농학석사학위논문

엇갈이배추 재배 중 살균제 Fluxapyroxad와 대사물의 동시 분석 및 물/토양 시료 중 PAHs의 동시분석

Simultaneous Determination of Fungicide Fluxapyroxad and its Metabolites in Ssam cabbage, and PAHs in Water and Soil

2018년 8월

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농생명공학부 응용생명화학전공

전 다 래

A Dissertation for the Degree of Master of Science

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2018년 6월

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전다래의 석사학위논문으로 인준함

2018년 6월

위 원 장 _____ 신 찬 석 (인)

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Abstract

In this study, we investigated the residual of fungicide fluxapyroxad and its metabolites M700F002 and M700F048 used in ssam cabbage cultivation and established an optimal analytical method of PAHs in water and soil samples, It was carried out to confirm application monitoring. Analysis of fluxapyroxad and metabolites ensured reliability through QuEChERS pretreatment method and analytical method validation of the sample. Fluxapyroxad+metalaxyl-M 8 (4+4)% (SC)was sprayed to each plot on planned preharvest spreading day and LC-MS/MS was passed through a pretreatment process after harvest and analyzed. In the ssam cabbage the residual amount of fluxapyroxad in terms of total treatment in each treatment group was 1.15-0.28 mg/kg, which showed a tendency to decrease over time. PAHs are used for analysis of existing PAHs by optimizing Ion source temperature, Detection mode, Electron energy etc. to ensure optimum sensitivity and selectivity in MS/MS analysis for about 25 species as environmental pollutants High sensitivity and improved signal-to-noise ratio could be obtained using GC-MS/MS rather than analyzed conditions. Improvement of pretreatment methods such as liquid-liquid distribution and concentration, verification of analytical limits of quantitative determination, linearity of calibration curves, recovery rate, etc for each component, established an easy and rapid simultaneous analysis method. We also confirmed the applicability to actual water and soil samples.

Key Words: Fluxapyroxad, M700F002, M700F048, LC-MS/MS, GC-MS/MS, PAHs, Water, Soil

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List of Abbreviations

ACN	acetonitrile
ACT	acetone
CV	coefficient of variation
ADI	acceptable daily intake
CV	coefficient of variation
DEG	diethylene glycol
DCM	dichloromethane
D.W	deionized Water
EI	electron impact mode
ESI	electrospray ionization
GC/MS	gas chromatography mass spectrometry
LC/MS	liquid chromatography mass spectrometry
ILOD	instrument limits of detection
ILOQ	instrument limits of quantification
LOD	limit of detection
LOQ	limit of quantitation
MeOH	methanol
MLOQ	method limit of quantitation
MRLs	maximum residue limits
MS/MS	tandem mass spectrometry
MRM	multiple reaction monitoring
NaCl	sodium chloride

$NaSO_4$	sodium sulfate
PSA	primary secondary amine
PTFE	polytetrapropylene
Q	quadrupole
QuEChERS	quick, easy, cheap, effective, rugged and safe
SIM	selected ion monitored
SRM	selected reaction monitoring
PAH	polycyclic aromatic hydrocarbons
PSA	primary secondary amine
PMRM	pseudo-multiple reaction monitoring

Part 1

Simultaneous Determination of Fungicide Fluxapyroxad and its Metabolites in Ssam cabbage

Introduction

Pesticide

Pesticides were widely used in agriculture to fight weeds, disease and pests there by increasing productivity. This positive effect pesticide of health should be considered along with the hazards. Pesticides promote health, directly and indirectly through the management of insect vector mediated diseases, through the increase and improvement of agricultural production of food. Thus, pesticides are important in improving the quality of life. But pesticides were compounds and they are also toxic to humans. To understand this toxicity, humans have been experimenting with many animals (William F. Durham, 1979). Currently, the pesticides widly used variety of compounds belonging to different chemical classes. More than 800 chemicals used worldwide such as pesticides, herbicides, and fungicide were available in a variety of formulations. Pesticides have been regarded as potential chemical mutagens. (C. Bolognesi, G. Morasso, 2000) Therefore, many products, including fruits and vegetables, must monitor and regulate the concentration of pesticide residues.

