농학석사학위논문

엇갈이배추의 재배 중 살충제 Metaflumizone, Chlorantraniliprole, Spinetoram, 및 Etofenprox의 소실 양상

Dissipation of Insecticide Metaflumizone, Chlorantraniliprole, Spintoram, and Etofenprox in Ssam Cabbages during Cultivation

2018년 8월

서울대학교 대학원

농생명공학부 응용생명화학전공

백 수 진

A Dissertation for the Degree of Master of Science

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Abstract

The metaflumizone, chlorantraniliprole, spinetoram and etofenprox of insecticides are registered in Korean cabbages(head) for control the striped flea beetle. This study was conducted with residual characteristic of these insecticides to obtain baseline data on pesticide registration and guidelines on safe use of pesticides in minor crop Korea cabbages (Brassica leafy vegetables). Metaflumizone, etofenprox in emulsifiable concentrate (EC) formula and Spintoram, Chlorantraniliprole in water dispersible granule formulation were treated with 4 plots (30/21 days, 21/14 days, 14/7 days, 7/0 days) on Korean cabbage. Metaflumizone, chlorantraniliprole and spinetoram were quantitative analyzed by liquid chromography-tandem mass spectrometry in the ESI (electrospray ionization) positive mode using MRM. Etofenprox was analyzed by HPLC equipped DAD. These insecticides of MLOQ were satisfied 0.01 – 0.02 mg/kg and the calibration curve coefficient (r^2) was ≧0.99 in the calibration range of 5-100 ng/mL using matrix matched method (metaflumizone, chloratraniliproe and etofenprox). For recoveries, metaflumizone and chlorantraniliprole and spinetoram conventional methods were used for the analysis, spinetoram was used QuEChERS method. Infield sample, the residual amount of metaflumizone and spinetoram was calculated as the sum. The maximum residues in Korean cabbages were measured from 0.15 to 4.17 mg/kg (metaflumizone), 0.05 to 0.95 mg/kg (chlorantraniliprole), <0.01 to 0.41 mg/kg (spinetoram) and 0.49 to 18.90 mg/kg (etofenprox). In Korea, these pesticides were established as below the 1.0 - 3.0 mg/kg of MRLs in the Korean cabbages. In Korea, these pesticides were established as below

the 1.0 - 3.0 mg/kg of MRLs in the Korean cabbages. All pesticides showed a tendency to decrease over the time. Based on these data, this study provides that metaflumizone, chlorantraniliprole, spinetoram and etofenprox is suitable to apply for Korean cabbage under recommended dosage as the PHI.

Key Words: Metaflumizone, Chlorantraniliprole, Spinetoram, Etofenprox, minor cops, PHI, LC-MS/MS, QuEChERS

Student Number: 2016-21727

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List of Abbreviations

ACN	acetonitrile
ADI	acceptable daily intake
DCM	dichloromethane
WG	Water dispersible granule
EC	emulsifiable concentrate
ESI	electrospray ionization
GAP	good agriculture practice
GC-MS/MS	gas chromatography mass spectrometry
LC-MS/MS	liquid chromatography mass spectrometry
ILOD	instrumental limit of detection
ILOQ	instrumental 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
SRM	selected reaction monitoring

Introduction

Minor crop

Crops are divided into two types according to cultivation area: major crops and minor crops. Generally, major crops mean crops with an area of over 1,000 hectares in Korea. Definitions for minor crops vary from each country, because the scale of crop cultivation in country is different. In Korea, minor crops are defined as cultivated area less than 1,000 ha in Food, agriculture, forestry and fisheries statistical yearbook and the use of pesticides for minor crop is regarded as minor use (Lee, 2013). The kinds of minor crop in Korea are listed in table 1. In USA, Minor crop is defined as fewer than 300,000 acres in production (EPA, 1996). In the EU, crops are classified as minor crop and very minor crop. Minor crop is defined as daily dietary intake contribution <7.5 >1.5 g/day, cultivation area <10,000 >600 ha and production <200,000 t/year. Very minor crop is defined as daily dietary intake contribution <1.5 g/day, cultivation area <600 ha and production very low (EC, 2011). In the Australia, The minor crop is distinguished with volume of commodity production, area under cultivation (Zhang K), dietary consumption (g/kg BW/day), value of crop and export quantities (APVMA, 2017)

In the whole world, since the use of pesticide such as about minor crops was low, pesticide manufacturing companies are not able to registration with pesticide in minor crops for economic reasons (Gerald M. Ghidiu, 1994; Jarvis, 2002). Small area of cultivation reflects upon low interest of pesticide

manufacturing companies which does not consider the investment needed for product registration. But farmers confronted a problem about the lack of registered pesticides for minor crops, MRLs and appropriated Good Agriculture Practice(GAP) (Perry, 2002). For this reason, registered pesticides for minor crops are low compared to major crops. Table 2 show the status of pesticides registered in representative minor crops from 2011 to 2017.

Table 1. List of minor crop

Type	Group	Commodity
Cereal grains	-	Prosomillet, Wheat, Sorghum, Foxtail millet, Quinoa
Potatoes	-	Sweet potato, Yam
Beans	-	Mung bean, Cowpea, Soybean, Red Bean
Nuts and Seeds	Oilseed	Perilla-seed, Sesame, Sunflower seed
	Peanut or nuts	Chestnut, Walnut
Fruits	Pome fruits	Quince, Pear, Pomegranate
	Citrus fruits	Korean lemon (Citrus junos)
	Stone fruits	Jujube, Japanese apricot, Apricot, Sweet cherry, Five-flavor magnolia vine, Plum, European plum
	Berries and other small fruits	Chinese matrimony vine, Strawberry, Wild grape, Fig, Rubi fructus, Blueberry, Wild berry, Black chokeberry, Mulberry
	Assorted tropical and sub-tropical fruits	Mango, Banana, Dragon fruit, Kiwifruit, Papaya, Passion fruit

Vegetables	Flowerhead brassicas	Broccoli, Brussels sprouts, Korean cabbage(Head), Cabbage
	Leafy vegetables	Mustard leaf, Beach silvertop, Gondre, Narrow-head ragwort, Fischer's ragwort, Chard, Shepherd's purse, Vitamin, Sedum, Perilla leaf, Butterbur, Dandelion, Amaranth, Beat leaves, Alpine leek, Lettuce, Oriental chaff flower, Spinach, Ssam cabbage, Foremost mugwort, Crown daisy, Marsh mallow, Small radish (leaf), Lettuce(head), East Asian hogweed, Brassica leafy vegetables, Young radish (leaf), Indian lettuce, Rape leaves, Chamnamul, Chinese vegetable, Chwinamul, Common chicory, Kale, Parsley
	Stalk and stem vegetables	Wild garlic, Water dropwort, Chinese chives, Celery, Kohlrabi, Asparagus, Shallot
	Root and tuber vegetables	Korean wasabi(root), Carrot, Balloon flower, Garlic, Beet(root), Small radish(root), Young radish(root), Burdock
	Fruiting vegetables, Cucurbits	Sweet pumpkin, Melon, Watermelon, Zucchini
	Fruiting vegetables other than Cucurbits	Eggplant, Bitter gourd, Sweet pepper, Tomato
Tea leaves	-	Tea
Other plants	-	Licorice, By-gone ostericum, Sicklepod seed, Korean go-bon (Slender angelica), Mountain germander, Dong quai leaf, Big blue lilyturf, Angelica dahurica root, Loquat, Ovate-leaf atractylodes, Korean ginseng, Peony, Three-leaf ladybell, Redroot gromwell, Rehmanniae radix, Snowparsley, Wilford's swallow-wort, Hwang-gi (Mongolian milkvetch)

* Rural Development Administration

Table 2. The status of registered pesticide about representative minor crops.

Crops /Year	Ssam Cabbages	Spinach	mustard green	Lettuce	Water dropwort	Shallot
2011	0	7	1	2	1	1
2012	0	16	3	10	8	9
2013	0	14	5	12	11	13
2014	0	6	3	6	4	3
2015	0	0	1	1	1	0
2016	0	7	0	7	4	2
2017	0	18	1	7	4	8
total	0	68	14	45	33	36

* Rural Development Administration

Ssam cabbage

Korean cabbage is the main vegetables which are consumed most Koreans. The Korean cabbage is classified into head type and non-head type according to the botanical type. Korean cabbage, which is used as material for Kimchi, is head type cabbage and ssam cabbage is non-head type cabbage. Since the morphological characteristics of Korean cabbage, the residual pattern of pesticides sprayed are different and domestic MRLs for Korean cabbage of head type and ssam cabbage (non-head type) have been set separately since 2007 (Kim, 2014a). The established MRLs of ssam cabbage is table 3. The National Agricultural Products Quality Management Service collects samples of agricultural products of distributions and sales every year, investigate whether exceed the MRLs and takes measure to prevent market shipment such as delaying shipment. In the past five years, the 124 of agricultural products were nonconformity judgment about exceed the MRLs. Among them, the ssam cabbage was found to be ineligible for 26 components, 10 of which were not set MRLs (NQAS, 2011-2015). The list of nonconforming ingredients for ssam cabbage during monitoring of pesticide residues is table 4.

