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소면적 재배작물 열무 및 알타리무의 재배 중 살충제 Spinetoram과 그 대사산물의 행적

Fate of Insecticide Spinetoram and Its Metabolites in Minor Crop Young and Small Radishes during Cultivation

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A Dissertation for the Degree of Master of Science

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Abstract

Fate of Insecticide Spinetoram and Its Metabolites in Minor Crop Young and Small Radishes during Cultivation

This study was aimed to optimize a residual analytical method of insecticide spinetoram (XDE-175-J and XDE-175-L) and its metabolites (N-demethyl-175-J/L, N-formyl-175-J/L) in minor crops (young and small radishes) using a QuEChERS (quick, easy, cheap, effective, rugged and safe) method combined with liquid chromatography tandem mass spectrometry (LC-MS/MS). For field trial, both minor crops were grown in separate greenhouse cultivation. The pesticides were applied to the plants with 7-day interval. Plants in plot 1 were treated with pesticides twice at 21 and 14 days before harvest and plot 2 were treated three times at 30, 21 and 14 days before harvest. Plants in plot 3 were also treated three times at 21, 14 and 7 days before harvest and plot 4 were treated three times at 14, 7 and 0 days before harvest. The samples were prepared with an optimized QuEChERS method for the LC-MS/MS analysis. Total concentration of spinetoram and metabolites in young radish was 0.40 mg L^{-1} for plot 1, 0.37 mg L^{-1} for plot 2, 0.61 mg L^{-1} for plot 3 and 0.96 mg L^{-1} for plot 4. Total concentration of spinetoram and metabolites in small radish were 0.69 mg L^{-1} for plot 1, 0.80 mg L^{-1} for plot 2, 1.22 mg L^{-1} for plot 3 and 1.86 mg L^{-1} for plot 4. For method validation, recovery tests were performed at two spiking levels (10MLOQ and 25MLOQ) in both radishes and limit of quantification (LOQ) and method limit of quantitation (MLOQ) were also determined. Average recoveries for young radish samples ranged 79.2 - 115.7% with <10% RSD (relative standard deviation). For small radish samples,

average recoveries ranged 74.1 - 114.9% with RSD <10%. For test of storage stability, recoveries were obtained at one fortification level (25MLOQ) in young radish samples (ranged 78.7 - 114.2%) and small radish samples (ranged 74.5 – 115.4%) with RSD <10%, regardless of any sample type. LOQ for spinetoram was 0.005 mg L^{-1} (S/N>10) and MLOQ was 0.01 mg L^{-1}. Based on the results from this study, pesticide registration status of spinetoram in young radish was established by Rural Development Administration (6th January 2016). The status specifies that, to control beet armyworm in young radish, the commercial insecticide product containing spinetoram should be diluted 2,000 times and can be applied twice to young radish anytime 7 days before harvest.

Key Words: Spinetoram, XDE-175-J, XDE-175-L, LC-MS/MS, LOQ, MLOQ, QuEChERS, Minor crops, Insecticide, Young radish, Small radish

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Table of Contents

Abstract……i

Table of Contents…….iii

List of Tables……... vi

List of Figures……vii

List of Abbreviations……viii

Introduction……1

Materials and Methods……7

Subject pesticides.....7

Chemicals and reagents.....12

Standard solutions.....12

Subject crops.....12

Apparatus and equipment.....12

Analytical instruments and conditions.....13

Field trials…….....13

Pesticide application and sample collection….....17

Standard and sample preparation.....21

Method validation (LOD, LOQ)…….....21

Calibration curve and linearity…….....22

Calculation of MLOQ…….....22

Recovery test of spinetoram and its metabolites in crop samples by using QuEChERS extraction method…….....22

Storage stability test of spinetoram and its metabolites in crop samples by using QuEChERS method…….....23

Determination of total concentration of compounds.....23

Results and Discussion…….……..……..25

Field condition temperature and humidity.....25

Pesticide application and sample collection…….....28

Residue analysis of spinetoram and its metabolites from two crop...30

Optimization of LC-MS/MS condition for spinetoram and its metabolites.....35

Method validation (LOD, LOQ)…….....37

Linearity of calibration curve of spinetoram and its metabolites.....40

Calculation of MLOQ…….....45

Recovery test of spinetoram and its metabolites from young and small radishes samples…….....45

Storage stability test of spinetoram and its metabolites in young and small radishes samples ……...…….....49

Establishment of guidelines on safe use of spinetoram and metabolites……...…….....……53

References…….……54

Abstract in Korean…….…..…60

List of Tables

Table 1. Analytical method of spinetoram described in literatures..4

Table 2. Physicochemical properties of spinetoram……...……..10

Table 3. MRLs of spinetoram in various crops…….11

Table 4. Pesticide application and collection dates for young and small radishes …….29

Table 5. Maximum residual amount of spinetoram and its metabolites in young and small radishes……..….31

Table 6. Total concentration of spinetoram and its metabolites in young and small radishes…...…….…...….34

Table 7. The optimal transition parameters of LC-MS/MS for Spinetoram and its metabolites…...…..…….…….....36

Table 8. Calibration curve and r^2 value in young radish (leaf and root) of spinetoram and its metabolites……...……..…..…..43

Table 9. Calibration curve and r^2 value in small radish (leaf and root) of spinetoram and its metabolites……..…..…..44

Table 10. Recovery test (10, 25MLOQ) of young and small radishes of spinetoram and its metabolites....…….….46

Table 11. Through storage stability test, recoveries with RSD in young and small radishes…….....……..…….....52

List of Figures

Figure 1. Structure of spinetoram and its metabolites …….9

Figure 2. Diagram of field trial in greenhouse young and small radishes.…….……..…….16

Figure 3. Pesticide application and sample collection….……..…20

Figure 4. Field condition temperature and humidity in greenhouse ……...27

Figure 5. Representative chromatogram of residue analysis of spinetoram and its metabolites in young and small radishes.....…...33

Figure 6. Chromatogram of LOD and LOQ of spinetoram…....….39

Figure 7. Representative calibration curve of spinetoram and its metabolites…….....….42

Figure 8. Representative chromatogram of recovery test of spinetoram and its metabolites in young and small radishes…...….48

Figure 9. Representative chromatogram of Storage stability test of spinetoram and its metabolites in young and small radishes…..….51

List of Abbreviations

ACN	acetonitrile
d-SPE	dispersive solid phase extraction
GC	gas chromatography
HPLC	high performance liquid chromatography
LC	liquid chromatography
LC/MS	liquid chromatography mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
MeOH	methanol
MLOQ	method limit of quantitation
MRLs	maximum residue limits
MS/MS	tandem mass spectrometry
PSA	primary secondary amine
QuEChERS	quick, easy, cheap, effective, rugged and safe
RSD	relative standard deviation
SRM	selected reaction monitoring
SC	suspension concentrate

Introduction

Minor Crops

Minor crop producers and public health program administrators typically have fewer pesticide options for pest management due to lack of economic return to registrants to develop and register and/or support reregistration of pesticides for minor uses (EPA report, 2009). Therefore, minor crops generally have no specific pesticide maximum residue limits (MRLs). In many cases, even though farmers grow minor crops using any pesticide available around in order to put them out on the market, their crops generally cannot pass the pesticide MRLs in food after the official and compulsory analysis of pesticide residue (Bae et al., 2012).

Minor crops are defined as crops that are cultivated on limited acreage, produced as a strain of major crops for niche markets, provide relatively low income for farmers, receive limited or no research investments in either the public or private sectors (Park et al., 2012). These are also referred to as ‘orphan crops’ or ‘underutilized crops’ because of their lack of global cultivation and utilization (Park et al., 2009). These crops should also be a matter of interest, but they are not always as actively monitored for food safety because human exposure to residues related with minor crops would be low due to relatively small amounts of minor crops consumed (IUPAC, 2011).