Fungicide

Fungicide refers to agricultural chemicals used mainly to kill filamentous fungi, bacteria, viruses and the like or to inhibit growth. Since these pathogens were organisms such as plants, which were hosts, there was no harm to the host and it was necessary to selectively control only pathogens, so it was difficult to control diseases with pesticides rather than pests. Spraying of the fungicide for prevention was necessary at the time when the plant is likely to be infected with the pathogen, and since only the part applied mainly by the fungicide was prevented, uniform coating on the target plant was necessary. In addition, Spraying of fungicide must be carried out repeatedly. Bacteria and fungi whose growth was suppressed by bactericidal agents can produce 12 to 25 generations during the growth period of 3 months and the effect of the fungicide was less effective due to dilution and plant growth (최신농약학, 2000).

Fluxapyroxad and its metabolites

Fluxapyroxad is a new active ingredient developed by BASF Corporation to control a broad spectrum of fungal diseases. IUPAC name is 3-(difluoromethyl)-1-methyl-N-(3′,4′,5′-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide. The Molecular formula is $C_{18}H_{12}F_5N_3O$. Fluxapyroxad belongs to the carboxamide class of chemicals and its mode of action is inhibition of succinate dehydrogenase in complex II of the mitochondrial respiratory chain, which results in inhibition of spore germination, germ tubes, and mycelial growth within the fungus target species (Figure 1) (Table 1) (EPA, 2012). Fluxapyroxad has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues. 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid (M700F002) and, 3-(difluoromethyl)-1-(ß-D-glucopyranosyl)-N-(3′,4′,5′-trifluorobiphenyl-2-yl)-1H-pyrazole-4- are the predominant metabolites of fluxapyroxad (M700F048) (Table 3). Fluxapyroxad is formulated as an emulsifiable concentrate (EC) or suspension concentrate (SC) and is foliar applied or used as a seed treatment. (S. Li et al., 2014) (EPA, 2012). According to Applicability of Pesticide MRLs for food in general MRLs of fluxapyroxad in various agricultural products in Korea were listed (Table 2)

QuEChERS method

QuEChERS is sample preparation where there was a trend to shift from laborious traditional methods to fast and simple approaches. The term QuEChERS is Quick, Easy, Cheap, Effective, Rugged and Safe multiresidue method. The most used approach for extraction of pesticides from agricultural products samples is nowadays the QuEChERS procedure. This method was replaces many complicated analytical steps. The QuEChERS method includes two steps. The first is the extraction and the second is the cleaned up. In the extraction step, use ACN as the extraction solvent, and add salt mixture for the partitioning. Cleaned up step was used by dispersive solid phase extraction (d-SPE). The final extract in acetonitrile is analysis by LC or GC (Anastassiades, Lehotay, Stajnbaher, and Schenck, 2003). The QuEChERS covers a very wide compounds, including polar compound as well as basic and acidic compound. Recently, there are two other method besides the original method: the acetate buffering version AOAC and the citrate buffering version CEN (AOAC official method Lehotay et al., 2007, CEN standard method EN 15662 Payá et al., 2007).

Figure 1. Structure of fluxapyroxad

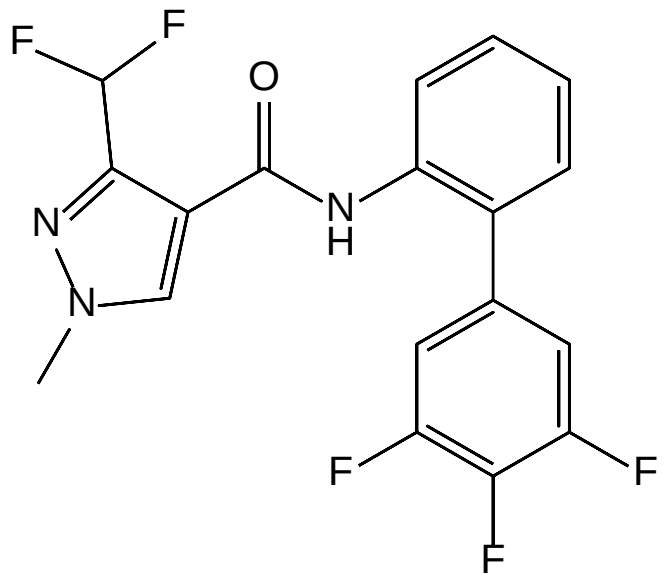
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Table 1. Chemical and physical properties of fluxapyroxad