Table 3. The MRL list of Korean cabbages

Compounds	MRL (mg/kg)	Compounds	MRL (mg/kg)	Compounds	MRL (mg/kg)	Compounds	MRL (mg/kg)
Abamectin	0.3	Cypermethrin	5	Imidacloprid	1	Cymoxanil	0.5
Acephate	5	Deltamethrin	1	Iprovalicarb	2	Pymetrozine	0.5
Acetamiprid	3	Diazinon	0.1	Kresoxim-methyl	0.1	Pyraclofos	0.1
Ametoctradin	5	Dichlorvos : DDVP	0.5	Lepimectin	0.05	Pyraclostrobin	0.5
Amisulbrom	2	Diflubenzuron	2	Lufenuron	1	Pyridaryl(Pyridalyl)	5
Benalaxyl	3	Dimethomorph	5	Malathion	0.5	Pyrifluquinazon	1
Benfuracarb	0.3	Dimethyl dithiocarbamates	5	Mancozeb	5	Sethoxydim	10
Benomyl	2	Diniconazole	0.3	Mandipropamid	3	Spinetoram	1
Benthiavalicarb-isopropyl	5	Dinotefuran	3	maneb	5	Spirotetramat	5
Bifenthrin	2	Dithianon	0.1	Metaflumizone	2	Sulfoxaflor	0.3
Bistrifluron	3	Dithiocarbamates	5	Metalaxyl	0.5	Tebufenozide	1
Cadusafos	0.05	Emamectin benzoate	0.05	Metaldehyde	1	Tebupirimfos	0.01
Captan	20	Ethaboxam	2	Methamidophos	2	Teflubenzuron	1
Carbendazim	2	Ethoprophos(Ethoprop)	0.02	Methomyl	3	Tefluthrin	0.1

Carbofuran	0.05	Etofenprox	2	Metiram	5	Thiacloprid	0.5
Carbosulfan	0.3	Famoxadone	1	Nabam	5	Thiamethoxam	1
Chlorantraniliprole	3	Fenitrothion : MEP	0.05	Napropamide	0.1	Thiobencarb	0.05
Chlorfenapyr	3	Fenvalerate	1	Novaluron	2	Thiodicarb	3
Chlorfluazuron	1	Ferbam	5	Oxolinic acid	3	Thiophanate-methyl	2
Chlorothalonil	5	Flonicamid	2	Pendimethalin	0.07	Thiram	5
Chlorpyrifos	0.2	Fluazifopbutyl	2	Phenthoate : PAP	0.1	Tralomethrin	1
Chlorpyrifos-methyl	0.2	Fluazinam	0.05	Phoxim	0.05	Trichlorfon(DEP)	0.5
Chromafenozide	5	Flubendiamide	3	Pirimiphos-methyl	2	Trifloxystrobin	0.5
Cyantraniliprole	2	Flufenoxuron	3	Probenazole	0.07	Trifluralin	0.05
Cyazofamid	2	Fluopicolide	1	Profenofos	2	Valifenalate	1
Cyclaniliprole	0.5	Flusulfamide	0.05	Prohexadione-Calcium	5	Zineb	5
Cyfluthrin	2	Fosetyl-aluminium	20	Propamocarb	3	Ziram	5
Cyhalothrin	0.5	Furathiocarb	0.3	Propineb	5	Zoxamide	3
Cymoxanil	0.5	Glufosinateammonium	0.05	Hydrogen cyanide	5		

*Pesticides and Veterinary Drugs Information (Safety) (2016) (Safety, 2017)

Table 4. List of nonconforming ingredients for ssam cabbage during monitoring of pesticide residues

year	2011	2012	2013	2014	2015
compounds	Azoxystrobin	Diazinon*	Carbendazim*	Carbofuran*	Azoxystrobin
	Chlorothalonil*	Diniconazole*	Carbofuran*	Chlorpyrifos*	Carbofuran*
	Chlorpyrifos	Endosulfan(Total)	Chlorothalonil*	Diazinon*	Chlorpyrifos*
	Dimethomorph*	EPN	Chlorpyrifos*	Flufenoxuron*	Diazinon*
	Diniconazole*	Ethoprophos	Diniconazole*	Lufenuron*	Diethofencarb
	Endosulfan(Total)	Ethoprop	Endosulfan(Total)	Novaluron*	Dimethomorph*
	Flubendiamide*	Flufenoxuron*	Fenpropathrin	Terbufos*	Flufenoxuron*
	Kresoxim-methyl	Fluopicolide*	Flufenoxuron*		Pencycuron
	Pencycuron	Mandipropamid*	Lufenuron*		
	Phorate*	Pencycuron	Pencycuron		
	Tebupirimfos*	Tebupirimfos*			
	Terbufos*				

* the Compounds of established MRL for Korean cabbages

Positive List System

Pesticides are widely used to variety of situation about control the pests such as hinder the growth of fungi or to prevent crop damage by insects. Pesticides has advantage to prolong storage life and improve quality of agricultural product . After World War II, the use of pesticides in agriculture soared steadily leading to increased world food production. The pesticide usage and environmental pollutant of their production process were caused the residues of these chemicals and their metabolites in food commodities, water and soil (Ahmed, 2001). But people recognize as consider pesticide residues in food a strong concern (Brewer, 2008). For pesticides, the maximum residue level (MRL) is very important indicator that minimized the exposure of the consumer to intakes of pesticides. Pesticides were established of MRLs using data of pesticide application rates and pre-harvested intervals and products are regulated by fixed MRLs (Nasreddine L, 2002).

For control the pesticide residue, the regulation is requirement. Each country has an agricultural product safety management system to manage the residual pesticide safety in the market. The department which is about safety use of pesticide regularly collects, investigates the agricultural product in market for check the residues of pesticide and set the MRLs using these data((주)에이비솔루션, 2017).

To better control safety use of pesticide, the PLS system will be introduced in 2019. It manages agricultural chemicals by establishing a uniform limit of 0.01

mg/kg, except for pesticides that are registered for domestic use of for which MRLs are established through the application for MRL setting in imported food. With increasing dependence on imported food, the foods that contain pesticides that are not registered for domestic use were imported. This new system prevents the influx of pesticides whose safety is not tested and prove, and import safe agricultural products (KFDA, 2016). Prior to introduction of PLS, the MRLs of agricultural products without Korean criterion was applied by CODEX standards. As a result, there were some limitation in the safety management of pesticide residues in imported agricultural products and control of usage of unregistered pesticides as domestic agricultural products. The PLS is applied not only in Korea but also in other countries. The PLS had started by Japan since 2006, EU, Taiwan and etc were applied and the other countries such as USA, Australia, Canada and etc were operated in "zero tolerance" which is applied non-detection if there is no criterion (김진숙, 2018).

For safety control plan, the Minister for Food, Agriculture, Forestry and Fisheries shall investigate whether the persistent noxious substances under relevant Acts and subordinate statues, such as the Food Sanitation Act exceed permissible levels concerned (MAFR, 2011). Most of the crops that were judged to be nonconformity were minor crops (table 5). Minor crops were often detected as inadequate products that exceed the MRLs of pesticides during distribution. If the PLS is implemented and the MRLs of pesticide of unregistered is set 0.01 mg/kg, it might be increased nonconformity judgment of agricultural products of pesticide residues. Therefore, it is important to establish safe use standards for minor crops.

Table 5. MRL excess crop list during monitoring of pesticide residues

Crops/Year	Ssam Cabbages (Brassica leafy vegetables)	Spinach	mustard green	Lettuce	Water dropwort	Shallot
2011	17	51	5	35	14	19
2012	17	38	8	25	17	17
2013	15	31	8	27	24	19
2014	12	26	3	24	36	29
2015	12	61	3	33	22	24
Total	73	207	27	144	113	108

Metaflumizone, Chlorantraniliprole, Spinetoram and Etofenpox

Metaflumizone is semicarbazone insecticide, it consisted of E isomer and Z isomer. Its MOA(mode of action) is voltage-dependent sodium channel blockers. Chlorantraniliprole is diamides insecticide, the MOA of it is ryanodine receptor modulators. Spinetoram is spinosyn insecticides. Spinetoram is spinosy insecticides, it has two of major compounds and four of metabolites. Its MOA is Nicotinic acetylcholine receptor (nAChR) allosteric modulators. etofenprox is Pyrethroid, Pyrethrin insecticides, it worked by Sodium channel modulators. The common point of these insecticides is that pesticide use for Korean cabbage(head) is registered to striped flea beetle (KCPA). The figure 1 is structure of each compounds and the table 6 is physical properties.

Figure 1. Structure of Metaflumizone, Chlorantraniliprole, Spinetoram and Etofenprox

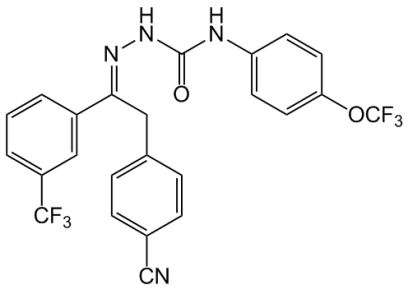
(A) Metaflumizone E isomer (B) Metaflumizone Z isomer

(C) Chlorantraniliprole (D) Etofenprox

(E) XDE-175-J (F) XDE-175-L (G) N-demethyl-J (H) N-demethyl-L

(I) N-formyl-J (J) N-formyl-L

(A)



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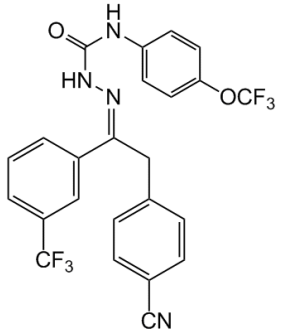

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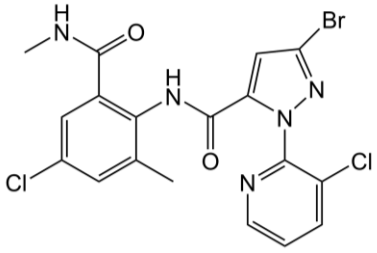

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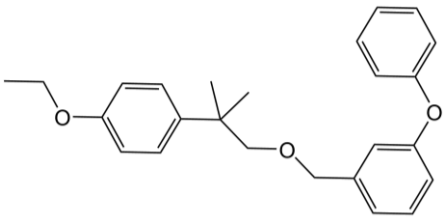