Ten major crops that farmers grow around the world, are wheat, maize, rice, barley, sugar cane, soybeans sunflower, potatoes, and pulses while ten minor crops are oats, grapes, orange, coffee, linseed, cocoa, olives, apple, buckwheat, and sweet potatoes (Leff et al., 2004). In Korea, in terms of productivity representative minor crops are angelica, celery, small radish, young radish, beet, red cabbage, horseradish, carnola, wild rocambol, and fig (RDA, 2013). In Korea, among these crops, small and young radishes are consumed by many Korean people as Kimchi. These radishes have been increasingly damaged by beet armyword attacks these days. Spinetoram is now available for control of this pest. Spinetoram has been registered for use on numerous agricultural crops, home gardens, commercial aquatic plant production, ornamentals grown outdoors, tree farms, and lawns since 2007 in the US (EPA, 2009) Spinetoram, a reduced-risk insecticide, is created by the chemical modification of spinosyn, which is derived from fermentation of actinomycete bacterium, Saccharopolyspora spinosa (DeAmicis et al., 2011). It acts in a unique manner at insect nicotinic acetylcholine receptors (Krämer and Schirmer, 2007). Spinetoram provides long-lasting control over a broad spectrum of pests, including Lepidoptera larvae, leaf miners, and thrips on a variety of crops, as well as having a low impact on most beneficial insects (AgroSciences, 2006). Since spinetoram was only recently developed and registered, there are not many analytical methods for spinetoram currently available. In the published

literature few methodologies have been developed for the determination of XDE-175-J and XDE-175-L in vegetable samples (Liu et al., 2011). (Table 1.)

Table 1. Analytical method of spinetoram described in the literatures

Pesticide	Sample	Instrument	Reference
Spinetoram	Amaranth Parsley	LC-MS/MS	(Park et al., 2012)
Spinetoram	Garland chrysanthemum Aster scaber	LC-UVD LC-MS/MS	(Liu et al., 2011)
Spinetoram	Tomato	LC-DAD	(Malhat, 2013)
Spinetoram	livestock	LC-MS/MS	(Ko et al., 2016)

Analytical Methods for Pesticide Residual Analysis

Liquid chromatography mass spectrometry (LC/MS) is an analytical chemistry laboratory technique for identification, quantitation and mass analysis of materials. This technique allows for the structural elucidation of unknown molecules through fragmentation. With recent advances in LC tandem mass spectrometry (LC-MS/MS) instrumentation, this technique is quickly gaining acceptance for pesticide residue testing. LC-MS/MS can be used to simultaneously monitor hundreds of potential contaminants—including those difficult to detect by GC. MS/MS technology also allows identification of the target pesticides through the selection of specific SRM transitions for each compound (Grimalt and Dehouck, 2016).

QuEChERS (quick, easy, cheap, effective, rugged and safe) Method

Combined with the instrumental techniques, the QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction method has been internationally accepted for pesticide residue approaches and thoroughly investigated by many researchers since it was first introduced by Anastassiades and coworkers in 2003 (Wilkowska and Biziuk, 2011). It is the method of choice for food analysis because it combines several steps and extends the range of pesticides recovered over older, more tedious extraction techniques (González-Curbelo et al., 2015).

The traditional methods often give poor quantitation and involve a single analyte or analytes from a single class of compounds. On the other hand,

QuEChERS methodology reduces sample size and quantities of laboratory glassware. Clearly, QuEChERS requires fewer steps (no blending, filtration, large volume quantitative transfers, evaporation/condensation steps, or solvent exchanges required): this is very significant, as every additional analytical step complicates the procedure and is also a potential source of systematic and random errors. It is widely recognized that the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method is relevant in pesticide residue analysis.

The Purpose of the Study

This study was conducted as a part of an effort to extend the applications of registered pesticide products to the minor crops, young radish and small radish. A suspension concentrate (SC) of spinetoram was applied to the two crops grown under the greenhouse conditions, and the presence of XDE-175-J and XDE-175-L and their four metabolites (N-demethyl-175-J/L and N-formyl-175-J/L) were determined using LC–MS/MS.

Materials and Methods

Subject pesticides

Standard material of spinetroram is the sum of XDE-175-J and XDE-175-L. Spinetoram (XDE-175) is a multicomponent tetracyclic macrolide developed for the control of Lepidoptera larvae, Leafminers, and Thrips on a variety of crops. It consists of two closely related active ingredients, XDE-175-J and XDE-175-L, present in an approximate 3:1 ratio. The structures of the target compounds are shown in Figure 1. The only difference between XDE-175-J and XDE-175-L is that XDE-175-L contains an extra methyl group at carbon 4 on the central ring. XDE-175-J (97.6%) and XDE-175-L (100%) was kindly provided by Dongbang AgroSciences (Seoul, Republic of Korea). Metabolites of Spinetoram (N-demethyl-175-J (96.0%), N-demethyl-175-L (98.0%), N-formyl-175-J (78.0%), N-formyl-175-L (96.0%) were also kindly provided by the same company. (Figure 1.)

