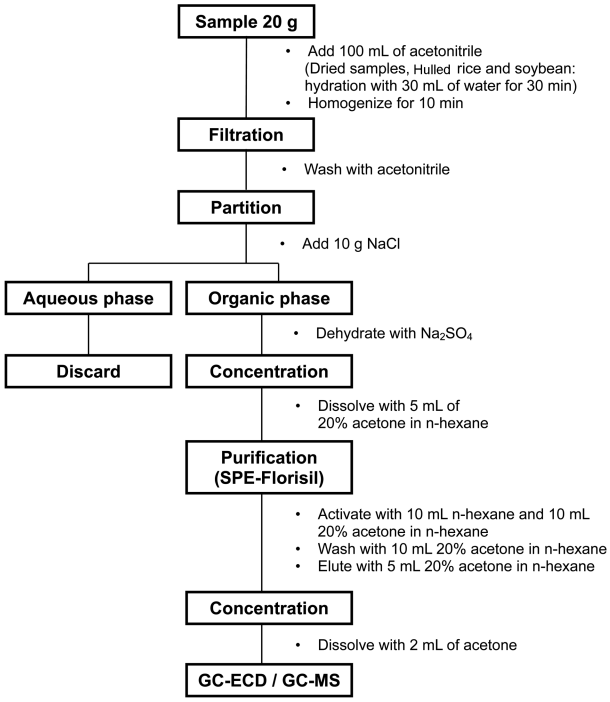

Fig. 1. Scheme of the acetonitrile extraction method for Picoxystrobin analysis.

Purification procedure

For conditioning the SPE-Florisil cartridge, 10 mL of n-hexane and 10 mL of 20% acetone in n-hexane were added and discarded. Extracted solution in 5 mL of 20% acetone in n-hexane was introduced into the florisil cartridge and the eluate was collected into a 50 mL-round bottomed flask. The analytes were then eluted with 5 mL of 20% acetone in n-hexane. The eluate was evaporated to dryness by a rotary evaporator and the concentrated residue was dissolved in 2 mL of acetone and filtered through a 0.45 μm GHP syringe filter. Extraction and purification procedures were illustrated as Fig. 1.

Chromatic Separation Parameters

The acetone solution obtained was analyzed by gas chromatography (Agilent 7890, USA) equipped with DB-5 (50 m × 0.25 mm i.d × 0.25 μm film thickness) column and

Table 2. Instrumental conditions for Picoxystrobin

Instrument	GC-ECD (7890A, Agilent Technologies, USA)
Column	DB-5 (50 m × 0.25 mm i.d, 0.25 μm film thickness)
Oven temperature	80°C
Injector temperature	260°C
Detector temperature	280°C
Injection volume	1 μL (Split Ratio 10 : 1)
Makeup flow (N_2)	1 mL/min (N_2)

Table 3. Confirmative conditions for Picoxystrobin

Instrument	GC-MS (Quattro microTM GC, Waters, USA)
Column	DB-5MS (30 m × 0.25 mm i.d, 0.25 μm film thickness)
Oven temperature	70°C(3 min) → 20°C/min → 180°C → 5°C/min → 300°C(7.5 min)
Column flow	1 mL/min (He)
Injector temperature	250°C
Interface temperature	280°C
Ion source temperature	230°C
Injection volume	1 μL (splitless mode)

electron capture detector (ECD). The injector and detector temperatures were operated at 260°C and 280°C, respectively. Nitrogen at the flow rate of 1 mL/min was used as carrier gas. Injection volume was 1 μL with split mode, split ratio of 10:1. The column was held at 80°C for 2 min after injection, and then programmed at 7°C/min to 250°C and then at 5°C/min to 280°C, which was held for 5 min (Table 2).

For confirming of Picoxystrobin in agriculture products, Quattro micro™ GC - mass spectrometry (Waters, USA) equipped with a DB-5MS (30 m × 0.25 mm and 0.25 μm film thickness) column was used. The GC-MS was operated by in the selected ion monitoring (SIM) mode (Table 3).