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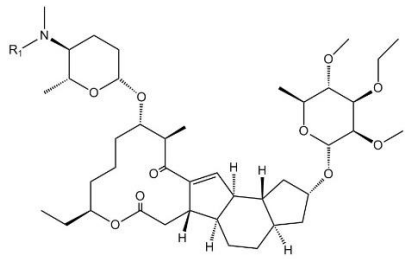

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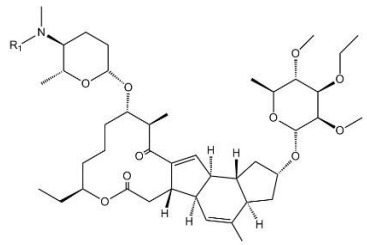

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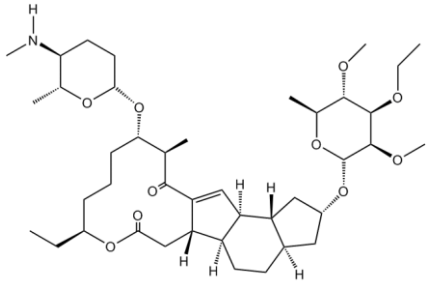

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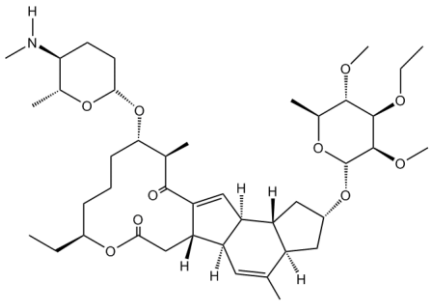

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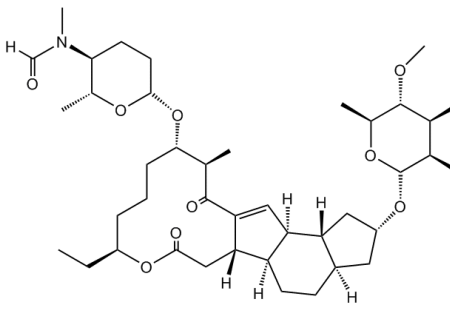

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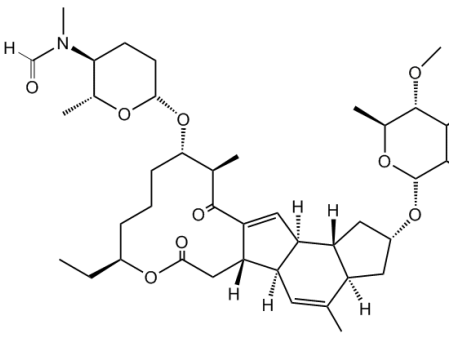


Table 6. Physical properties of each compounds

Property	Metaflumizone		Chlorantraniliprole	Etofenprox	Spinetoram	
Common name	Metaflumizone (E) isomer	Metaflumizone (Wilkowska) isomer			Spinetoram J (major component)	Spinetoram L (minor component)
CAS No.	852403-68-0	139970-56-2	500008-45-7	*80844-07-1*	187166-40-1	187166-15-0
Molecular formula	$C_{24}H_{16}F_6N_40_2$		$C_{18}H_{14}BrCI_2N_50_2$	$C_{25}H_{28}0_3$	$C_{42}H_{69}NO_{10}$	$C_{43}H_{69}NO_{10}$
Molecular weight	506.4		483.2	376.5	748.0	760.0
Vapor pressure	7.94 X 10-7 (20 °C)	2.42E-4 (20 °C)	6.3 X 10-9 (20 ℃)	8.13E-4 (25℃)	5.3 × 10−2 (20℃)	2.1 × 10−2 (20℃)
K_{ow}	5.1	4.4	2.76 (pH 7)	6.9	2.44 (pH 5) 4.09 (pH 7) 4.22 (pH 9)	2.94 (pH 5) 4.49 (pH 7) 4.82 (pH 9)

Pesticide residue analysis

The history of residual pesticide analysis is derived from investigate and assessment about side effect of residual pesticide in agricultural product and environment (NIFDS, 2017). Residual analysis of pesticides of food and environmental samples has been performed in numerous government and approximately 40 years. Some residue monitoring laboratories still use method developed 30 years ago, which is a lot of usage of solvent, time of extended analysis and labor (Anastassiades et al, 2003). Some disadvantages traditionally attributed to these methods were complexity, time expenditure, amounts of sample and organic solvent and difficulty in automation(Pico, 2015)

Conventional method

The procedure of pesticides residues analysis in agricultural products is following as.

1) Preparation of analytical sample: It is a process of preparation of samples that were changed into the form of analysis. The analysis part of agricultural samples is mostly edible part, but it is unnecessary to match with edible and analysis part (NIFDS, 2017).
2) Extraction: It is a extracting the compounds to be analyzed from samples, The organic solvent such as acetone, acetonitrile or methanol was extracted by solvent agricultural product used due to they are easily miscible with the agricultural products (Pico, 2015)

3) Purification of the extract: It is a process of purification to enable analysis using analytical instrument in sample extracts, called cleanup.

Currently, Because it is hard to selected only analytes for purification, a mount of interfering substances with analytes are coexisted. Substances of interference affects sensitivity of analytical instrument, so it is essential to separate and remove it, which requires the most time and effort in analysis (NIFDS, 2017)

4) Determenation: This is the step of analyzing the target compounds. Qualitative- and quantitative analyzes are performed, the most used analyzer is GC and HPLC. (NIFDS, 2017).

The conventional method has some disadvantage such as complexity, time expenditure, amounts of ample and organic solvent and difficulty in automation (Zhang K, 2011).

QuEChERS method

QuEChERS means quick, easy, cheap, effective, rugged and safe and this method is new, more rapid and effective analytical approaches and improve analytical quality and laboratory efficiency. The QuEChERS method provided with use of $MgSO_4$ for salting out extraction/partitioning and dispersive-SPE (dSPE) for cleanup, It was contrived a highly simplyfied sample preparation method with good reults (Anastassiades, 2003). The QuEChERS method provided good results in the most pesticides by use of different amounts and type of solvent and different sorbent of dSPE (Lehotaya S.J. et al, 2010). The original QuEChERS method was designed to analyze residues of pesticides in fruits and vegetables, It was used acetonitrile for solvent extraction, PSA sorbent and anhydrous $MgSO_4$ for subsequent cleanup to remove water. In addition to the original QuEChERS method, this method was undergone

modification and improvements. For example, the Method EN 15662, including weaker citrate buffer, and the AOAC, including strong acetate buffer were made (Wilkowska, 2011).

The QuEChERS method used LC-MS/MS or GC-MS/MS in analytical instruments because they are much more sensitive and selective than conventional HPLC or GC using the planned selective response monitoring (SRM) mode (Lee, 2015).

Method validation

Analysis of pesticide residue is quantitatively process an analysis of trace pesticide residues present among samples in which many unknown interfering substances coexist, it is likely to occur error of quantitative analysis. The analysis methods of pesticide residue such as extraction, purification and instrumental analysis are required to verified (NIFDS, 2017). For validation of developed method, LOD, LOQ, linearity, recovery tests and matrix effects were considered.

1) ILOD: The ILOD is the smallest quantity of analytes that can be detected, it means senesitivity of instruments and S/N ratio is more than three (Silvial L. et al, 2015).

2) ILOQ: The LOQ is the lowest amounts of analytes that can be quantitation, defined as 10 times the S/N ratio (Somenath, 2003).

3) MLOQ: The MLOQ is definition of the ability of the method to detect the analyte at the concetration (Fong, 1999).

4) Calibration curve and linearity: The calibration curve is relationship between signal of detector and concentration of standard, whose range is expected in the sample (Somenath, 2003).

5) Accuracy & Precision: The definition of accuracy is deviation from true value and precision is reproducibility of replicate measurements (Somenath, 2003).

6) Recovery: It can be obtained by adding the pesticide to the appropriate concentration range and then analyzing and calculating the recovered amount (Fong, 1999).

PHI (Pre-Harvest Interval)

The PHI(pre-harvest interval) is the time between spraying of pesticide and harvesting of the crops (NPIC, 2018). The PHI is provided as data to allow about pesticide residues on the crop at harvest. The pesticides on the crop is easy to degrade by UV, rain and etc. So, the PHI should be long enough to degrade pesticides to safety agricultural products (PMRA, 2007)

The purpose of studies

This study was conducted for spraying pesticides on the Ssam cabbage (Brassica leafy vegetables) and confirm of residual pattern. With residual characteristic of these insecticides to obtain baseline data on pesticide registration and guidelines on safe use of pesticides in minor crop Ssam cabbages (Brassica leafy vegetables).

Materials and Methods

Analytical standard and pesticide for spraying

Standard material of metaflumizone E (Purity : 98.0 %) and metaflumizone Z (Purity : 98.0%) were purchased from Wako (Osaka, Japan). Chlorantraniliprole (Purity : 98.7%) was provided by HPC (Borsdorf, Germany). Major compound of spinetoram (XDE-175-J (Purity : 97.6%) and XDE-175-L (Purity : 100%)) and its metabolites (XDE-175-N-demethyl-J (Purity : 96.0%), XDE-175-N-demethyl-L (Purity : 98.0%), XDE-175-N-formyl-J (Purity : 78.0%) and XDE-175-formyl-L (Purity : 92.0%)) were purchased from Dow agrosciences (Seoul, Korea). Etofenprox (Purity : 99.6%) was provided by Sigma-aldrich (Missouri, USA)

Chlorantraniliprole 5% Water dispersible granule (WG) and Spinetoram 5% Water dispersible granule (WG) were obtained from Framhannong. Metaflumizone Emulsifiable concentrate (EC) and Etofenprox 20% Emulsifiable concetrate (EC) were purchased from Kyung nong at pesticide market (Seoul, Korea).