Figure 1. Structure of Spinetoram and its metabolites

(A) XDE-175-J (B) XDE-175-L (C) N-demethyl-J (D) N-demethyl-L

(E) N-formyl-J (F) N-formyl-L

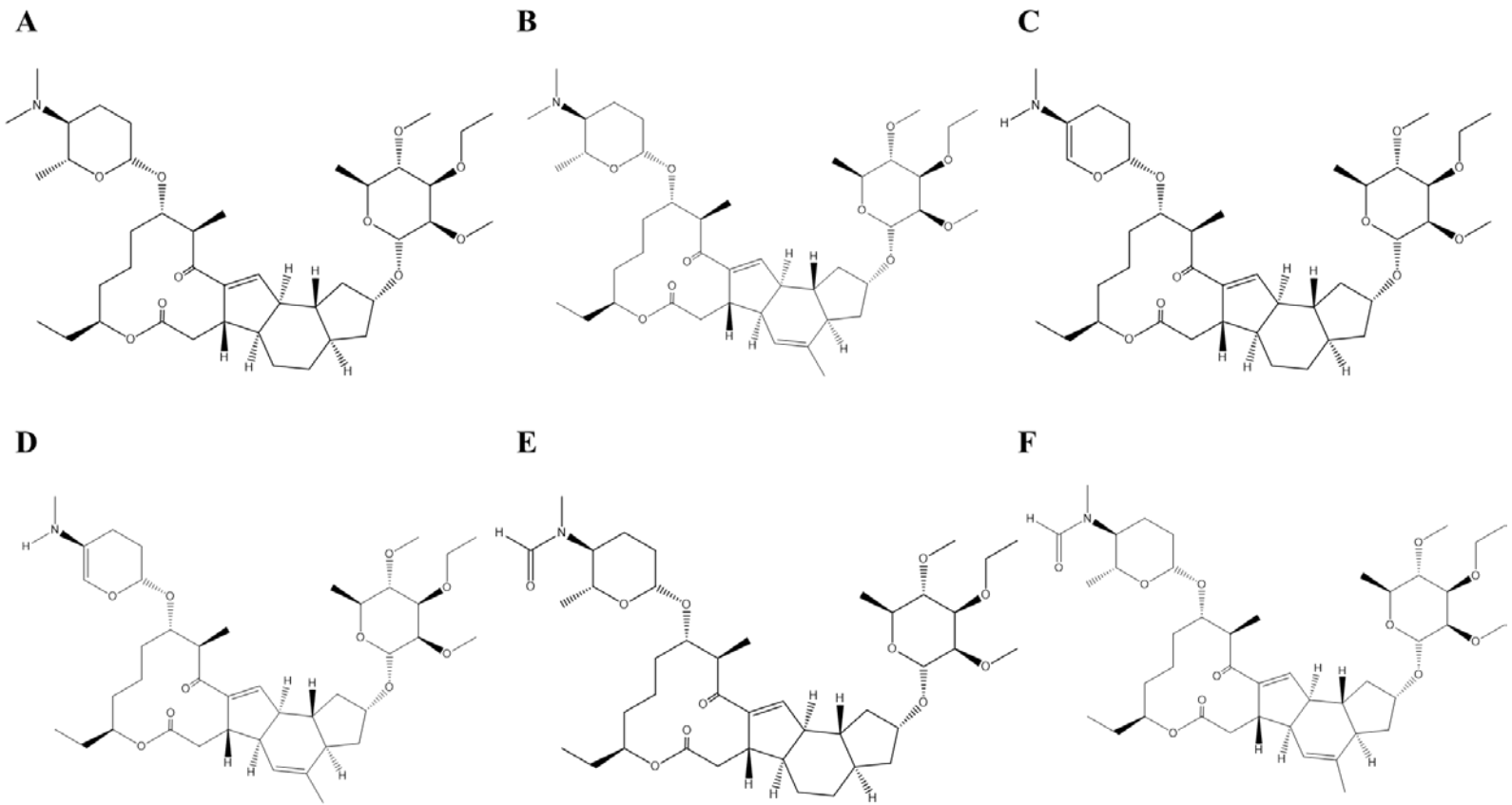

A
B
C
D
E
F

Table 2. Physicochemical properties of spinetoram

Property	**Spinetoram**	
Composition	Spinetoram J (major component)	Spinetoram L (minor component)
IUPAC name	Mixture 50-90% (2R, 3aR, 5aR, 5bS, 9S, 13S, 14R,16aS, 16bR)-2-(6-deoxy-3-O-eth yl-2,4,-di-O-methyl-α-L-mannopyranosyloxy)-13-[(2R, 5S, 6R)-5-(dimethylamino)tetrahydro-6-methylpyran-2-yloxy]-9-ethyl-2, 3, 3a, 4, 5, 5a, 6, 9, 10, 11, 12, 13, 14, 16a,16b-hexadecahyd ro-14-methyl-1H-as-indaceno[3,2-d] oxacycl ododecine-7-15-dione	Mixture 50-10% (2R, 3aR, 5aR, 5bS, 9S, 13S,14R, 16aS, 16bR)-2-(6-deoxy-3-O-ethyl-2,4,-di-Omethyl-α-L-mannopyranosyloxy)-13-[(2R, 5S, 6R)-5-(dimethylamino)tetrahydro-6-methyl pyran-2-yloxy]-9-ethyl-2,3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a,16b-tetradecahydr o-4,14-di methyl-1H-as-indaceno-[3,2-d]oxacyclododecine-7-15-dione
CAS No.	187166-40-1	187166-15-0
Classification	Insecticide	
Mode of action	Active by contact and ingestion; causes paralysis.	
Molecular formula	$C_{42}H_{69}NO_{10}$	$C_{43}H_{69}NO_{10}$
Molecular weight	748.0	760.0
Log Pow	2.44 (pH 5) 4.09 (pH 7) 4.22 (pH 9)	2.94 (pH 5) 4.49 (pH 7) 4.82 (pH 9)
pKa	7.86 (25℃)	7.59 (25℃)
Vapor pressure	5.3 × 10−2 mPa (20℃)	2.1 × 10−2 mPa (20℃)
Solubility in water	423 (pH 5), 11.3 (pH 7), 6.27 (pH 10) all in mg/L, 20℃	1630 (pH 5), 46.7 (pH 7), 0.706 (pH 10) all in mg/L, 20℃
Toxicology	LD_{50} for rats >5000 mg/kg LC_{50} (96 h) for rainbow trout >3.46	
Residue	Rapidly degraded in soil, field dissipation DT_{50} 3-5 days Aquatic field dissipation DT_{50} <1 day MRLs : (Radish root) 0.3 mg L^{-1}, (Radish leaf) 2.0 mg L^{-1} (Table 3.)	

Table 3. MRLs of spinetoram in various crops

Crop	MRLs (mg L^{-1})	Crop	MRLs (mg L^{-1})
Radish (root)	0.3	Beet (root)	0.05
Radish (leaf)	2.0	Beet (leaf)	0.05
Mandarin	0.5	Lettuce	7.0
Mustard leaf	0.3	Leaf beet	1.0
Korean Cabbage	0.3	Cabbage	0.05

Chemicals and reagents

Acetonitile (ACN) and methanol (MeOH) were HPLC grade and purchased from Fisher ChemAlert® (Fisher Scientific, USA). The QuEChERS materials were obtained from commercial suppliers. For extraction crops, 'Ultra QuECh extract kit' (Ultra Scientific, USA), containing 4 g of magnesium sulfate ($MgSO_4$), 1 g of NaCl, 1 g of $Na_3Citrate \cdot H_2O$ and 0.5 g of $Na_2HCitrate \cdot 1.5H_2O$ was used. For the dispersive SPE (d-SPE) cleanup of crop extracts from young radish and small radish samples, 'Ultra QuECh dSPE-General' (2 mL centrifuge tubes containing 150 mg of $MgSO_4$ and 25 mg of primary secondary amine (PSA), Ultra Scientific, USA) was used.

Standard solutions

Each analytical standard was dissolved in ACN to make concentrated stock solution at concentration of 1000 mg L^{-1}. The working solutions were prepared by serial dilutions of the stock solutions with ACN.

Subject crops

Yong radish and small radish of "pesticide-free (i.e. no pesticide residues are present above the detection limits of the multi-residue method)" grade were harvested from field trials. After being chopped, macerated, the sample were kept in polyethylene bags in a freezer (-20℃) until experiment.

Apparatus and equipment

Combi 408 centrifuge and micro 17TR high speed centrifuge (Hanil, Repubilc of Korea) were used. Vortex equipment vortex-genie®2 (Scientific Industries,

USA) was used.

Analytical instruments and conditions

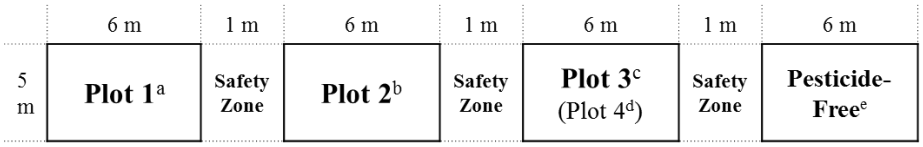
LC–MS/MS analysis was performed on LCMS-8040 (Shimadzu, Japan) coupled to Nexera UHPLC (Shimadzu, Japan) with electrospray (ESI, positive mode). The analytical column was a Kinetex C18 (100 × 2.1 mm i.d., 2.6 μm, Phenomenex®, USA) column and the column oven temperature was set at 40ºC. The injection volume was 2 μL and the mobile phases were eluted at a 0.2 mL min^{-1}. Mobile phases were 0.1% formic acid (CH_2O_2), 5 mM ammonium formate (NH_4HCO_2) in water (A) and 0.1% CH_2O_2, 5mM NH_4HCO_2 in MeOH (B). For gradient elution, the initial combination was 30 : 70 (A : B, v/v) and the B solution was increased to 95 % in duration of 2 min, held for 50 s. To establish the selected reaction monitoring (SRM) condition on LCMS-8040, precursor ions, product ions, Q1 and Q3 pre bias voltage, and collision voltage were optimized through the flow injection of spinetoram (XDE-175-J/L), metabolites (N-demethyl-175-J/L and N-formyl-175-J/L) and standard solutions (0.005 mg L^{-1}).

Field trials

Field trials on young and small radishes were carried out in a separate greenhouse cultivation respectively, located in 540, Migok-ri, Daeso-myeon, Eumseong-gun, Chungcheongbuk-do, Republic of Korea. The greenhouse sizes were 27 m (length) x 5 m (width) and each treatment was made in triplicate in each plot (10 m^2). Safety zone (1 m) between each plot (Figure 2.)

was designed to prevent cross contamination when applying pesticides. The temperature and humidity in a greenhouse were continuously every hour measured during young and small radish cultivation. The field trial was conducted from September to November of 2014.

Figure 2. Diagram of field trial in greenhouse young and small radishes



[a] Plot 1: Treated twice at 21-14 days before harvest with 7 days interval.
[b] Plot 2: Treated thrice at 30-21-14 days before harvest with 9, 7 days interval.
[c] Plot 3: Treated thrice at 21-14-7 days before harvest with 7 days interval.
[d] Plot 4: Treated thrice at 14-7-0 days before harvest with 7 days interval.
[e] Pesticide-Free : No treated.

Pesticide application and sample collection

Spinetoram 5% SC (suspension concentrate) with product name of ‘Ariexcert’ (Dongbang AgroSciences Co., Ltd.) was diluted 2,000 times with water and sprayed on to the two crops (young radish, small radish) grown at the plot 1, 2 and 3 (4) in order using a pressurized handgun sprayer. When application pesticides, everytime prepared from pesticides to each plot by using 20L hand sprayer; diaphragm pump, lithium-ion battery, three nozzles, injection quantity per hour 1,750mL.