Method Validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity, and limit of detection (LOD) and quantification (LOQ) were considered. The accuracy if method was determined by recovery test, using samples spiked at concentration levels of 0.02, 0.05, and 0.5 mg/kg. Linearity was determined by different known concentrations (0.1, 0.5, 1.0, and 5.0 μg/mL) were prepared by diluting the stock solution. The LOD (μg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The LOQ (μg/mL) was determined as the lowest concentration of Picoxystrobin a response of 10 times the baseline noise.

Results and Discussion

Sample preparation

Acetonitrile, acetone, and ethyl acetate are commonly used organic solvents to extract pesticide residues in crops for the solid-liquid extraction step[16]. In this study acetonitrile was selected for hydrophobic pesticide Picoxystrobin ($logP_{ow}$ = 3.6) due to not only its miscibility with the water derived from the sample, but also the relatively poor solubility of the lipophillic plant materials[17]. In addition, acetonitirle extraction could reduce co-extracts effectively compare to acetone extraction.

Most studies regarding pesticide analysis from crops contain liquid-liquid extraction and column chromatography, or combination of both. However, some studies used only SPE without liquid-liquid partitioning because sample extract was diluted during the sample preparation so that the SPE was capable of cleaning up the extract[18-19]. In this study the introduction of saline water-acetonitrile partitioning resulted in good recovery and higher sensitivities. Moreover, saline water-acetonitrile partitioning could reduce costs by reducing the use of other organic solvent such as hexane and dichloromethane.

Linearity

Different known concentration of Picoxystrobin (0.1, 0.5, 1.0, and 5.0 μg/mL) were prepared in acetone by diluting the stock solution. Each solution was prepared in triplicate. Standard solutions were injected into GC column and peak area was measured for calculating Picoxystrobin residue. A calibration curve has been plotted of concentration of the standards injected versus peak area observed and the linearity of method was evaluated by analyzing four solutions. The peak area obtained from different concentrations of Picoxystrobin was used to calculate the residues (r^2 = 0.999) (Figs. 2 and 3).

Selectivity

The selectivity (matrix effect) of the analytical method was judged from the absence of interfering peaks at the retention times of the analytes. The retention times of Picoxystrobin was constant at 28.7 ± 0.2 min, and there were no interfering peaks at the retention time of Picoxystrobin [Fig. 4 (apple), 5 (hulled rice), 6 (mushroom), 7 (pepper), 8 (soybean), and 9 (mandarin)].

Accuracy and Precision

Recovery studies was carried out at 0.02, 0.05, and 0.5 mg/kg fortification levels for Picoxystrobin in apple, hulled rice, mushroom, pepper, soybean, and mandarin (Figs. 4-9). The recovery data and relative standard deviation obtained by

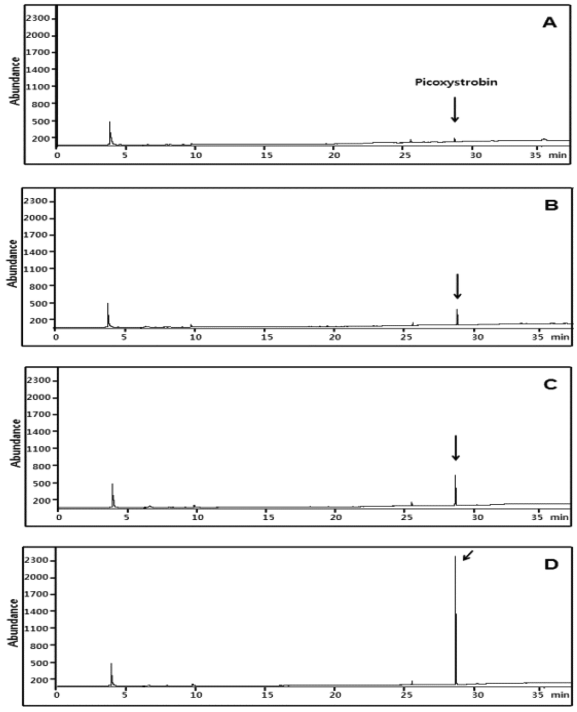

Fig. 2. GC-ECD chromatogram of Picoxystrobin standard at 0.1 mg/kg (A), 0.5 mg/kg (B), 1.0 mg/kg (C) and 5.0 mg/kg (D).