Standard solutions

The stock solution of metaflumizone (E/Z), Chlorantraniliprole, Etofenprox, XDE-175-J/L, XDE-175-N-demethyl-J/L and XDE-175-formyl-L were prepared 500 mg/kg. XDE-175-formyl-J were prepared 1000 mg/kg. Each standard stock solutions were made from acetonitrile. One mixture working solution of Metafluzmizone was prepared by mixing appropriate volumes of Metaflumizone (E, Z) standard solutions. The mixture working solution of

Spinetoram was prepared by mixing appropriate volumes of major compound of Spinetoram and its metabolites standard solutions. These standard solutions and working solutions were stored at -20℃.

Subject crops

A variety of Ssam cabbages was "Sil-lok", used for field experiment.

Field trials

Field experiment was performed in 243, Munhyeon-ro 357beon-gil, Mohyeon-eup, Cheoin-gu, Yongin-si, Gyeonggi-do, Republic of Korea. Korean cabbages were planted in greenhouse and sectionalized of non-pesticide plot and four plots about pesticide spraying date. Plot 1 was processed at 30/21 before the harvest, plot 2 was processed at 21/14 before the harvest, plot 3 was processed at 14/7 before the harvest and plot 4 was processed at 7/0 before the harvest. The size of plots whose were consist of three replicate were 30 m^2. The buffer zones were set to avoid cross contamination between plots.

Metaflumizone 20% EC, Chlorantraniliprole 5% WG, Spintoram 5% WG and Etofenprox 20 % EC were diluted 2,000 times with water. Korean cabbages were sprayed enough until diluted solution were shed on leaves using a pressurized 20 L handguns sprayer.

Figure 2. Experimental plots in field

Fig2. Experimental plots in field

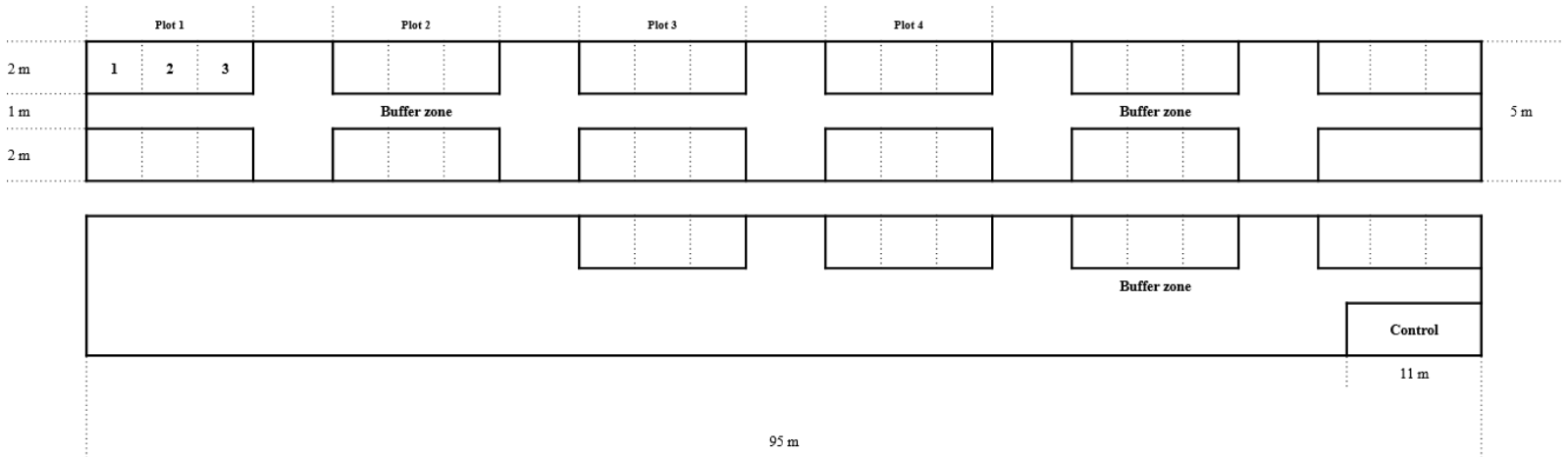


Figure 3. Preparation of spray solution and spray in field

(A) **(B)**

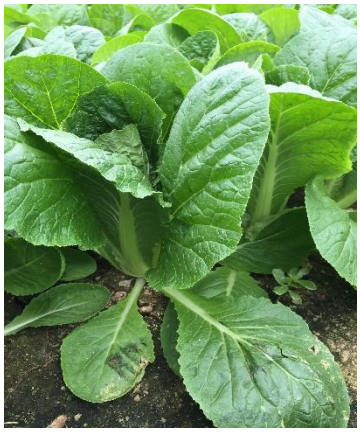
(A) Dilution of pesticide product
(B) Application of pesticide on Korean cabbages

Sampling

The harvest of Ssam cabbages was performed in September 13, 2017. Ssam cabbages were collected at random, making sure the samples were representative. More than 2 kg of Ssam cabbages was harvested in each plots. After the samples were harvested and transferred directly to laboratory using ice pack. They were removed roots and dead leaves, chopped, macerated using dry ice and stored in a freezer at a temperature below -20ºC in polyethylene bags.

Figure 4. Sample collection

A) **(B)**



(A) Ssam cabbages

(B) Harvest

Figure 5. Sample preparation

※ Cut to 3 - 4 cm

※ Grinding by adding dry ice

Analytical instruments and conditions

1. Metaflumizone

Chromatographic separation of two compounds was performed with a Shimadzu LCMS-8040 with UHPLC Nexera equipped with MS/MS detector (Kyoto, Japan). The chromatographic column was an Kinetex C18 (2.1 × 100 mm, 2.6 μm, Phenomenex, California, USA) and kept at 40°C. The mobile phase was consisted of 0.1% formic acid, 5 mM ammonium formate in water (solvent A) and 0.1% formic acid, 5 mM ammonium formate in methanol (solvent B). The mobile phase gradient started at 20% B (0-2 min), increased to 90% B (2-8 min), maintained at 90% B (8-10 min), decreased to 20% B (10-12 min) and maintained at 20% B (12-15 min). The rate of mobile phase was 0.2 mL/min and injection volume was 5 uL. Electrospray (ESI) positive mode was used for confirmation of metaflumizone (E) and (Wilkowska). The parameters of MS/MS condition used for instrument analysis were as follow. DL temperature 230 °C, nebulizing gas 3 L/min, heat block temperature 400°C and capillary voltages 6kV.

2. Chlorantraniliprole

The analysis of chlorantraniliprole was performed with a Shimadzu LCMS-8050 with UHPLC Nexera equipped with MS/MS detector (Kyoto, Japan). The chromatographic column was an Kinetex C18 (2.1 × 100 mm, 2.6 μm, Phenomenex, California, USA) and kept at 40°C. The mobile phase was consisted of 0.1% formic acid, 5 mM ammonium formate in water (solvent A) and 0.1% formic acid, 5 mM ammonium formate in methanol (solvent B). The mobile phase gradient started at 5% B (0-1 min), increased to 90% B (1-6 min),

maintained at 90% B (6-9 min), decreased to 5% B (9-11 min) and maintained at 5% B (11-13 min). The rate of mobile phase was 0.2 mL/min and injection volume was 2 uL Electrospray (ESI) positive mode was used for confirmation of chlorantraniliprole. The parameters of MS/MS condition used for instrument analysis were as follow. DL temperature 250 °C, nebulizing gas 3 L/min, heat block temperature 400°C, drying gas flow 10 L/min and capillary voltages 6kV.

3. Spinetoram

Chromatographic separation of six compounds was performed with a Shimadzu LCMS-8050 with UHPLC Nexera equipped with MS/MS detector (Kyoto, Japan). The chromatographic column was an Kinetex C18 (2.1 × 100 mm, 2.6 μm, Phenomenex, California, USA) and kept at 40°C. The mobile phase was consisted of 0.1% formic acid, 5 mM ammonium formate in water (solvent A) and 0.1% formic acid, 5 mM ammonium formate in methanol (solvent B). The mobile phase gradient started at 50% B (0-0.5 min), increased to 90% B (0.5-7 min), maintained at 90% B (7-10 min), decreased to 50% B (10-10.5 min) and maintained at 20% B (10.5-15 min). The rate of mobile phase was 0.2 mL/min and injection volume was 2 uL..Electrospray (ESI) positive mode was used for confirmation of major compound of spinetoram and its metabolites. The parameters of MS/MS condition used for instrument analysis were as follow. DL temperature 250 °C, nebulizing gas 3 L/min, heat block temperature 400°C, drying gas flow 10 L/min and capillary voltages 6kV.

4. Etofenprox

The analysis of etofenprox was performed with an Agilent 1100 HPLC

equipped with diode array detector (California, USA). The chromatographic column was an Kinetex C18 (4.6 × 250 mm, 5 μm, Phenomenex, California, USA) and kept at 40°C. The mobile phase was consisted water (solvent A) and acetonitrile (solvent B). The mobile phase gradient started at 60% B (0-5 min), increased to 95% B (5-20 min), maintained at 95% B (20-30 min), decreased to 60% B (30-35 min) and maintained at 60% B (35-40 min). The rate of mobile phase was 1 mL/min and injection volume was 20 uL. The wavelength of detector was 235 nm.

Method validation

Validation of the analytical method

1. ILOQ (Instrumental Limit of Quantitation)

Standard solutions of Metaflumizone, Chlorantraniliprole, Spinetoram (0.005, 0.01, 0.02 mg/kg LC-MSMS) and Etofenprox (0.05, 0.1, 0.2 mg/kg HPLC, 0.005, 0.01, 0.02 mg/kg GC-MSMS) were analyzed and calculated for LOD (S/N ≥3) and LOQ (S/N ≥ 10).