Young and Small radishes had the same treatment at greenhouse. The pesticides were applied to the plants with 7-day interval. Plants in plot 1 was treated twice at 21 and 14 days before harvest and plot 2 was treated three times at 30, 21 and 14 days before harvest. Plot 3 was also treated three times at 21, 14 and 7 days before harvest and plot 4 was treated three times at 14, 7 and 0 days before harvest. Plot 3 and 4 was the same plot but they were harvested in a different time. The plants from plot 4 were obtained to measure the initial concentration when the pesticide was applied last day planned.

Young radish and small radish samples were collected at plot 1, 2, 3 (4) and pesticide-free area. Over 2.0 kg of young and small radishes from 4 different plots of each treatment were randomly collected. Radishes with the similar size were selected. (Figure 3.)

As soon as the plants were harvested, they were put in polyethylene bags and transported with ice to the laboratory. When the samples were transported,

each radish was carefully separated into two parts; root and leaf. All the samples from the same plot were mixed and homogenized using a homogenizer (Hanil, Repubic of Korea) and then placed into polyethylene bags in a freezer (-20℃) until analysis.

Figure 3. Pesticide application and sample collection

Standard and sample preparation

Each analytical standard was dissolved in ACN to prepare concentrated stock solution at concentration of 1000 mg L^{-1}. The working solutions were prepared by serial dilutions of the stock solutions with ACN. Samples were prepared using the modified QuEChERS extraction method previously described (Anastassiades, 2003). Young or small radish sample (10 g) homogenized was placed into a 50 mL propylene tube and 10 mL ACN was added to the tube. And then, the tube was vigorously shaken for 2 min by hand, followed by 'Ultra QuECh extract kit'. The tube was vigorously shaken for 2 min by hand and placed into the ice bath to prevent breakdown of the target analytes. And then the tube was centrifuged for 5 min at 3500 rpm. Approximately 1 mL of the upper layer was transferred to 2 mL d-SPE tubes. The tube was vortexed for 1 min followed by centrifugation for 5 min at 13000 rpm. The supernatant (0.5 mL) was transferred into an analytical LC vial and 0.5 mL of ACN was added for the LC–MS/MS analysis.

Method validation (LOD, LOQ)

Matrix matched standard solutions (0.001 and 0.005 mg L^{-1}) prepared for both radishes were analyzed using LC-MS/MS. LOD and LOQ were determined as the minimum concentration of analyte providing S/N ratio of 3 and 10, respectively.

Calibration curve and linearity

Matrix matched standard solutions (0.005, 0.0125, 0.0250, 0.0500 and 0.2500 mg L^{-1}) were prepared by serially diluting standards of spinetoram (XDE-175-J/L) and its metabolites (N-demethyl-J/L, N-formyl-J/L) with ACN (0.010, 0.025, 0.050, 0.100 and 0.500 mg L^{-1}) for quantitative analysis using LC-MS/MS. For preparation of the matrix matched standard, 500 µL of each level of standards diluted with ACN were mixed with 500 µL of unfortified crop samples which were processed by QuEChERS method. The linearity was examined by r^2 value.

Calculation of MLOQ

MLOQ is calculated by Equation according to the sample amount, extraction procedure, rate of dilution and instrumental system.

$$\text{MLOQ (mg L}^{-1}) = \frac{\text{LOQ (ng)} \times \text{Final volume (mL)} \times \text{Dilution factor}}{\text{Injection volume (µL)} \times \text{Initial sample weight (g)}}$$

Recovery test of spinetoram and its metabolites in crop samples by using QuEChERS extraction method

After harvest, homogenized the pesticide-free grade two crops (young radish, small radish separated into two parts; root and leaf) samples (10 g was placed into a 50 mL propylene tube) were fortified with spinetoram (XDE-175-J/L) and its metabolites (N-demethyl-J/L, N-formyl-J/L) standard solution at spiking level of 0.1 and 0.25 mg L^{-1} (10 MLOQ and 25 MLOQ). All the samples were prepared using the analytical methods paltaroposed in this study for LC-

MS/MS analysis.

Storage stability test of spinetoram and its metabolites in crop samples by using QuEChERS method

After harvest, homogenized the pesticide-free grade two crops (young and small radish) samples (10 g was placed into a 50 mL propylene tube) were fortified with spinetoram (XDE-175-J/L) and its metabolites (N-demethyl-J/L, N-formyl-J/L) standard at 0.1 and 0.25 mg L^{-1} (10MLOQ and 25MLOQ) spiking level. And the samples were left for 30 min, they were placed into polyethylene bags in a freezer (-20℃) until analysis. In this experiment, the young radish sample was stored in a freezer (-20℃) for 39 days (Nov 1 ~ Dec 9, 2014) and small radish sample in a freezer (-20℃) for 42 days (Nov. 1 ~ Dec. 12, 2014) before sample analysis. All the samples were prepared using the analytical methods proposed in this study when the real samples were prepared for the LC-MS/MS analysis.

Determination of total concentration of compounds

The residue of spinetoram (XDE-175-J/L) and its metabolites (N-demethyl-175-J/L and N-formyl-175-J/L) were determined from the young and small radishes samples after harvest. The residual concentration was determined using matrix-matched calibration curves since isotope-labelled internal standards for the analytes were not available. The field-incurred samples with residues above the linear range were diluted accordingly with ACN.

The total residue was calculated by summing the spinetoram and its

metabolite residues, as follows:

$$\text{Residue of P} + [\text{residue of M} \times \frac{\text{MW of P}}{\text{MW of M}}]$$

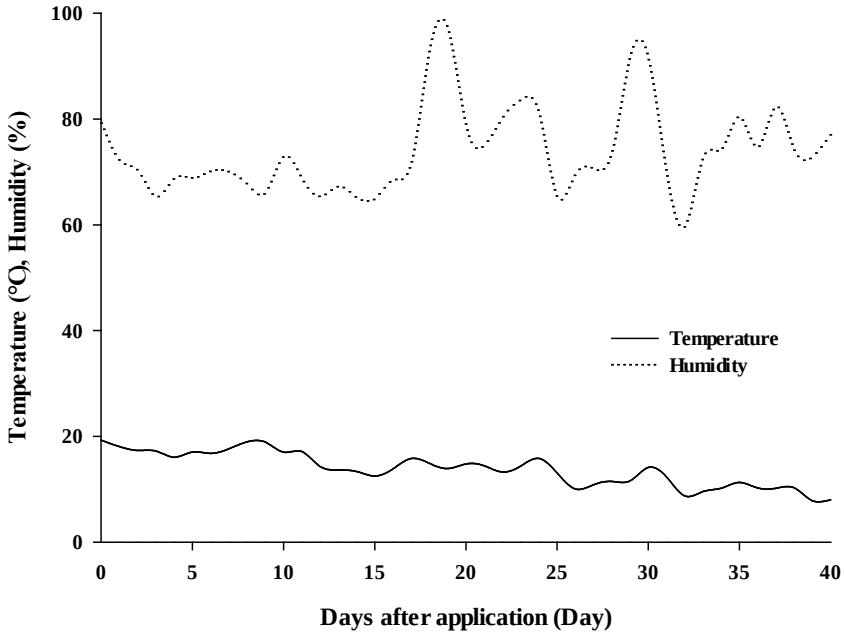
where P, M and MW refer to the parent compound, metabolite and molecular weight, respectively (Pihlström et al., 2009)

Results and Discussion

Field condition temperature and humidity

During young radish and small radish cultivation, the green house air temperature range was 7.8 - 19.3℃ and humidity was 59.7 - 97.0%. (Figure 4.)

Figure 4. Field condition temperature and humidity in greenhouse


100
80
60
40
20
0
Temperature (°C), Humidity (%)
0
5
10
15
20
25
30
35
40
Days after application (Day)
Temperature
Humidity

Pesticide application and sample collection

Method of prepared from pesticide was ‘Ariexcert’ 2,000 times dilution foliage application. Spent dosage was plot 1 10.7 L, plot 2 17.7 L, plot 3 (4) 20.2 L in young radish greenhouse and plot 1 10.9 L, plot 2 19.4 L, plot 3 (4) 20.3 L in small radish greenhouse. In addition, application on fixed day which shows table.