Property	Information	Reference
Common name (Company Experimental name)	Fluxapyroxad (BAS 700 F)	The Pesticide Fact Sheet (EPA, 2012)
Pesticide Type	Fungicide	
Chemical Class	Carboxamide	
Company (Year of Initial Registration)	BASF Corporation (2012)	
IUPAC name	3-(difluoromethyl)-1-methyl-N-(3′,4′,5′-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide	
CAS No.	907204-31-3	
Molecular formula	$C_{18}H_{12}F_5N_3O$	
Molecular weight	381.3	
Mode of action	Inhibition of succinate dehydrogenase in complex II of the mitochondrial respiratory chain	
Toxicology	**Mammalian toxicology** Oral Acute oral LD50 for female rats ≥2000 mg/kg. **Skin and eyes** Acute skin LD50 for male and female rats > 2,000. Acute irritation to skin and eyes (rabbits). Slightly irritating to skin and eyes (rabbits). No sensitization to skin (guinea pig) **Inhalation** LC50 for rats > 5.1 mg/L air.	(BASF Corporation., 2010) (Hammer, S., 2009)

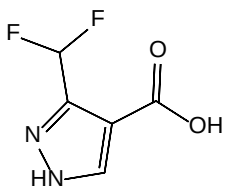
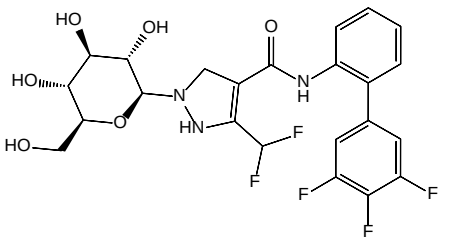
Table 2. MRLs of fluxapyroxad in various agricultural products

Crop	MRL (mg/kg)	Registered date
Eggplant	0.5	2016-12-29
Persimmon	0.3	2014-09-11
Citrus Fruits	1.0	2016-12-29
Potato	0.02	2014-04-24
Prune	3.0	2016-12-29
Raisin	5.7	2016-12-29
Nuts	0.05	2016-12-31
Green &red pepper(Fresh)	1.0	2014-09-11
Soy bean	0.15	2014-04-24
Strawberry	2.0	2016-05-31
Peanut	0.01	2014-04-24
Melon	0.5	2014-09-11
Wheat	0.3	2014-04-24
Banana	3.0	2016-12-29
Pear	0.8	2013-11-12
Korean cabbage	0.05	2016-05-31
Barley	2.0	2014-04-24
Peach	0.3	2016-12-31
Leek	5.0	2016-12-29
Apple	0.5	2013-11-12
Sugar cane	3.0	2016-12-31

Crop	MRL (mg/kg)	Registered date
Celery	10	2016
Watermelon	0.1	2014-09-11
Sorghum	0.8	2014-04-24
Onion	0.05	2015-10-29
Cucumber	0.2	2014-09-11
Corn	0.15	2014-04-24
Pea	0.4	2014-04-24
Rape seed	0.8	2014-04-24
Plum	1.5	2016-12-29
Korean melon	0.3	2014-09-11
Cherry	3.0	2014-04-24
Beans	0.3	2016-12-31
Welsh Onion	2.0	2016-05-31
Grape	2.0	2014-09-11
Sweet pepper	1.0	2014-09-11
Sunflower seed	0.2	2014-04-24