2. MLOQ (Method Limit of Quantitation)

MLOQ was calculated as follows.

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

3. Calibration curve and linearity

A five concentration points of each compounds were injected into instrument for calibration curve.

3.1 Metaflumizone: Mixture working solutions of metalumizone (E,Z) were diluted by using acetonitrile and added extracts of blank (non-pesticide) sample to make matrix-matched standard solution.

3.2 Chlorantraniliprole: Working solutions of Chlorantraniliprole were diluted by using acetonitrile and added extracts of blank (non-pesticide) sample to make matrix-matched standard solution.

3.3 Spinetoram: Mixture working solutions of major compound of Spinetoram and it metabolites were diluted by using acetonitrile and added extracts of blank (non-pesticide) sample to make matrix-matched standard solution.

3.4 Etofenprox: Working solutions of Etofenprox were diluted by using acetonitrile

4. Recovery test of analytical method

4.1 Metaflumizone

The 20 g of homogenized non-pesticide sample were weighed into 175mL PTFE-lined bottle. The samples were fortified with working solution of metaflumizone to spike level of 0.01 and 0.1 mg/kg (MLOQ and 10 MLOQ) and 80 mL methanol was added. The samples were extracted by shaking at 250 rpm for 30 minutes. The extracts were suction filtered through Buchner funnel and methanol (20 mL) was added to wash the filter cake. The filtrates were transferred into a 1,000 mL separatory funnel and saturated sodium chloride solution (20 mL) and water (100 mL) were added. Then partitioned twice with 100 mL, 50 mL dichloromethane each time. The organic solvent layer was passed through anhydrous sodium sulfate and evaporated to dryness at below 40°C using rotary evaporator. The SPE cartridge was conducted using SPE-silica (NIFDS, 2017). The residue was dissolved in 10 mL of n-hexane. Each SPE cartridge were conditioned with 5 mL of n-hexane. The samples were loaded onto the SPE cartridge, washed with 10 mL of acetone/n-hexane (10:90, v/v) and eluted with 10 mL of acetone/n-hexane (20:80, v/v). The eluates were received in a test tube and evaporated in a stream of nitrogen and re-dissolved in 5 mL of acetonitrile. The samples was passed through 0.2 um PTFE filter,

transferred 200 μL into autosampler vial and acetonitrile 200 μL was added.

4.2 Chlorantraniliprole

The 20 g of homogenized non-pesticide sample were weighed into 175mL PTFE-lined bottle. The samples were fortified with working solution of chlorantraniliprole to spike level of 0.01 and 0.1 mg/kg (MLOQ and 10 MLOQ) and 100 mL acetonitrile was added. The samples were extracted by shaking at 250 rpm for 30 minutes. The extracts were suction filtered through Buchner funnel and acetonitrile (50 mL) was added to wash the filter cake. The filtrates were evaporated to qbout 1 mL at below 40°C using rotary evaporator and transferred into a 1,000 mL separatory funnel. Saturated sodium chloride solution (50 mL) and water (50 mL) were added. Then partitioned twice with 100 mL, 50 mL dichloromethane each time. The organic solvent layer was passed through anhydrous sodium sulfate and evaporated to dryness at below 40°C using rotary evaporator. The comparison of different SPE cartridge was conducted using SPE-silica (NIFDS, 2017) and GCB-NH_2. 1) SPE-silica : The residue was dissolved in 10 mL of ethyl acetate/n-hexane (20:80, v/v). SPE cartridge were conditioned with 10 mL of n-hexane. The samples were loaded onto the SPE cartridge, washed with 15 mL of ethyl acetate/n-hexane (30:70, v/v) and eluted with 10 mL of ethyl acetate/n-hexane (40:60, v/v). The eluates were received in a test tube and evaporated in a stream of nitrogen and re-dissolved in 10 mL of acetonitrile. 2) SPE-GCB/NH_2 : The residue was dissolved in 4 mL of methanol/dichloromethane (1:99, v/v). SPE cartridge were conditioned with 5 mL of dichloromethane. The samples were loaded onto the SPE cartridge, eluted with 7 mL of methanol/dichloromethane (1:99, v/v). Both were received in a test tube and evaporated in a stream of nitrogen and re-

dissolved in 2 mL of acetonitrile. The samples was passed through 0.2 um PTFE filter, transferred 200 μL into autosampler vial and acetonitrile 200 μL was added.

4.3 Spinetoram

The 10 g of homogenized non-pesticide sample were weighed into 50mL PTFE centrifuge tube. The samples were fortified with working solution of spinetoram (XDE-175-J/L) and its metabolites (XDE-175-N-demethyl-J/L, XDE-175-N-formyl-J/L) to spike level of 0.01 and 0.1 mg/kg (MLOQ and 10 MLOQ) and 10 mL acetonitrile was added. The samples were extracted by shaking for 10 minutes. Four gram of $MgSO_4$ and 1 g NaCl were added and vortexed for 1 min. The samples were extracted by shaking for vigorously 1 minutes and centrifuged at 3,000 rpm for 10 minutes. Aliquot of 1 mL of the supernatant were transferred into 2 mL dSPE (PSA 150 mg + GCB 7.5 mg) containing each sorbents, vortexed for 1 min and centrifugated at 14,000 rpm for 4 minutes. The samples were transferred 200 μL into autosampler vial and acetonitrile 200 μL was added.

4.4 Etofenprox

The 10 g of homogenized non-pesticide sample were weighed into 50mL PTFE centrifuge tube. The samples were fortified with working solution of etofenprox to spike level of 0.02 and 0.2 mg/kg (MLOQ and 10 MLOQ) and 10 mL acetonitrile was added. The samples were extracted by shaking for 10 minutes. Four gram of $MgSO_4$ and 1 g NaCl were added and vortexed for 1 min. The samples were extracted by shaking for vigorously 1 minutes and centrifuged at 3,000 rpm for 10 minutes. Aliquaot of 5 mL of the supernatat were transferred

into 100 mL round-bottom flask and evaporated to dryness at below 40°C using rotary evaporator. The comparison of different SPE cartridge was conducted using SPE-NH_2 (NIFDS, 2017) and SPE-GCB/NH_2 (1000 mg). The residue was dissolved in 4 mL of methanol/dichloromethane (1:99, v/v). SPE cartridge were conditioned with 5 mL of dichloromethane. The samples were loaded onto the SPE cartridge, eluted with 7 mL of methanol/dichloromethane (1:99, v/v). Both were received in a test tube and evaporated in a stream of nitrogen and re-dissolved in 1 mL of acetonitrile. The samples were passed through 0.2 um PTFE filter, transferred into autosampler vial.

5. Storage stability test

Non-pesticide samples were fortified with Metaflumizone, Chlorantraniliprole, Spinetoram (0.1 mg/kg) and Etofenprox (0.2 mg/kg) at 10 MLOQ level. These samples were stored at -20℃ until analytical date of each compounds. The analysis of samples was performed through the established method.

Residue analysis of Ssam cabbages

Homogenized sample of 10 g (Spinetoram, etofenprox) were weighed into 50 mL sastard tube and 20 g (Chlorantraniliprole, metalumizone) were weighed into 170 mL PTFE bottle. These samples were analyzed using established method.

Method development: Etofenprox

1) Analytical instruments and conditions: The analysis of Etofenprox was performed with a Shimadzu GCMS-TQ8040 equipped with MS/MS detector (Kyoto, Japan). The analytes were separated though capillary column, BPX5 (30 x 0.25 mm, i.d., film 0.25 um). The initial temperature of oven was 120 ℃ and programed as follows: 120℃ (hold time 0.5 min), raised to 320℃ (50℃/min) and hold 5.5 min (total program time 10.00 min). The carrier gas was helium and flowed constantly at 1.5 mL/min in the column. Samples were injected 2 μL into the mass spectrometer using splitless mode. The mass spectrometer was carried out used EI(electron impact) ionization. The parameters of MS/MS condition used for instrument analysis were as follow. Ion source temperature 230℃, interface temperature 280℃, solvent cut time 5 min, detector voltage 1.4 kV and collision gas argon.

2) Calibration curve: Mixture working solutions of major compound of Spinetoram and it metabolites were diluted by using acetonitrile and added extracts of blank (non-pesticide) sample to make matrix-matched standard solution.

3) Recovery test: The 10 g of homogenized non-pesticide sample were weighed into 50mL PTFE centrifuge tube. The samples were fortified with working solution of etofenprox to spike level of 0.01 and 0.1 mg/kg (MLOQ and 10 MLOQ) and 10 mL 0.1% formic acid acetonitrile solution was added. The samples were extracted by shaking vigorously for minutes. Four gram of $MgSO_4$ and 1 g NaCl were added and vortexed for 1 min. The samples were extracted by shaking for vigorously 1 minutes and centrifuged at 3,000 rpm for

10 minutes. Clean-up method were compared by used dSPE (PSA150 mg + GCB 7.5 mg). Aliquot of 1 mL of the supernatant were transferred into 2 mL dSPE kit containing each sorbents, vortexed for 1 min and centrifugated at 14,000 rpm for 4 minutes. The samples were transferred 400 μL into autosampler vial and acetonitrile 100 μL was added.

Results and Discussion

Subject crops

There have been only 0 cases of pesticides registered in Ssam cabbage for the past five years (2011 - 2015) (table 2), but the number of cases of nonconformity in monitoring inspection for safety management was 12-17 cases per year during same periods (table 5). This is a poor condition compared to other minor crops. With the introduction of PLS, when the MRLs are set at 0.1 mg / kg for all crops, the safe use of pesticides is further strengthened. If MRLs is set and the appropriate PHI is not set, there is a high probability that the crop nonconformity will increase. So, registration of pesticide use is necessary.