Samples were collected by each plot over 2.5 kg in young radish and 2.0 kg in small radish. Two radishes pesticide-free samples were collected over 8.0 kg at November 1, 2014 except plot 4. (Table 4.)

Table 4. Pesticide application and collection dates for young and small radishes

Sample	Plot	Before Harvest Application	No. of Application	Application Date	Application (L Plot^{-1})	Pesticide Concentration (mL L^{-1})	Samples Weight (kg)	Harvest Date
Young radish	No Pesticide	-	0	-	-	-	8.4	Nov 1
	1	21-14	2	Oct 11, & 18	10.7	0.5	2.9	
	2	30-21-14	3	Oct 2, 11, & 18	17.7		2.7	
	3	21-14-7	3	Oct. 11, 18, & 25	20.2		2.6	
	4	14-7-0	3	Oct 11, 18, & 25	20.2		2.8	Oct 25
Small radish	No pesticide	-	0	-	-	-	8.5	Nov. 1
	1	21-14	2	Oct. 11, & 18	10.9	0.5	2.1	
	2	30-21-14	3	Oct. 2, 11, & 18	19.4		2.0	
	3	21-14-7	3	Oct. 11, 18, & 25	20.3		2.1	
	4	14-7-0	3	Oct. 11, 18, & 25	20.3		2.0	Oct. 25

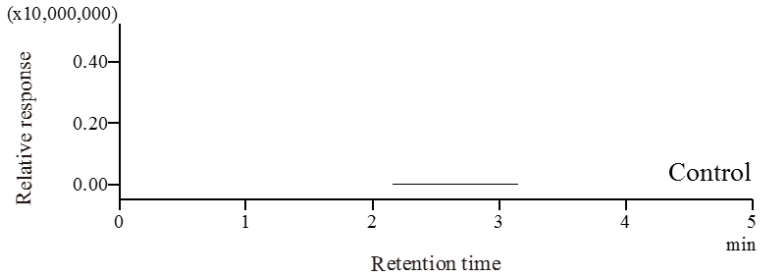
Residue analysis of spinetoram and its metabolites from two crops

No residues were detected in any untreated sample. The residual concentrations of spinetoram and metabolites in both minor crops were respectively the overall residues of the J-form analytes were higher than those of the L-form analytes in both treated crops. This was possibly due to the large proportion of XDE-175-J in the active ingredients. N-formyl-175-L was not quantified in all the treated samples. The results in Table 2 confirmed that the L-metabolites are an extremely minor component of the total residue and confirm their non-inclusion in global residue definitions. Overall, spinetoram (as total residue) had a tendency to remain more in small radish rather than young radish in Table 3. It should be noted that the total residues exceeded the existing maximum MRL value (0.3 mg L^{-1} radish root, 2.0 mg L^{-1} radish leaf) Republic of Korea (KFDA, 2016) in all samples. Although the MRLs of spinetoram for amaranth and parsley are not established yet, the residual pattern of spinetoram showed that the dissipation rate was low (0.49-4.49 mg L^{-1}) of both crops. Therefore the MRLs should be established based on the observed residues.

Table 5. Maximum residual amount of spinetoram and its metabolites in young and small radishes

Sample	Section	Plot	Residual maximum amount of spinetoram and Metabolites (mg L^{-1})					
			XDE-175-J	XDE-175-L	N-demethyl-XDE-J	N-demethyl-XDE-L	N-formyl-XDE-J	N-formyl-XDE-L
Young Radish	Root	1	0.03	0.01	0.02	<0.01	0.01	<0.01
		2	0.02	0.01	0.01	<0.01	0.01	<0.01
		3	0.05	0.02	0.03	0.01	0.01	<0.01
		4	0.04	0.01	0.01	<0.01	<0.01	<0.01
	Leaf	1	0.14	0.04	0.07	0.01	0.06	<0.01
		2	0.15	0.05	0.06	0.01	0.05	<0.01
		3	0.25	0.07	0.10	0.01	0.07	<0.01
		4	0.48	0.19	0.17	0.05	0.07	<0.01
Small Radish	Root	1	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		2	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		3	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		4	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Leaf	1	0.28	0.13	0.14	0.01	0.11	<0.01
		2	0.31	0.14	0.16	0.01	0.13	<0.01
		3	0.57	0.29	0.16	0.01	0.17	0.01
		4	0.94	0.56	0.18	0.03	0.14	0.01

Figure 5. Representative chromatogram of residue analysis of spinetoram and its metabolites in young and small radishes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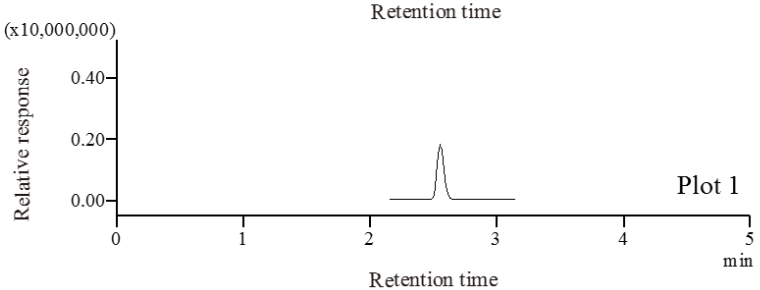
2
3
4
5
min
Retention time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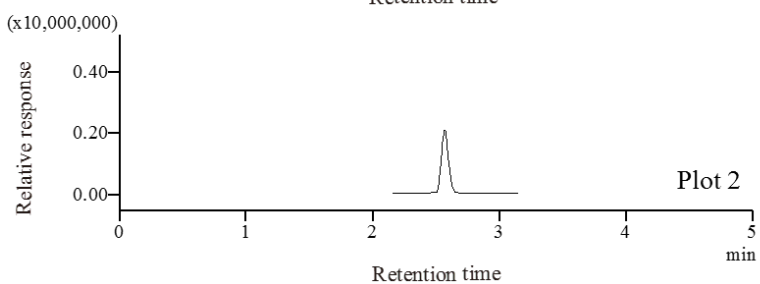
min
Retention time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1
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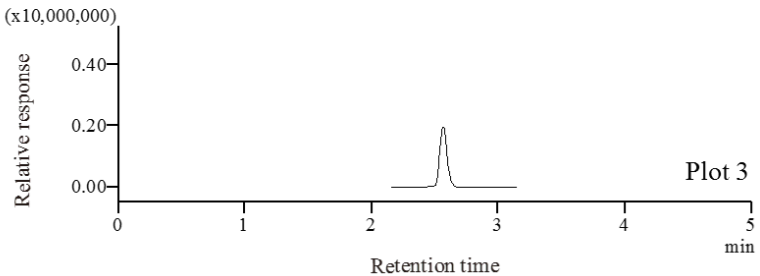
4
5
min
Retention time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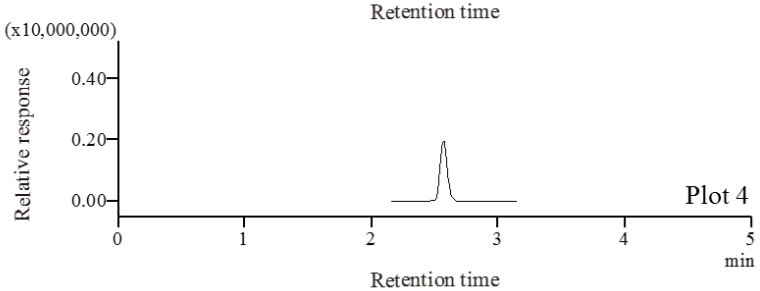
1
2
3
4
5
min
Retention time


(x10,000,000)
Relative response
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0.20
0.00
Plot 4
0
1
2
3
4
5
min
Retention time

Table 6. Total concentration of spinetoram and its metabolites in young and small radishes

Plot	Total concentration of Spinetoram and Metabolites (mg L^{-1})						MLOQ (mg L^{-1})
	Young radish			Small radish			
	Root	Leaf	Total	Root	Leaf	Total	
1	0.07	0.33	0.40	0.01	0.68	0.69	0.01
2	0.05	0.32	0.37	0.02	0.78	0.80	
3	0.11	0.50	0.61	0.01	1.21	1.22	
4	0.06	0.90	0.96	0.01	1.85	1.86	

Optimization of LC-MS/MS condition for spinetoram and its metabolites

LC–MS/MS offers very sensitive, selective and rapid analysis compared with the conventional HPLC. When SRM mode was used in this study, low concentrations (ppb level) of the target compounds in crop samples were observed as MLOQs.