*Pesticides and Veterinary Drugs Information (Ministry of food and drugs safety)
(Korean Pesticides MRLs in Food; 2016;, 2016) (Safety, 2017)

Table 3. Fluxapyroxad and metabolites found in metabolism and environmental fate studies

	M700F002	**M700F048**
Chemical Structure		
IUPAC Name	3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid	3-(difluoromethyl)-1-(ß-D-glucopyranosyl)-N- (3′,4′,5′-trifluorobiphenyl-2-yl)-1H-pyrazole-4-carboxamide
Found in	Traces in Rat, Soya bean, Wheat, Confined Rotational, Soil	Rat, Tomato, Soya bean, Wheat, Confined Rotational

The purpose of studies

The purpose of this study is to investigate the crop residue of fluxapyroxad and to utilize it as basic data for the safety evaluation of residual pesticide. Flxapyroxad+Metalaxyl-M 8(4+4)% Suspension concentrate (SC) was applied to the ssam cabbage according to the scheduled time (35/28 days, 35/28/21 days, 35/28/21/14 days, 28/21/14/7 days, and 21/14/7/0 days before harvest) and the residue ssam cabbag were analyzed to find out the maximum residue level.

Materials and Methods

Chemical and reagents

Acetonitrile (ACN) were HPLC grade and purchased from Fisher ChemAlert® (Fisher Scientific, USA). The QuEChERS materials were usted by extract kit ’ (Ultra Scientific, USA), containing 4 g of magnesium sulfate ($MgSO_4$), 1 g of NaCl, 1 g. For the dispersive SPE (d-SPE) cleanup of crop extracts from sample, ‘Ultra QuECh dSPE-General’ (2 mL centrifuge tubes containing $MgSO_4$ 150 mg, primary secondary amine (PSA) 25 mg) (2 mL centrifuge tubes containing $MgSO_4$ 150 mg, C18 25 mg) (Ultra Scientific, USA) was used.

Analytical standard and pesticide for spraying

Standard material of fluxapyroxad (Purity: 99%) was purchased from Sigma-aldrich (Buchs, Switzerland). M700F002 (Purity: 98.5%)and M700F002 (Purity: 94%) were obtained from Nonghyup Chemical (Seongnam, Korea) Flxapyroxad+Metalaxyl-M 8(4+4)% Suspension concentrate (SC) were from Farmhannong (Seoul, Korea).

Standard solutions

Each analytical standards were dissolved in acetonitrile to make concentrated stock solution at the concentration of 1,000 mg/L. The working solutions were prepared by serial dilution of stock solution with acetonitrile.