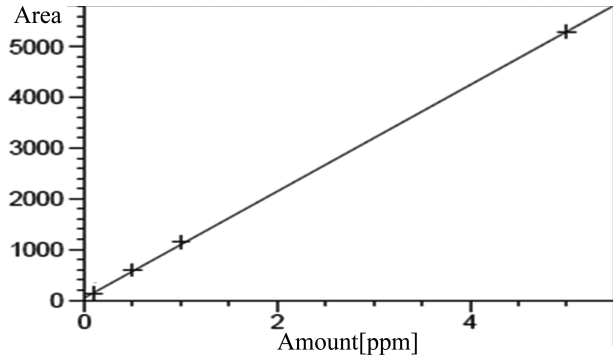

Fig. 3. Calibration curve of Picoxystrobin.

this method is summarized in Table 4. These numbers were calculated from three replicate analyses of given samples made by a single analyst on one day. The repeatability of method was satisfactory (RSDs < 6%). However, there were variations in average recoveries of each sample, this is because of presenting variety of components and remained co-extracts such as water, protein, fat, sugar, and so on. In this study, pepper (high chlorophyll content), hulled rice and soybean (high fat content), and mandarin (high acid and wax contents) gave lower average recoveries compared to mushroom and apple.

In addition, specific confirmation of Picoxystrobin residue was achieved in the GC-MS system. The confirmative condition of GC-MS for Picoxystrobin was set in selected-ion monitoring (SIM) mode with transitions of *m/z* 367 →

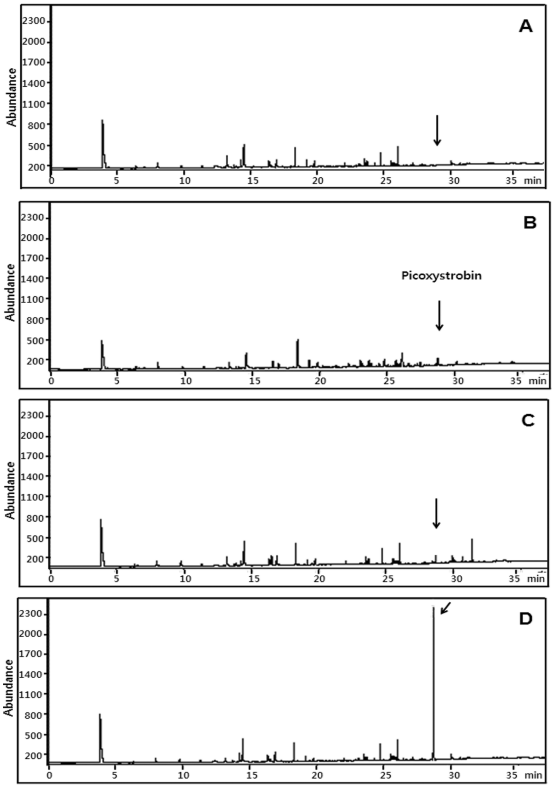

Fig. 4. GC-ECD chromatograms of Picoxystrobin, control apple (A), apple at 0.02 mg/kg (B), 0.05 mg/kg (C) and 0.5 mg/kg (D).