On LC-MS/MS, the protonated molecular ion $[M + H]^+$ at *m/z* 748.0 and 760.5 for spinetoram (XDE-175-J/L), and *m/z* 734.0, 746.0, 762.0 and 774.0 for N-demethyl-175-J/L and N-formyl-175-J/L at the positive ESI mode. And product ions of spinetoram and its metabolites were selected in product scan during the SRM optimization of Q1 pre bias, Q3 pre bias and collision voltages (Table 7.). The other conditions such as DL temperature, nebulizing gas flow, heat block temperature were set at the recommended values of the instrument. Good and clear separation was observed on SRM for spinetoram and metabolites in a variety of samples. Method limit of quantitation (MLOQ) were 0.01 mg L^{-1} (spinetoram) and 0.01 mg L^{-1} (metabolites) for radishes samples.

Table 7. The optimal transition parameters of LC-MS/MS for spinetoram and its metabolites

Compound	Exact Mass	Ionization	Precursor ion (m/z)	Product ion (m/z)		Fragmentor (v)		RT (min)
				Quantitation	Qualification			
XDE-175-J	748.0	$[M+H]^+$	748.5	142.2	98.2	-32	-55	2.633
XDE-175-L	760.5	$[M+H]^+$	760.5	142.1	98.1	-33	-55	2.799
N-demethyl-175-J	734.0	$[M+H]^+$	734.5	128.1	84.2	-27	-50	2.656
N-demethyl-175-L	746.0	$[M+H]^+$	746.5	128.1	84.2	-27	-50	2.793
N-formyl-175-J	762.0	$[M+H]^+$	762.5	156.1	560.3	-20	-12	20224
N-formyl-175-L	774.0	$[M+H]^+$	774.5	572.3	156.2	-11	-20	3.543

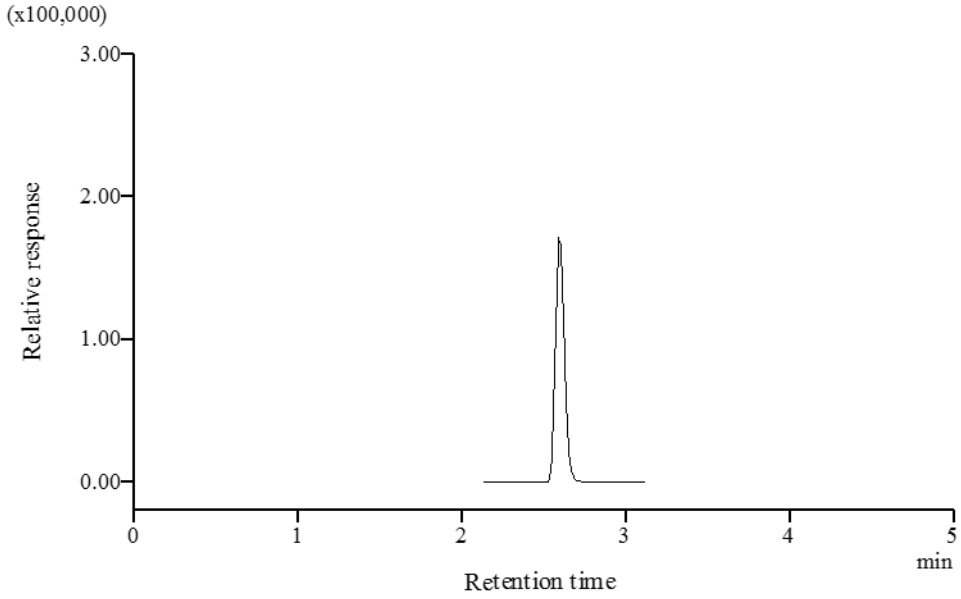
Method validation (LOD and LOQ)

LOD and LOQ express the sensitivity of instruments (Fong et al., 1999; Miller, 2005). From the results of analysis of several concentrations, 0.005 mg L^{-1} was observed as practicable LOQ. However, in the light of many interfering substance from various crops, and further research, 0.001 mg L^{-1} was determined as LOD. LOQ could be calculated by multiply LOD by 5. (Figure 6.)

Figure 6. Chromatogram of LOD and LOQ of spinetoram

(A) LOD 0.001 mg L^{-1} (B) LOQ 0.005 mg L^{-1}

(A)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Relative response
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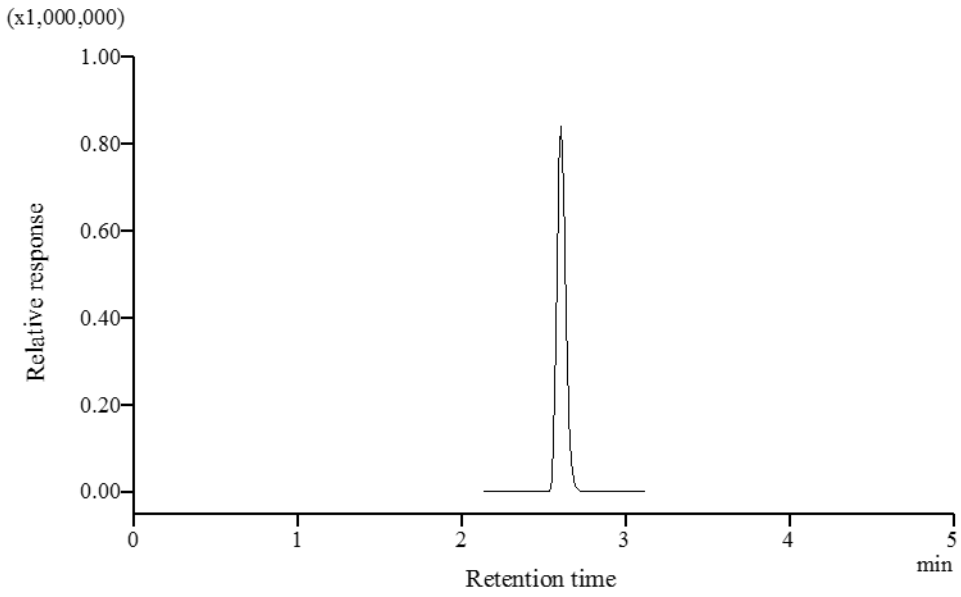
2
3
4
5
min
Retention time

(B)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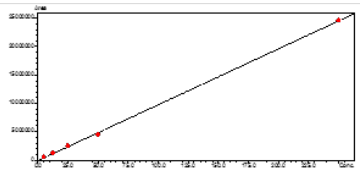
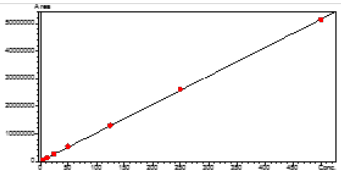
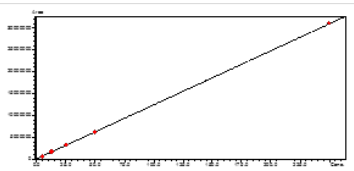
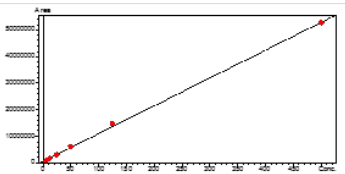
0.80
0.60
0.40
0.20
0.00
Relative response
0
1
2
3
4
5
min
Retention time

Linearity of calibration curve of spinetoram and its metabolites

For matrix matched standard curves of both young and small radishes, good linearity was achieved between 0.005 and 0.5 mg L^{-1} of spinetoram and its metabolites standard solutions. The regression equations were 24 calibration curves and coefficients of determination r^2) were over 0.999. (Figure 7, Table 8-9.)