Analytical instruments and conditions

The one of conventional equipment is HPLC, which is very efficient to separate non-volatile analytes and matrices of vegetables (Mangia, 1996). Most of HPLC analysis method is performed with UV, diode array detector (DAD). Sensitivity and selectivity of HPLC equipped DAD are so low that It can be hard to detect pesticides residue at low concentration (I. Liška, 1996). Today use of HPLC equipped tandem mass spectrometry (MS/MS) is increased because of performing more effective quantitation analysis in small amounts of pesticide residues than HPLC equipped DAD (Reemtsma, 2001). In HPLC equipped MS/MS was carried out selected reaction monitoring (SRM) mode, which was provided high selective and sensitive of detection(VanVyncht, 2001). For using SRM mode, the standard solution of pesticide compounds was used for optimizing of ionization condition (Silvial L. et al, 2015). In LC-MSMS, the ionization was occurred protonated molecular ion of major compound $[M+H]^+$ in positive mode, occurred deprotonated molecular ion $[M-H]^-$ in negative mode. Molecular of ionization $[M+Na]^+$ was possible to occur in positive mode (Leandro C.C. et al, 2007). Standard solutions of the compound were injected into LC-MSMS and full scan mass spectra data were obtained to check precursor ion (SANTE/11813, 2017). The product ions were obtained by the precursor ions which were fragmented at a series of collision energies

intensity. The optimal precursor ions, product ions and collision energy were chosen for selected reaction monitoring (Petrović M et al, 2005). The analysis of Metaflumizone, Chlorantraniliprole and Spinetoram were performed with HPLC equipped MS/MS by using LC analytical method of each compounds. Mass spectra data for Metaflumizone, Chlorantraniliprole and Spinetoram were obtained at mass range of 50-900 m/z. In the ESI (+) mode, most of all compounds were formed protonated molecules of $[M+H]^+$ and part of compounds were generated protonated molecules of $[M+Na]^+$.

Metaflumizone: The isomers of Metaflumizone were same MS/MS condition and separated by LC program for analyze severally. The precursor ion were selected protonated molecule of $[M+H]^+$ at 507.0 m/z. The production ion and collision energies were 507.0 > 178.1 (-27 eV) to quantitation and 507.0 > 287.1 (-25eV) to identification.

Chlorantraniliprole: Chlorantraniliprole has several isotopes by two of chlorine and one of bromine. The precursor ion were selected protonated molecule of $[M+4+H]^+$ at 486.0 m/z. The production ion and collision energies were 486.0 > 454.9 (-19 eV) to quantitation and 486.0 > 286.0 (-14 eV) to identification.

Spinetoram: Spinetoram was consisted of major compounds and its metabolite, each compounds were established as a MS/MS conditions

separately.

Etofenprox: The analysis of Etofenprox was conducted with HPLC equipped DAD, after then proposed developed method used GC-MS/MS. Etofenporx is highly non-polar compound and thermally stable, allowing GC analysis. But, since it is a hydrocarbon compound containing no halogen or nitrile group, it is hard to analysis using GC detector. Original method of Etofenprox analysis is performed GC equipped ECD by changing molecular structure using iodinated derivative to increase sensitivity (NIFDS, 2017). Other thesis was used analytical instrument as HPLC equipped DAD (Farag M. et al, 2012), etofenprox was injected into HPLC to check sensitivity of detector. The optimal wavelength of Etofenprox was investigated in HPLC equipped DAD. In the previous paper, Etofenprox was selected as 225 nm for analysis (Kim, 2014b), but 235 nm was used as a wavelength for better selectivity in this study.

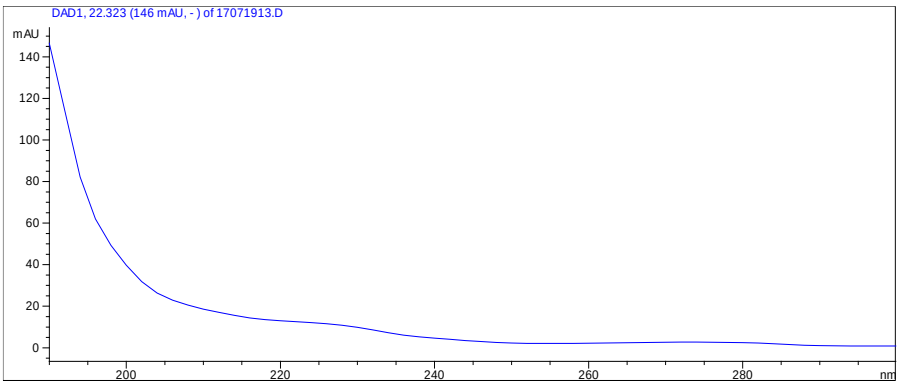


Table 7. MRM condition

Compound	M.W	Ionization	Precursor ion>Product ion(CE, eV)	
			Quantifier ion	Qualifier ion
Metaflumizone (Z)	507	$[M+H]^+$	507.00>178.15 (-27)	507.00>287.15 (-25)
Metaflumizone (E)	507	$[M+H]^+$	507.00>178.15 (-27)	507.00>287.15 (-25)
Chlorantraniliprole	481.0	$[M+4+H]^+$	486.0>454.9 (-19)	486.0>286.0(-14)
XDE-L	760	$[M+H]^+$	760.00>142.20 (-30)	760.00>98.20 (-55)
XDE-J	748	$[M+H]^+$	748.00>142.15 (-32)	748.00>98.15 (-50)
Demethyl-175-L	746	$[M+H]^+$	746.00>128.10 (-29)	746.00>84.10 (-54)
Demethyl-175-J	734	$[M+H]^+$	734.00>128.10 (-26)	734.00>84.05 (-55)
Formyl-175-L	774	$[M+H]^+$	774.00>156.10 (-21)	774.00>125.15 (-15)
Formyl-175-J	762	$[M+H]^+$	762.00>156.15 (-22)	762.00>560.35 (-12)

Clean-up method

In pesticides residue analysis, SPE is generally used for clean-up of extracts mainly obtained from solid or liquid matrices. The basic principle which governs the SPE process with these polar sorbents is adsorption chromatography (Hennion, 1999).

Adsorbents such as aminopropyl (-NH2) and primary secondary amine (PSA) have strong polar organic entities such as sugars, caustic acids and fats in food and environmental samples. GCB is notable when it strongly retains planar molecules such as carotenoids, chlorophyll and sterols. Another SPE format is dSPE, which removes matrix interference by adding adsorbents to the extract and then separates most of the extract by centrifugation (Pico, 2015).

1. Chlorantraniliprole & Etofenprox: In order to remove chlorophyl from Korean cabbages, SPE include GCB (GCB-NH2) SPE catridge was used instead of conventional SPE(SPE-silica). In this test, result from chlorantraniliprole was not good, recovery rate 36.7%. Etofenprox had good recovery, recovery rate 91.8%, so this cartridge used only etofenprox.

2. Spinetoram: The presence of GCB to remove chlorophyll was not disturbed the recovery results and the experiment was

conducted with the highest pigment dSPE containing the most GCB.

Method validation

For validation of developed method, LOD, LOQ, linearity and range of calibration, recoveries, matrix effects and storage stability were performed (FAO, 1993).

Instrumental LOD and LOQ are determination of the sensitivity of instrument (Silvial L. et al, 2015). Under several concentration of standard solution, 0.05 ng(Metaflumizone), 0.01 ng(Chlorantraniliprole), 2 ng(Etofenprox) and 0.01 ng(Spinetoram) were observed as ILOQ.

MLOQ (Method limit of quantitation) is defined as the smallest concentration of the analyte that can be quantified with acceptable precision and accuracy (FAO, 1993) and obtain using equation. MLOQ of metaflumizone was 0.01 mg/kg, chlorantraniliprole was 0.01 mg/kg, etofenprox was 0.02 mg/kg and spinetoram was 0.01 mg/kg. The results were accomplished in the criteria of Korea Food and Drug Administration which are below 1.0 - 3.0 mg/kg or half of MRLs.

The regression equation of each compounds and coefficient of determination was summarized in table 7 and figure 5. It was used matrix matched standard solution for calibration curves that metaflumizone, chlorantraniliprole and spinetoram. Etofenprox, which was analyzed using HPLC equipped DAD, was written as calibration curves with

standard solutions. All of compounds were satisfied as good linearity and coefficient of determination.

To determine the accuracy and precision of the established method, the recovery test was conducted using the Ssam cabbage untreated pesticide. Samples were spiked with two levels as MLOQ, 10MLOQ of each pesticide standard solutions and analyzed using established methods. All components satisfied the 70-120% recovery range. The detailed recovery is shown in table 9. Figure 6 is representative chromatogram of metaflumizone.