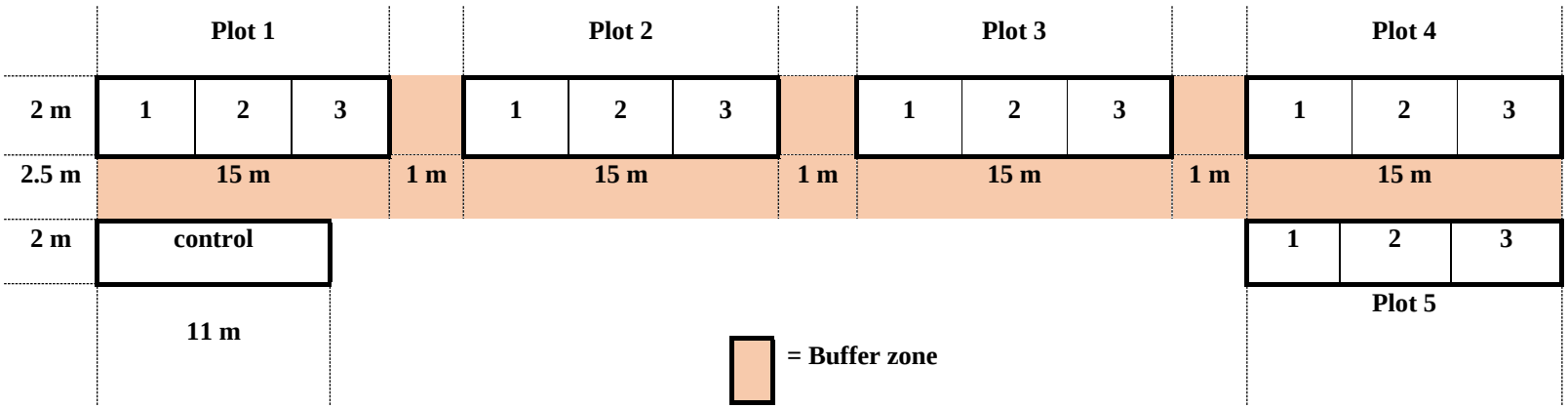
Subject crops

Ssam cabbage ‘Sil-lok’ of “pesticide-free (i.e no pesticide residues are present above the detection limits of the multi-residue method)” were harvested for the field trials. After being chopped, macerated, the sample were kept in polyethylene bags in a freezer (-20℃) until experiment.

Field trials

Test field was located in Yongin-si (Kyeonggi-do, Korea) and the field size was 180 m^2 (Figure 2). The field trial was divided into five plots depending on the date of pesticide treatment. The size of each plot was 30 m^2 containing 3 replicates of 2 m^2. Each plot was treated with the pesticide by several times as follows: Plot 1 was treated at 35/28 days before harvest, plot 2 was 35/28/21 days before harvest, plot 3 was 35/28/21/14 days before harvest, plot 4 was 28/21/14/7 days before harvest and plot 5 was 21/14/7/0 days before harvest. To prevent cross contamination during spraying the pesticide, the buffer zones were installed between buffer zone was made between control and treated plots. The arrangement of field trial is illustrated in (Figure 3). Flxapyroxad+Metalaxyl-M 8(4+4)% SC was sprayed by 2,000 times dilution with water using a pressurized 20 L handgun sprayer. Before using the handgun sprayer, reproducibility test for spraying. Spray quantity 983 mL per min. The crop was treated with the diluted pesticide solution until crop was wetted sufficiently (Figure 3).