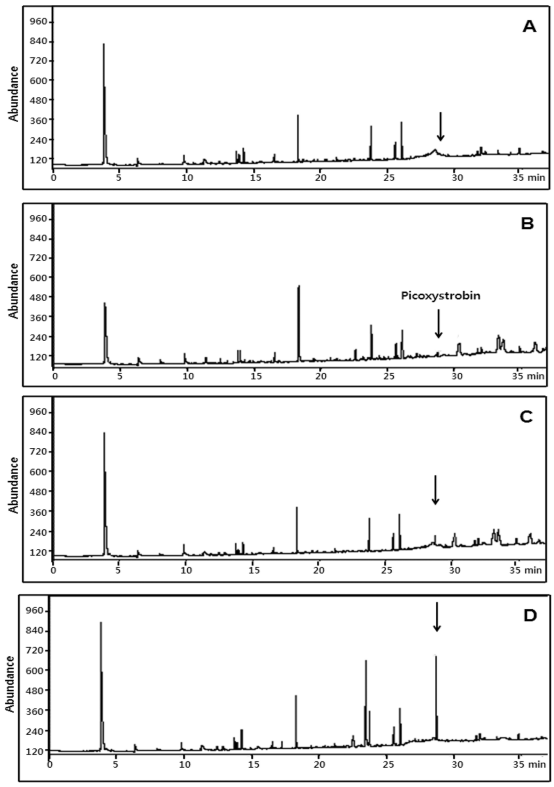

Fig. 5. GC-ECD chromatograms of Picoxystrobin, control hulled rice (A), hulled rice at 0.02 mg/kg (B), 0.05 mg/kg (C) and 0.5 mg/kg (D).

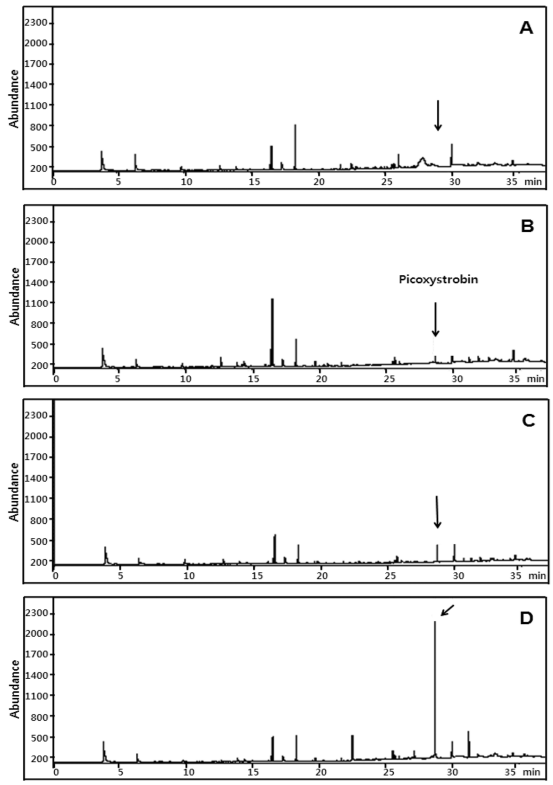

Fig. 6. GC-ECD chromatograms of Picoxystrobin, control mushroom (A), mushroom at 0.02 mg/kg (B), 0.05 mg/kg (C) and 0.5 mg/kg (D).

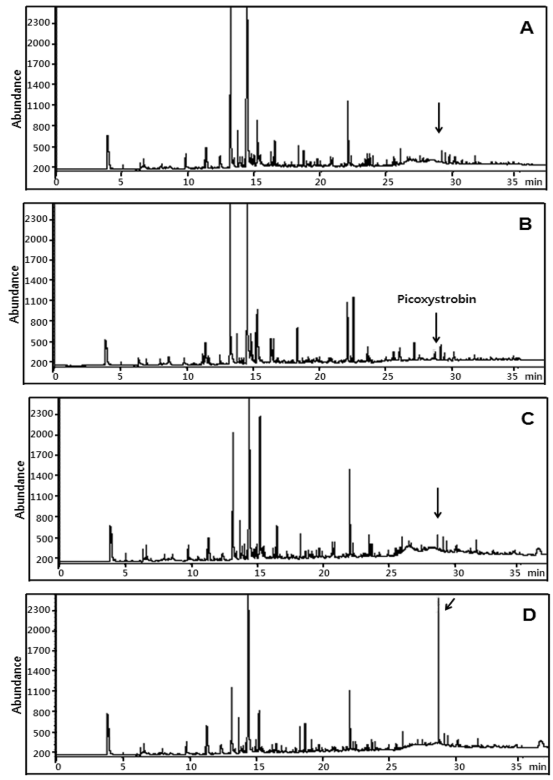

Fig. 7. GC-ECD chromatograms of Picoxystrobin, control pepper (A), pepper at 0.02 mg/kg (B), 0.05 mg/kg (C) and 0.5 mg/kg (D).