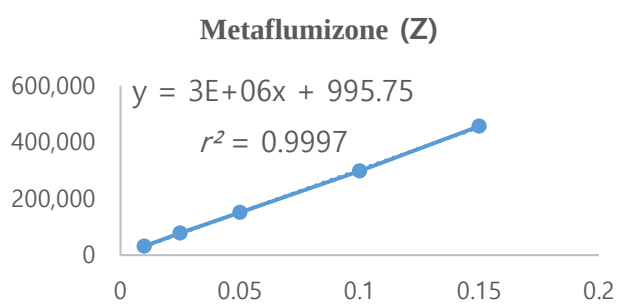
Table 8. Calibration curves and linearity

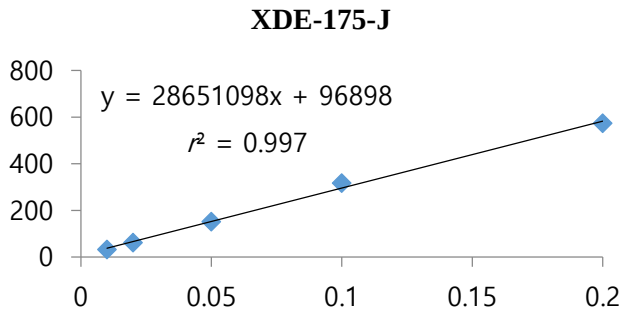
Compound		Calibration range (mg/kg)	Regression equation	r^2
Metaflumizone	Z isomer	0.01 - 0.15	y =3015804 x + 996	0.9997
	E isomer		y =2212868 x + 191	0.9982
Chlorantraniliprole		0.005 – 0.1	y = 22726052 x + 82357	0.9995
Spinetoram	XDE-175-J	0.005 - 0.1	y = 58227290 x + 73184	0.9985

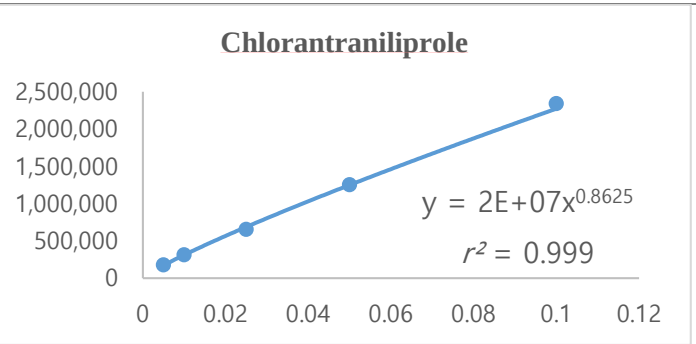
	XDE-175-L		y = 30967237 x + 45840	0.9986
	XDE-175-N-demethyl-J		y = 26006073 x + 35575	0.9985
	XDE-175-N-demethyl-L		y = 34535409 x + 20286	0.9995
	XDE-175-N-formyl-J		y = 4242328.9 x + 2917.3	0.9992
	XDE-175-N-formyl-L		y = 3710976.5 + 6337.7	0.9958
Etofenprox		0.1 - 5	y = 32.8292 x -0.0448	0.9999

Figure 6. Calibration curves

Figure 6. Calibration curves







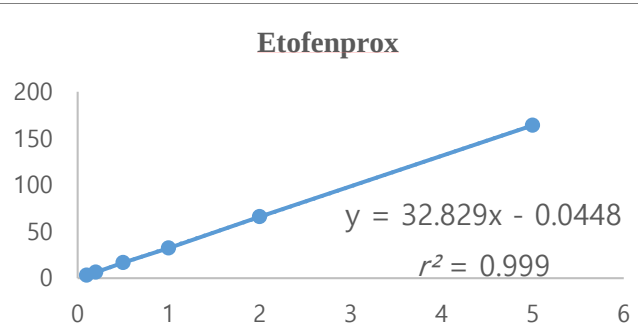


Table 9. Recovery test

Compounds	Fortified level (ppm)	Recovery rate(%) n=5	
		Average (%)	RSD (%)
Metaflumizone (Z)	0.01	86	6.2
	0.1	94.5	5
Metaflumizone (E)	0.01	92.8	5.4
	0.1	95.9	3.9
Chlorantraniliprole	0.01	70.3	7.8
	0.1	88	2.7
XDE-175-J	0.01	90.7	4.8
	0.1	100.4	4.8
XDE-175-L	0.01	86.1	6.2
	0.1	99	3
N-demethyl-175-J	0.01	88.3	7
	0.1	96.1	5
N-demethyl-175-L	0.01	95.3	3.7
	0.1	92.7	7.4
N-formyl-175-J	0.01	94	5.8
	0.1	102	2.5
N-formyl-175-L	0.01	81.5	10.6
	0.1	109.8	6
Etofenprox	0.02	81.8	6.7
	0.2	89.1	1.4

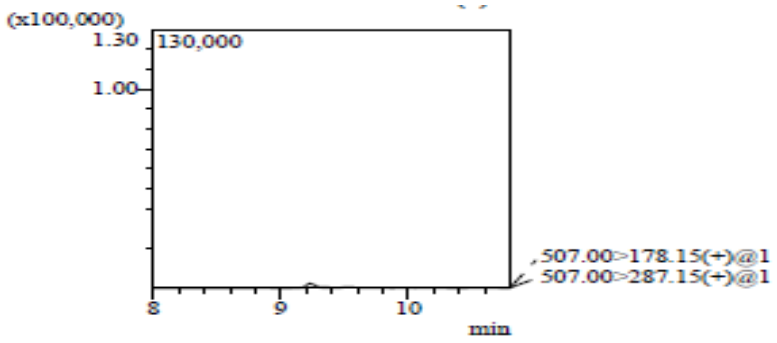
Figure 7. Representative chromatogram of recovery test (metaflumizone)

(A) Control

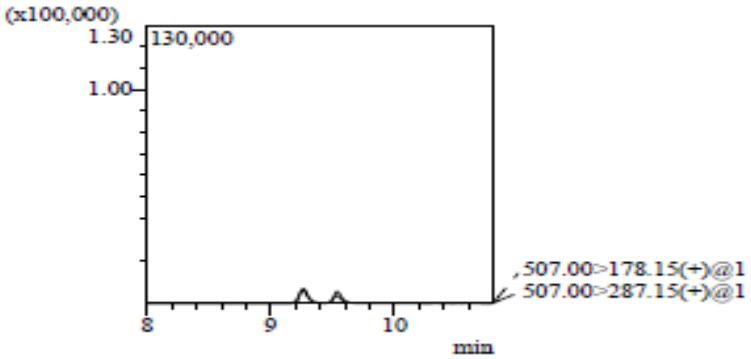
(B) MLOQ

(C) 10MLOQ

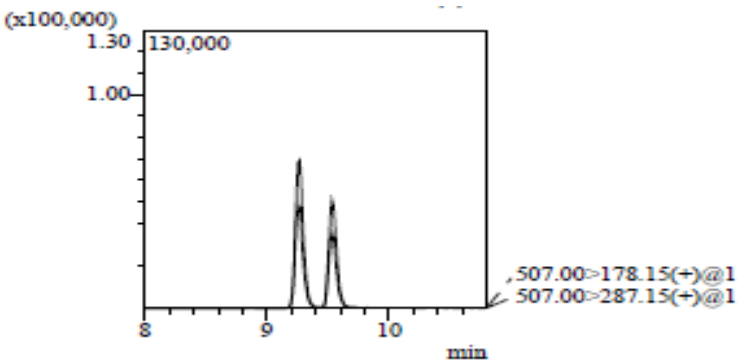
(A)


(x100,000)
1.30
1.00
130,000
507.00>178.15(+)@1
507.00>287.15(+)@1
8
9
10
min

(B)


(x100,000)
1.30
1.00
130,000
507.00>178.15(+)@1
507.00>287.15(+)@1
8
9
10
min

(C)


(x100,000)
1.30
1.00
130,000
507.00>178.15(+)@1
507.00>287.15(+)@1
8
9
10
min

Storage stability

The harvested samples should be analyzed immediately, but if cannot, they should be stored at -20°C until analysis. Then, non-pesticide samples were fortified with each pesticide working solutions, stored at same condition and analyzed in analysis date. The stored samples were analyzed using established method. The results showed that recoveries of average of each compounds were 87.7 - 108.0%, RSDs were below 10%. The detailed results of were table 10.

Table 10. The results of storage stability

Crop	Section	Fortified level (mg/kg)	Recovery (%)	RSD (%)
Metaflumizone	Z isomer	0.1	87.9	1.9
	E isomer	0.1	94.1	4.5
Chloratraniliprole		0.1	108.0	5.1
Spinetorma	XDE-175-J	0.1	106.4	4.0
	XDE-175-L	0.1	104.0	8.6
	XDE-175-N-demethyl-J	0.1	104.6	5.7
	XDE-175-N-demethyl-L	0.1	100.3	2.1
	XDE-175-N-formyl-J	0.1	104.8	1.5
	XDE-175-N-formyl-L	0.1	107.0	2.7
Etofenprox		0.2	87.7	1.8