Figure 2. Experimental plots in field



Control : Pesticide-free; No treated

Plot 1 : Treated twice at 35/28 days before harvest

Plot 2 : Treated thrice at 35/28/21 days before harvest

Plot 3 : Treated quater at 35/28/21/14 days before harvest

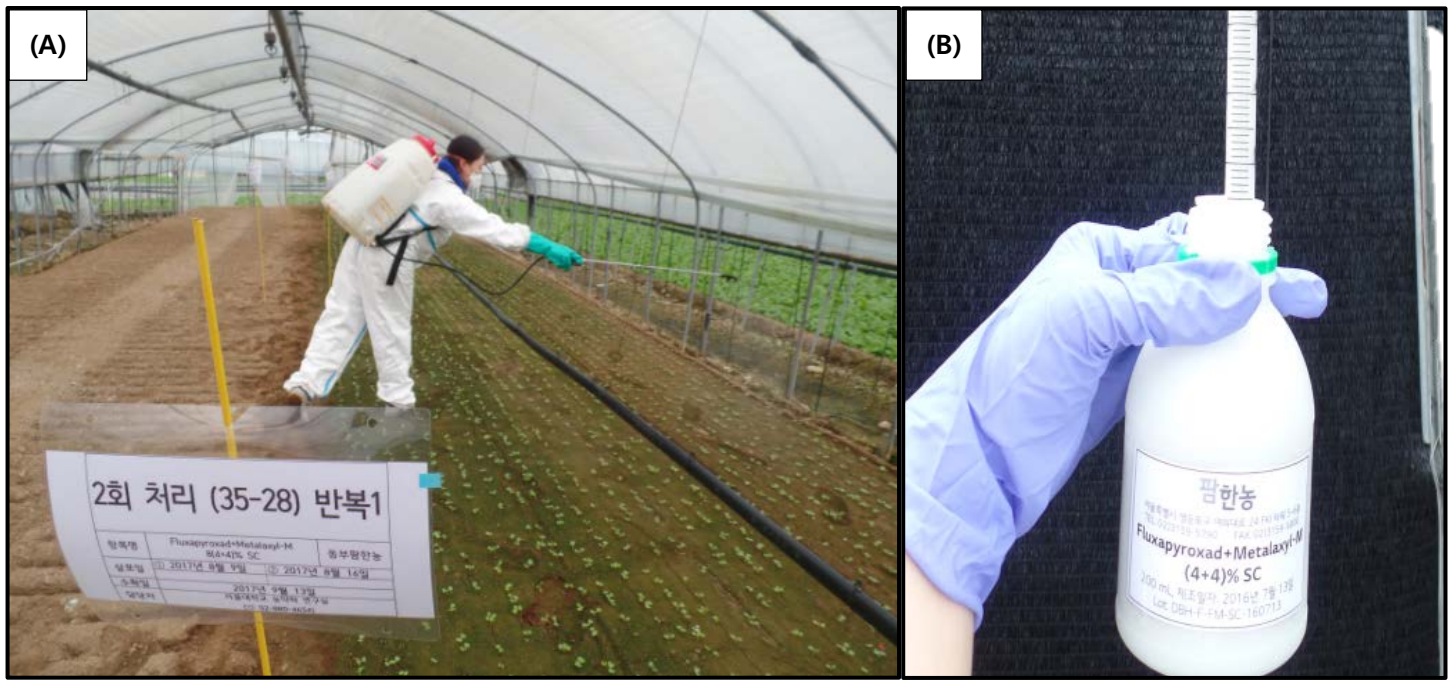
Plot 4 : Treated quater at 28/21/14/7 days before harvest

Plot 5 : Treated quater at 21/14/7/0 days before harvest

Figure 3. Preparation of spray solution and spray in field

(A) Application of pesticide on ssam cabbage

(B) Dilution of pesticide product (Flxapyroxad+Metalaxyl-M 8(4+4)% (SC))



Sampling

The harvest of ssam cabbage was conducted on September 13, 2017. Control plot was firstly harvested as prevent contamination prior to the pesticide-treated plots. Other samples of plot 1, 2, 3, 4 and 5 were randomly collected over 3.0 kg (Tabel 4). The samples were immediately transferred to laboratory after harvest. The collected ssam cabbage was homogenized with dry ice (Figure 4). Every sample were kept in a freezer -20 ℃ in polyethylene bags.

Table4. Yield of ssam cabbage

Plot	Yield (kg)		
	1	2	3
Pesticide-free plot	4.5		
Plot 1 35-28	4.3	4.5	4.4
Plot 2 35-28-21	4.5	5.2	4.7
Plot 3 35-28-21-14	4.2	4.0	4.3
Plot 4 28-21-14-7	3.9	3.7	3.3
Plot 5 21-14-7-0	3.6	4.8	4.5

Figure 4. Sample collection and preparation

(A)

(B)