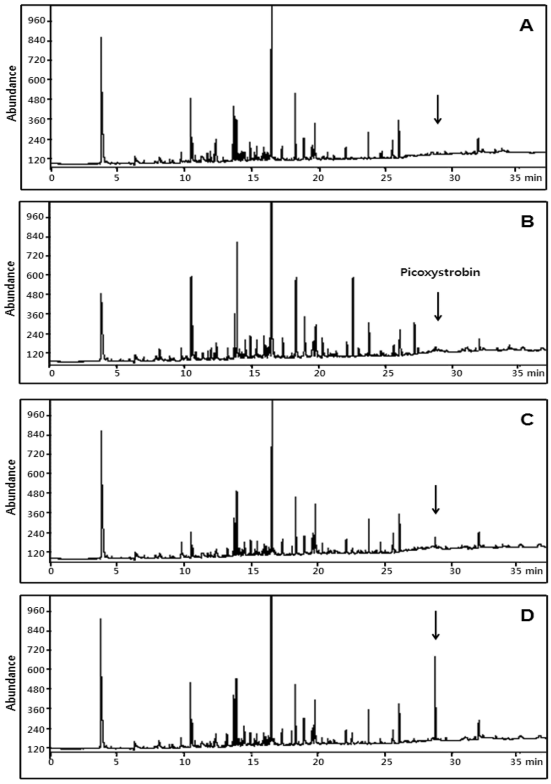

Fig. 8. GC-ECD chromatograms of Picoxystrobin, control soybean (A), soybean at 0.02 mg/kg (B), 0.05 mg/kg (C) and 0.5 mg/kg (D).

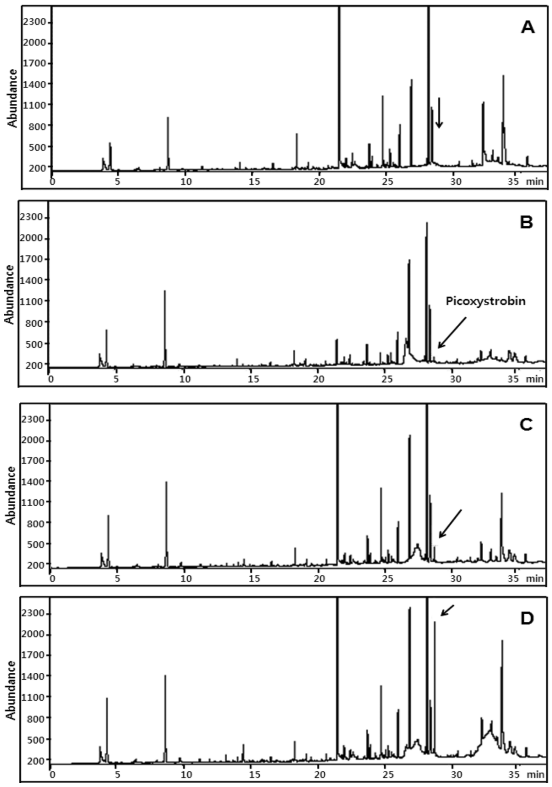

Fig. 9. GC-ECD chromatograms of Picoxystrobin, control mandarin (A), mandarin at 0.02 mg/kg (B), 0.05 mg/kg (C) and 0.5 mg/kg (D).

Table 4. Validation results of analytical method for Picoxystrobin

Sample	Fortification (mg/kg)	Recovery (%)	Average recovery (%)	LOD (mg/kg)
Apple	0.02	96.5 ± 7.8	94.8 ± 3.9	0.02
	0.05	95.4 ± 2.7		
	0.5	92.6 ± 1.2		
Hulled rice	0.02	64.0 ± 4.1	81.7 ± 2.8	
	0.05	85.0 ± 2.4		
	0.5	96.0 ± 1.9		
Mushroom	0.02	83.2 ± 6.7	90.4 ± 3.0	
	0.05	98.3 ± 0.3		
	0.5	89.8 ± 1.9		
Pepper	0.02	85.0 ± 5.5	78.7 ± 5.1	
	0.05	75.1 ± 4.1		
	0.5	76.1 ± 5.6		
Rice	0.02	76.2 ± 4.1	85.8 ± 2.8	
	0.05	85.0 ± 2.4		
	0.5	96.1 ± 1.9		
Soybean	0.02	75.0 ± 2.6	85.0 ± 3.0	
	0.05	88.4 ± 3.8		
	0.5	91.6 ± 2.6		