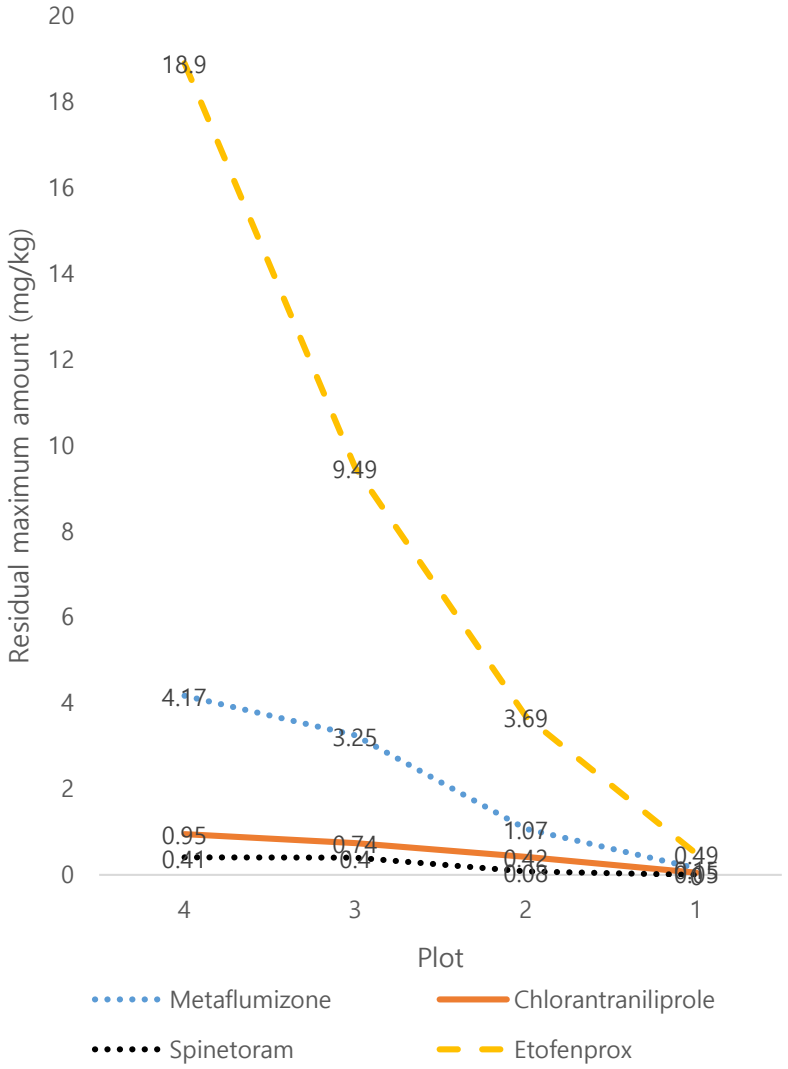
Dissipation Field Study

No pesticides were remained in untreated samples and detailed maximum residues amounts were table. In the analysis, samples, beyond the calibration curves range, were diluted. The residues of major compound and it metabolites were calculated and obtained as maximum residual amounts. The maximum residues in Korean cabbages were measured from 4.17 to 0.15 mg/kg (metaflumizone), 0.95 to 0.05 mg/kg (chlorantraniliprole), 0.41 to <0.01 mg/kg (spinetoram) and 18.90 to 0.49 mg/kg (etofenprox). In Korea, these pesticides were established as below the 1.0 - 3.0 mg/kg of MRLs in the Ssam cabbages. All pesticides showed a tendency to decrease over the time and were residual amounts of below the half of MRLs. Spinetoram and chlorantraniliprole were remained less than half an MRL 7days before harvest, and it is judged that it will not affect spraying 7 days before harvest. The MRLs of metaflumizone and etofenprox is set at 2 mg/kg, metaflumizone remained less than half an MRL 14days before harvest. However, etofenprox remained below MRL only 21 days ago. In the previous paper (Kim, 2009), the half-life of etofenprox EC in Korean cabbage was 3.2 days, it seems that the pesticide decomposition is affected by environment.

EC formula pesticides, metaflumizone and etofenprox, were remained high amounts of residues more than WG formula pesticides,

chlorantraniliprole and spinetoram. Based on these data, this study provides that metaflumizone, chlorantraniliprole, spinetoram and etofenprox is suitable to apply for Ssam cabbage under recommended dosage as the PHI.

Figure 8. Dissipation pattern of pesticides



	Metaflumizone	Chlorantraniliprole	Spinetoram	Etofenprox
1(30/21)	0.15	0.05	<0.01	0.49
2(21/14)	1.07	0.42	0.08	3.69
3(14/7)	3.25	0.74	0.40	9.49
4(7/0)	4.17	0.95	0.41	18.90

Method development: Etofenprox

In the former experiments, the etofenprox was analyzed was analyzed by LC-UVD using developed multiresidue method which is one of the conventional experimental methods. This method had been improved, but still consumed a lot of time and chemicals. These days, green chemistry is focused on for save time and save more the hazardous substance (EPA, 2006). Therefore, differently about conventional method, the new analysis method of etofenprox was established using QuEChERS method for easy, quick analysis and save the solvents and analyzed by GC-MS/MS for reduce the analysis time.

1) Analytical instruments and conditions

The GC conditions were set up through oven temperature programs to good sensitivity and good peak shapes. The SRM condition of etofenprox was established for high sensitivity analysis. The precursor ion were selected at 163.1 m/z. The product ion was obtained through different CE. The production ion and collision energies were 163.1 > 135.0 (-10 eV) to quantitation and 163.1 > 107.0 (-20 eV) to identification.

2) Clean-up method

The purification experiments were conducted to compare original dSPE kit and dSPE-GCB kit to remove chlorophyll. The original dSPE kit was consists of MgSO4 + PSA and the dSPE-GCB kit was contained in the original kit with GCB which was capable of removing chlorophyll. The etofenprox was selected dSPE-GCB kit that showed good recovery and removed most of the matrices of chloropyll.

3) Method validation

The etofenprox was observed to 0.002 ng as ILOQ. The ILOQ is lower than that analyzed by using HPLC, this means more sensitive analysis can be performed. The MLOQ of etofenprox was calculated to 0.001 mg/kg using ILOQ, weight of sample and so on. The calibration curve was made using matrix matching standards and the regression equation of etofenporx and coefficient of determination was y = 5053x + 1174.1 (r^2 = 0.999) in the concentration range of 0.4 – 20 ppb. The recovery test was conducted The recovery test was performed using untreated sample about pesticides. The samples were fortified to MLOQ, 10MLOQ as two levels and analyzed using established method and GC-MS/MS.

Recovery of 85.7% (RSD 3.69%) for the MLOQ concentration and 100.4% (RSD 0.67%) for the 10MLOQ concentration obtained acceptable data.

4) data compared with former data

The most noticeable feature is the difference in instrument analysis time and sensitivity (table 9) . In the conventional HPLC method, the analysis

time was 40 minutes, and the GC-MS/MS was 10 minutes. It was able to shorten it by 30 minutes, using GC-MS/MS. In addition, when using GC-MS/MS than HPLC, especially the sensitivity of ILOQ and MLOQ was excellent.

The conventional method and new method (QuEChERS) were compared with consumed solvents and analysis time. Although this varies from person to person by experimental proficiency, it was roughly compared the analysis time with the solvent used per sample (table 12). In the conventional method, 11 mL of ACN, 10 mL of MeOH and 4.11 mL of DCM per samples were consumed and about 40 minutes are consumed by the time of instrument analysis. On the contrary, the new method was used only 10 mL of ACN, it was consumed about 10 minutes by the time of instrument analysis. The consumed solvent was reduced by about 57 percent and the total analysis time, including instrumental analysis, was reduced by about 58 percent. The new method was satisfied to the green chemistry that consider the environment, and it is judged to be an efficient analytical method because the analysis time required is short and the experimental procedure is simple.

Table 11. compare instrument sensitivity

	HPLC	GC-MS/MS
ILOQ (ng)	2	0.002
MLOQ (mg/kg)	0.02	0.001
Analysis time	40	10

Table 12. compare the analysis time and solvent per sample

<step>	**former method**		**New method (using QuEChERS method)**	
	solvent	time	solvent	time
Extraction	ACN 10 mL	10 min	0.1 % formic acid in ACN 10 mL	10 min
Seperation	4 g MgSO4, 1 g NaCl	1 min	4 g MgSO4, 1 g NaCl	1 min
centrifugation		10 min		10 min
Evaporate		7 min		
Clean-up	MeOH/DCM(1/99) 11 mL DCM 4 mL ACN 1 mL SPE-NH2	12 min	150 mg MgSO4, 25 mg PSA, 7.5 mg GCB	2 min
Nitrogen stream	20 min			
Analysis		40 min		10 min`

Conclusion

The insecticide metaflumizone, chlorantraniliprole, spinetoram and etofenprox were established analytical method. The real sample was analyzed using established method, and checked residue amounts of pesticides. EC formula pesticides, metaflumizone and etofenprox, were remained high amounts of residues more than WG formula pesticides, chlorantraniliprole and spinetoram. All pesticide residues remained below the MRL value set in Korea. Through this study, base data of PHI for register of pesticide use Korean cabbages was obtained.

In addition, the new analysis of etofenprox using QuEChERS instead of the conventional method, and it was easy and simple to obtain good result compared with time and effort

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Abstract in Korean

서울대학교 대학원
농생명공학부 응용생명화학전공
백수진

현재 우리나라에는 배추 좀 벌레를 방제하기 위해서 살충제인 메타플루미존, 클로란트라닐리프롤, 스피네토람 그리고 에토펜프록스의 배추 사용이 등록되어있다. 본 연구는 유사작물인 엇갈이배추의 사용 농약 등록과 안전 사용 기준의 기초자료로 사용하기 위하여 실시되었다. 유제인 메타플루미존과 에토펜프록스 그리고 입상수화제인 스피네토람과 클로란 트라닐리프롤을 각각 4개의 처리구로 나눠서 엇갈이배추에 처리하였다. 메타플루미존, 클로란트라닐리프롤 그리고 스피네토람은 LC-MS/MS를 이용하여 분석하였고, 에토펜프록스는 HPLC-UVD를 이용하여 분석하였다. 이 성분들은 모두 MLOQ를 0.01-0.02 mg/kg을 만족하였으며, 검량선 범위에서 모든 성분이 0.99를 만족하였다. 메타플루미존, 클로란트라닐리프롤 그리고 에토펜프록스는 기존의 방법을 통하여 분석을 진행했지만, 스피네토은 QuEChERS를 이용해서 빠른 분석을 할 수 있었따. 실제 시료 분석에서는 잔류분의 정의에 대사물이 존재하는 메타플루미존과 스피네토람은 환산계수를 이용하여 전체 최대 잔류량을 계산했다. 엇갈이배추에서 최대잔류량은 0.15-4.17 mg/kg(메타플루미존), 0.05-0.95 mg/kg(클로란트라닐리프롤), <0.01-0.41 mg/kg(스피네토람) 그리고 0.49-18.90 mg/kg(에토펜프록스)였다. 시간이 지남에 따라 모든 농약은 감소하는 경향을 보였고, 한국에서 이들 농약에 대한 MRL이 1-3 mg/kg으로 잡혀있는 걸 생각했을 때 MRL 수준 미만으로 잔류하기 때문에 PHI 설정에 도움이 되는 자료라고 사료된다.

주요어: Metaflumizone, Chlorantraniliprole, Spinetoram, Etofenprox, minor cops, PHI, LC-MS/MS, QuEChERS

학 번: 2016-21727