(A) Sample collection

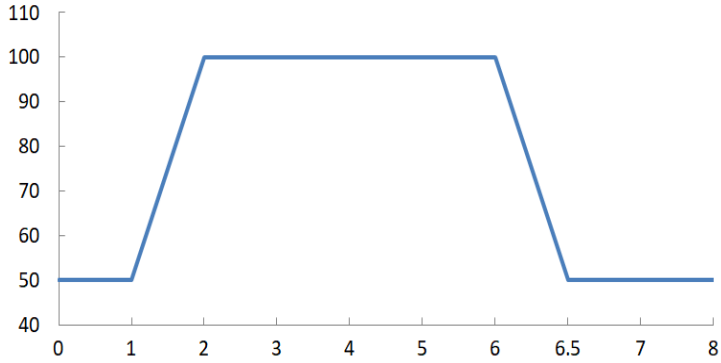
(B) Sample preparation

Analytical instruments and conditions

LC-MS/MS analysis for ssam cabbage was performed on LCMS-8040 (Shimadzu, Japan) coupled to Nexera UHPLC (Shimadzu, Japan) with electrospray ionization mode (ESI, positive mode). The analytical column were Kinetex C18 (100 mm × 2.1 mm i.d., 2.6 μm particle size, Phenemenex®, USA) column and Hypercarb (100 mm × 2.1 mm i.d., 3 μm particle size, Thermo, USA) column when analyzed M700F002, M700F048. The column temperature was 40ºC. Mobile phase was (A) 0.1 % formic acid in distilled water and (B) 0.1 % formic acid in ACN. The flow rate of mobile phase was 0.2 mL/min. Fluxapyroxad gradient system was programmed as follows: Initially, the organic solvent mobile phase (B) was hold at 50% for 1 min and ramped to 100% (B) in 1 min, held for 4 min. Finally, the mobile phase (B) was decreased to 50% in duration 1.5 min and maintained for 1.5 min (A total run time was 8 min) (Figure 5) and M700F002, M700F048 gradient system was programmed as follows: Initially, the organic solvent mobile phase (B) was hold at 20% for 1 min and ramped to 60% (B) in 1 min. Then, raised to 95% (B) in 5 min held for 2 min. Finally, the mobile phase (B) was decreased to 20% in duration 0.5 min and maintained for 2 min (A total run time was 12 min) (Figure 5). The injection volume was 5 μL. The temperature parameters for ESI were desolvation line (DL) temperature of 250℃, and heat-bock temperature of 400℃. The MRM transitions were optimized by injection of fluxapyroxad, M700F002 and M700F048 standard solution (1 μg/mL) and the best quantifier, qualifier ion, and collision energies (eV) were selected.

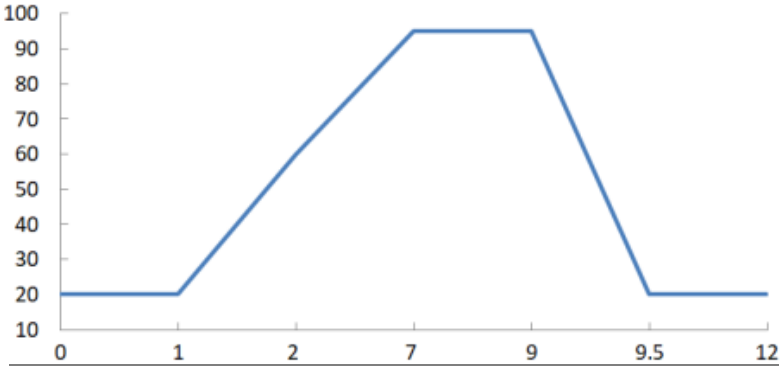
Figure 5. Gradient system of Fluxapyroxad and its metabolites

(A) Fluxapyroxad gradient



Time(min)	**0**	**1**	**2**	**6**	**6.5**	**8**
A(%)	50	50	0	0	50	50
B(%)	50	50	100	100	50	50

(B) M700F002 and M700F048 gradient



Time(min)	**0**	**1**	**2**	**7**	**9**	**9.5**	**12**
A(%)	80	80	40	5	5	80	80
B(%)	20	20	60	95	95	20	20

Method validation

1) ILOQ (Instrumental Limit of Quantitation)

After matrix matched standard solutions fluxapyroxad (0.2 and 0.005 mg/kg) and M700F002, M700F048 (0.1 and 0.01 mg/L) were analyzed by LC-MS/MS. The ILOQ was settled as the concentration where the signal-to-noise ratio was higher than 10.