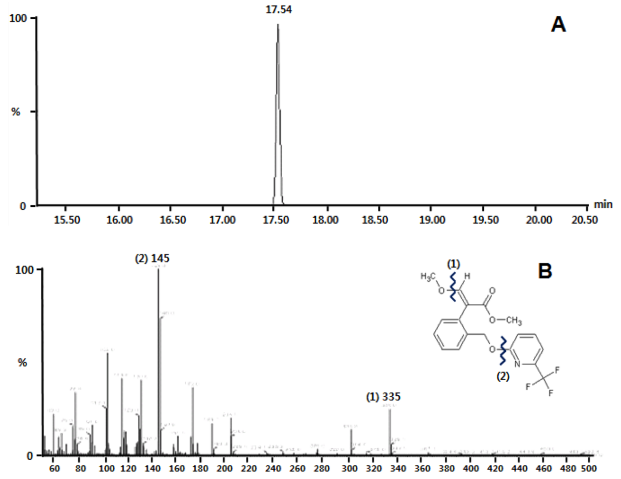

Fig. 10. Total ion chromatogram and MS spectrum of Picoxystrobin.

Table 5. Selected-ion of GC-MS for Picoxystrobin

Retention time (min)	Molecular weight	Fragment monitored (m/z)
17.54	367.3	145, 335

335 and *m/z* 367 → 145 (Fig. 10 and Table 5).

Detection and Quantification Limits

The limit of quantification was determined to be 0.02 μg/mL. The quantification limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (64~98%, RSDs < 6%) were achieved. This quantification limit also reflects the fortification level at which an analyte peak is consistency generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.005 μg/mL at a level of approximately three times the background of control injection around the retention time of the peak of interest.

Conclusions

This study described for the fast, simple, and sensitive analytical method based on GC-ECD and GC-MS was developed and validated for the determination of Picoxystrobin residue in agricultural products.

The Picoxystrobin extraction procedure of the described method is based on the Korean Food Standards Codex as multi class pesticide multiresidue methods with an acetonitrile. SPE-Florisil cartridge was used for sample preparation and the process is simple and easy, providing adequate clean-up of the matrix. Most of the sample extracts are very clean, with no interfering peaks at the retention time of the target compound, indicating good sensitivity of the proposed method.

Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained according to the Korean Food Standards Codex (2011)[20].

For Picoxystrobin the sensitivity of the method was suitable enough to ensure reliable determination levels lower than the respective MRLs. Therefore, the proposed analytical method could satisfactorily be useful for regular monitoring of Picoxystrobin residue in agricultural products.

Acknowledgement

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요 약

농산물 중에 있는 Picoxystrobin 잔류량을 검사하기 위해 가스크로마토그래프-전자포획검출기와 가스크로마토그래프-질량분석기를 이용한 간단하고 감도가 좋은 분석방법을 개발하였다. Picoxystrobin 잔류물은 acetonitrile 추출, 포화식염수를 이용한 액-액 분배, florisil 카트리지 정제 과정을 거쳐 기기분석을 수행하였다. 개발된 방법으로 Picoxystrobin 잔류허용기준이 설정된 식품인 고추와 사과 외 대표 농산물 시료 현미, 대두, 버섯, 감귤을 대상으로 농약을 0.02, 0.05, 0.5 mg/kg 농도로 처리하여 회수율을 시험한 결과는 64.0~98.3%이었으며, 상대표준편차는 10% 미만이었다. 검량선 작성을 위해 0.1~5 mg/L 범위로 표준품을 만들어 실험한 결과 상관계수(r^2)는 0.999로 높은 직선성을 보였으

며, 분석방법의 검출한계와 정량한계는 각각 0.005와 0.02 mg/kg이었다. 또한 확인시험을 위하여 질량분석기를 사용하였으며, 145와 335를 특성이온으로 선정하였다.

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