Figure 7. Representative calibration curve of spinetoram and its metabolites

Spinetoram (XDE-175-J)

Young radish - Leaf	Young radish - Root	Small radish - Leaf	Small radish - Root
			
y = 92252.9x - 63294.7	y = 102710x+119371	y = 107842x + 145493	y = 29712.7x - 127.085
0.9996	0.9999	0.9999	0.9999

Spinetoram (XDE-175-L)

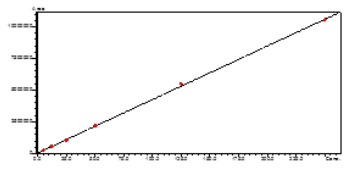
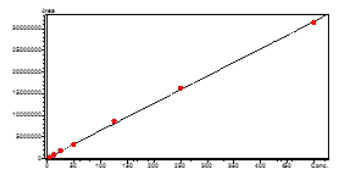
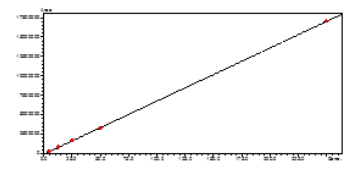
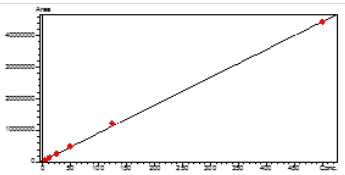
Young radish - Leaf	Young radish - Root	Small radish - Leaf	Small radish - Root
			
y = 42163.3x + 48310.9	y = 63019.8x + 217491	y = 131793x - 86599.7	y = 82997.3x - 25487.1
0.99995	0.9991	0.9999	0.9996

Table 8. Calibration curve and r^2 value in young radish (leaf and root) of spinetoram and its metabolites

Compound	Young radish leaf		Young radish root	
	The regression equations	r^2	**The regression equations**	r^2
XDE-175-J	y = 92252.9x - 63294.7	0.9996	Y=104208x+29241.5	0.9998
XDE-175-L	y = 42163.3x + 48310.9	0.9995	y = 65104.3x + 15341.2	0.9998
N-demethyl-175-J	y = 123300x + 117095	0.9999	y = 116640x + 152553	0.9999
N-demethyl-175-L	y = 68406.4x - 56761.2	0.9998	y = 98650.7x - 1179.83	0.9998
N-formyl-175-J	y = 13227.2x - 9515.99	0.9997	y = 15291.3x - 8360.61	0.9999
N-formyl-175-L	y = 9990.49x + 3993.65	0.9998	y = 12461.8x - 8573.96	0.9997

Table 9. Calibration curve and r^2 value in small radish (leaf and root) of spinetoram and its metabolites

Compound	Small radish leaf		Small radish root	
	The regression equations	r^2	The regression equations	r^2
XDE-175-J	y = 107842x + 145493	0.9999	y = 131793x - 86599.7	0.9999
XDE-175-L	y = 29712.7x - 127.085	0.9999	y = 82997.3x - 25487.1	0.9996
N-demethyl-175-J	y = 94337.0x + 386278	0.9998	y = 137146x + 212414	0.9999
N-demethyl-175-L	y = 53113.9x - 49769.8	0.9999	y = 120371x + 13409.5	0.9998
N-formyl-175-J	y = 10330.2x - 22050.3	0.9999	y = 14925.9x + 5757.57	0.9994
N-formyl-175-L	y = 9724.66x - 17647.4	0.9999	y = 12329.6x + 3662.89	0.9999

Calculation of MLOQ (Method Limit of Quantitation)

Based on MLOQ calculating equation, MLOQs were 0.01 mg L^{-1} in young and small radishes respectively.

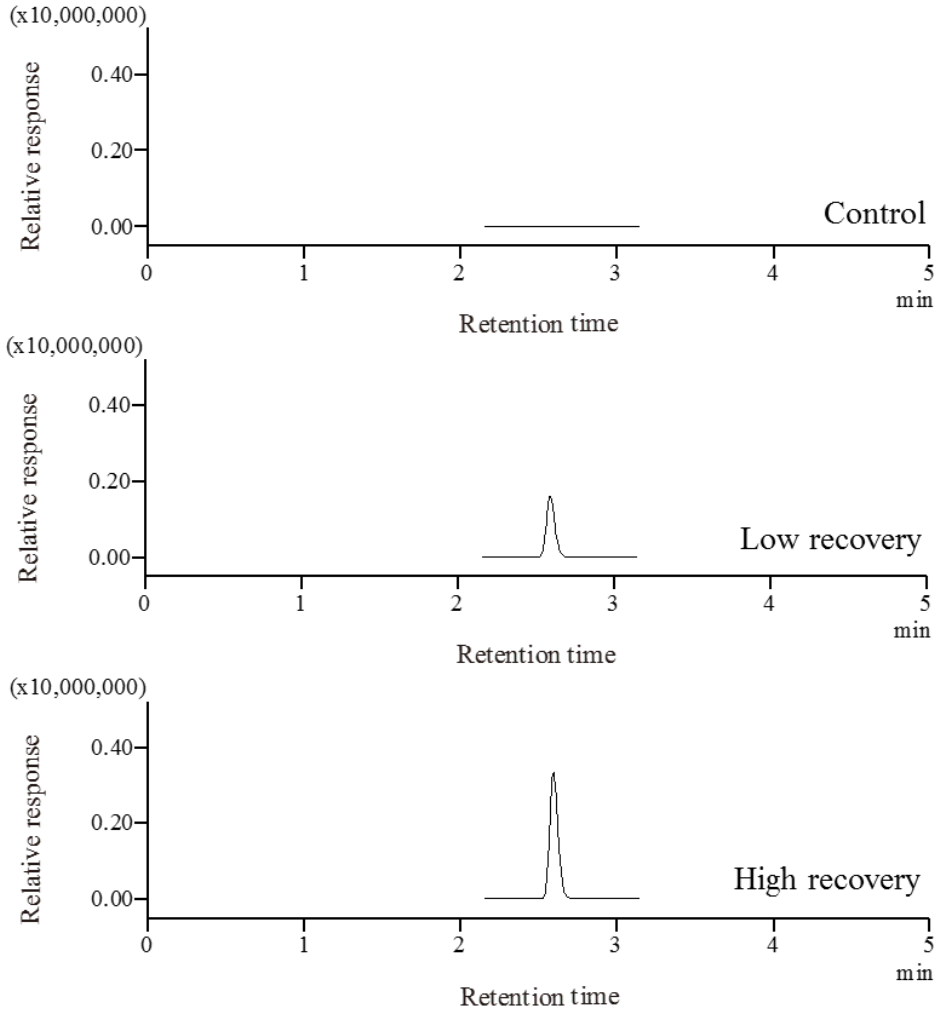
Recoveries of spinetoram and its metabolites from young and small radishes samples (accuracy and precision)

Recovery test can provide accuracy and precision of method validation by recovered rate (%) and RSD (relative standard deviation, %) (Fong et al., 1999). No pesticide samples were spiked with 10MLOQ and 25MLOQ (0.1, 0.25 mg L^{-1}) of spinetoram and its metabolites standard solutions, and the analysis was performed using the QuEChERS method of extraction, partitioning, and clean-up to give reasonable recoveries (%) and RSD (%). Young and small radishes sample recoveries were satisfied 70-120% and RSD value also satisfied less 10%. (Table 5.) The results showed that recoveries of young radish root samples ranged from 97.2 to 110.5 % with RSD 1.5 to 8.2 %, leaf samples ranged from 79.2 to 115.7 % RSD 0.6 to 9.0 %. And recoveries of small radish were ranged from 94.9 to 114.8 % with RSD 0.2 to 4.7 % and leaf samples ranged from 74.1 to 114.9 % with RSD 1.4 to 9.9 %. (Table 10.) These accuracy and precision tests indicated that the target compound and its metabolites were check recoveries.

Table 10. Recovery test (10, 25MLOQ) of young and small radishes of spinetoram and its metabolites

Crop	Section	Fortified level (mg L^{-1})	Recovery (%) / RSD (%)					
			XDE-175-J	XDE-175-L	N-demethyl-XDE-J	N-demethyl-XDE-L	N-formyl-XDE-J	N-formyl-XDE-L
Young Radish	Root	0.1	105.0/3.0	99.5/8.2	107.8/1.7	104.7/1.9	107.0/1.5	110.5/3.4
		0.25	97.7/3.9	103.4/3.2	100.8/2.7	97.2/2.5	97.7/3.5	102.2/2.7
	Leaf	0.1	104.9/1.0	114.8/3.8	89.8/3.5	115.7/0.9	97.9/1.4	108.2/9.0
		0.25	94.8/0.6	111.0/3.0	79.2/2.8	109.9/5.8	88.6/6.6	94.2/4.0
Small Radish	Root	0.1	102.1/1.8	114.8/1.9	104.0/1.0	105.2/0.8	107.0/1.1	103.1/0.9
		0.25	94.9/0.7	109.3/4.7	96.5/0.3	98.9/1.1	102.3/0.2	98.5/2.0
	Leaf	0.1	113.6/2.4	85.7/9.7	114.9/3.6	95.4/7.6	95.9/5.0	98.6/9.9
		0.25	104.3/2.2	82.7/5.8	105.8/1.9	87.5/1.4	74.1/1.5	87.4/1.7

Figure 8. Representative chromatogram of recovery test spinetoram and its metabolites in young and small radishes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0.20
0.00
Control
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1
2
3
4
5
min
Retention time
(x10,000,000)
Relative response
0.40
0.20
0.00
Low recovery
0
1
2
3
4
5
min
Retention time
(x10,000,000)
Relative response
0.40
0.20
0.00
High recovery
0
1
2
3
4
5
min
Retention time

Storage stability test of spinetoram and its metabolites

In pesticide residual analysis, it is generally difficult to carry out sample preparation immediately after sampling. Therefore samples have to be stored in the laboratory. Although samples usually are deep frozen, the question arises whether residues are degraded during storage. In this experiment, the fortified samples of young and small radishes were analyzed using the optimized method. The results showed that recoveries of young radish root samples ranged from 101.5 to 112.5 % with RSD 1.0 to 2.2 %, leaf samples ranged from 78.7 to 114.2 % RSD 0.9 to 4.1 %. And recoveries of small radish were ranged from 97.1 to 111.9 % with RSD 0.7 to 4.3 % and leaf samples ranged from 74.5 to 115.4 % with RSD 1.9 to 5.5 % (Table 11). These accuracy and precision tests indicated that the target compound and its metabolites were not degraded during the storage period.

Figure 9. Representative chromatogram of Storage stability test spinetoram and its metabolites in young and small radishes

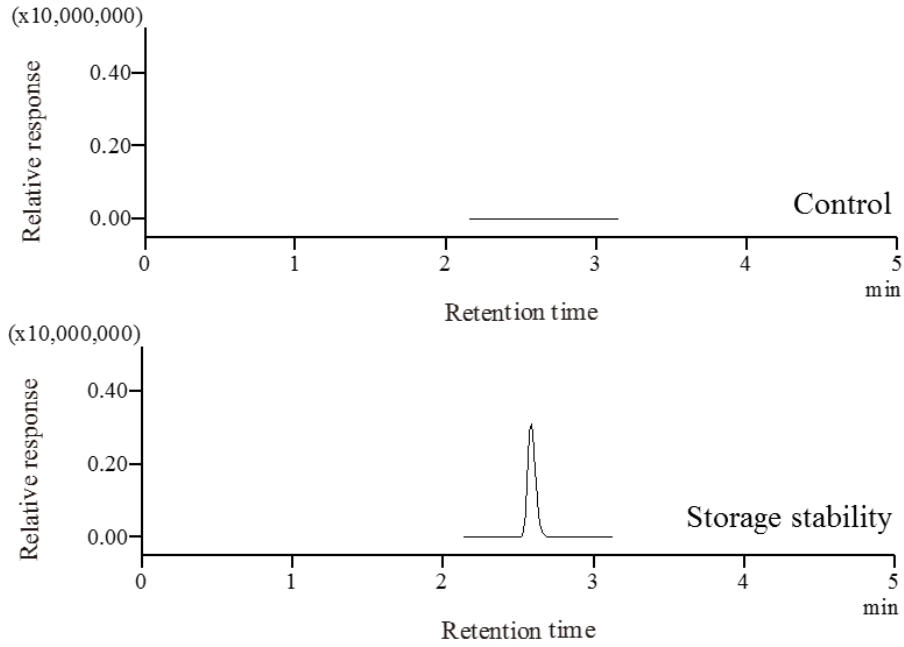
(x10,000,000)
Relative response
0.40
0.20
0.00
Control
0
1
2
3
4
5
min
Retention time
(x10,000,000)
Relative response
0.40
0.20
0.00
Storage stability
0
1
2
3
4
5
min
Retention time

Table 11. Through storage stability test, recoveries with RSD in young and small radishes

Sample	Section	Storage period (days)	Fortified level (mg L^{-1})	Recovery (%) / RSD (%)					
				XDE-175-J	XDE-175-L	N-demethyl-XDE-J	N-demethyl-XDE-L	N-formyl-XDE-J	N-formyl-XDE-L
Young Radish	Root	39	0.25	101.5/1.3	112.5/1.9	105.8/1.0	107.0/1.5	101.8/1.7	103.0/2.2
	Leaf			95.5/2.8	114.2/1.1	78.7/1.0	105.9/4.1	87.4/0.9	95.8/3.7
Small Radish	Root	42		97.1/2.2	111.9/4.3	100.0/3.0	101.6/3.3	103.0/0.7	101.5/0.9
	Leaf			75.7/1.9	115.4/2.9	95.2/2.3	111.0/4.4	74.5/5.5	82.5/3.4

Establishment of guidelines on safe use of spinetoram and metabolites

Based on the results from our study, pesticide registration status of spinetoram in young radish was established by Rural Development Administration (6th January 2016). According to the status, to control beet armyworm in young radish, spinetoram should be diluted 2,000 times and can be applied twice to young radish 7 days before harvest.

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Abstract in Korean

소면적 재배작물 열무 및 알타리무의 재배 중 살충제 Spinetoram과 그 대사산물의 행적

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성정희

본 연구는 소면적 재배작물인 열무 및 알타리무의 재배 중 살충제 spinetoram과 그 대사산물에 대해 QuEChERS 방법을 사용하여 LC-MS/MS로 분석하여 행적을 알아보고자 하였다. 포장실험은 열무와 알타리무 따로 시설재배에서 수행하였다. 농약살포는 수확 전 7일 간격으로 처리구 1은 21-14일전 2회처리, 처리구 2는 30-21-14일전 3회처리, 처리구 3은 21-14-7일전 3회처리 그리고 처리구 4는 14-7-0일전 3회처리 하였다. 수확 후 시료 분석에서 열무에서는 처리구별로 0.40, 0.37, 0.62, 0.95 mg L^{-1}, 알타리무에서 처리구별로 0.68, 0.77, 1.22, 1.87 mg L^{-1}의 잔류량을 확인하였다. 열무와 알타리무 무처리시료에 spinetoram와 대사산물 표준용액을 2수준(10MLOQ, 25MLOQ) 3반복으로 처리하여 회수율을 산출한 결과는 각각 열무에서 79.2-115.7%이었고, 알타리무 에서 74.1-114.9%이었으며 분석오차는 10% 미만이었다. 저장안정성에서는 spinetoram과

대사산물 표준용액을 1수준(25MLOQ) 3반복으로 처리하여 회수율을 산출하였을때, 열무 78.7-114.2%, 알타리무 74.5-115.4%이었으며 분석오차는 10% 미만이었다.

Spinetoram과 대사산물의 정량한계(LOQ)는 0.005 mg L^{-1} 이었고, 분석정량한계(MLOQ)는 0.01 mg L^{-1} 이었다. 이를 통하여 농촌진흥청 농약등록현황에 살충제 spinetoram을 열무 재배 시 파밤나방 방제에 수확 7일전 2,000배 희석하여 2회 사용할 수 있도록 등록이 되었으며(2016.01.06등록), 열무에 대한 안전사용기준이 확립되었다.

주요어: Spinetoram, XDE-175-J, XDE-175-L, LC-MS/MS, LOQ, MLOQ, QuEChERS, Minor crops, Insecticide, Young radish, Small radish

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