2) MLOQ (Method Limit of Quantitation)

MLOQ was calculated by equation below

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

3) Calibration curve and linearity

a) Fluxapyroxad

Matrix matched standard solution	MSTD 1 (0.2 μg/mL)	MSTD 2 (0.1 μg/mL)	MSTD 3 (0.05 μg/mL)	MSTD 4 (0.02 μg/mL)	MSTD 5 (0.01 μg/mL)	MSTD 6 (0.005 μg/mL)
Standard solution	0.4 μg/mL 200 μL	0.2 μg/mL 200 μL	0.1 μg/mL 200 μL	0.04 μg/mL 200 μL	0.02 μg/mL 200 μL	0.01 μg/mL 200 μL
Sample matrix	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL

A series of matrix-matched fluxapyroxad standard solutions with concentration of 0.4, 0.2, 0.1, 0.04, 0.02 and 0.01 μg/mL were prepared with a blank extract. The relative standard deviation (RSD) was calculated at the calibration curve.

b) M700F002, M700F048

Matrix matched standard solution	MSTD 1 (0.2 μg/mL)	MSTD 2 (0.1 μg/mL)	MSTD 3 (0.05 μg/mL)	MSTD 4 (0.02 μg/mL)	MSTD 5 (0.01 μg/mL)
Standard solution	0.4 μg/mL 200 μL	0.2 μg/mL 200 μL	0.1 μg/mL 200 μL	0.04 μg/mL 200 μL	0.02 μg/mL 200 μL
Sample matrix	200 μL	200 μL	200 μL	200 μL	200 μL

A series of matrix-matched M700F002, M700F048 mixture standard solutions with concentration of 0.4, 0.2, 0.1, 0.04 and 0.02 μg/mL were prepared with a blank extract. The relative standard deviation (RSD) was calculated at the calibration curve.

4) Recovery test of fluxapyroxad and its metabolites analytical method

Sample preparation was conducted by QuEChERS method (Xixi Chen, 2016). Homogenized samples (10 g) in a 50 mL propylene tube were fortified with fluxapyroxad at 0.01 and 0.1 mg/kg (MLOQ and 10 MLOQ) and metabolites mixture solution at 0.02 and 0.2 mg/kg (MLOQ and 10 MLOQ). And the samples left for 30 minutes. 0.2% formic acid in ACN (10 mL) was added to each tube and shaken (1500 rpm) using a Geno Grinder (1600 MiniG™, SPEX® SamplePrep, New Jersey, USA) for 1 min. Then, added the sodium chloride (NaCl) 1 g and $MgSO_4$ 4 g. After the mixture was shaken vigorously for another 1 min, the tube was centrifuged at 3,500 rpm for 5 minutes (Combi 408, Hanil Science industrial, Korea). The supernatant (1 mL) was transferred into dSPE tube ($MgSO_4$ 150 mg, C18 25 mg) and vortexed (1 min) on a Multi Speed Vortex (MSV-3500, Biosan, Riga, Latvia) before centrifugation at 15000 rpm for 5 min. Finally, the supernatant 200 μL, ACN 200μL. 5 μL of final sample was injected into LC-MS/MS.

5) Storage stability test

The homogenized pesticide-free samples were fortified with fluxapyroxad spiking at 0.1 mg/kg (10 MLOQ) and M700F002, M700F048 mixture at 0.2 mg/kg (10 MLOQ). This samples were placed in a freezer (-20℃) until analysis. Samples were stored for 62 days (Sep 6 ~ Nov 7, 2017).

6) Residue analysis of ssam cabbage sample

Homogenized sample (10 g) was weighted into a 50 mL propylene tube. The samples were prepared by established method and analyzed using established LC-MS/MS conditions.

Matrix effect

MS in the determination of pesticide residues in food, mainly fruits and vegetables (Pico, Blasco, & Font, 2004). The most common way to avoid matrix effects in instrument is to use matrix-matched calibration standards. The Matrix effects (ME, %) was calculated using the following equation: (Lehotay et al., 2010; Rajski, Lozano, Uclés, Ferrer, & Fernández-Alba, 2013).

$$\text{ME}, \% = \left(\frac{\text{slope of matrix matched calibration curve}}{\text{slope of solvent standard calibration curve}} - 1\right) \times 100$$

Matrix effects were a major challenge existing in the MS detection, which is a suppression or enhancement of the analytes response caused by matrix co extractives (Liu, X. G et al., 2011). In instrument, different types of matrices or the sample pretreatment procedure were influential factors of the matrix effect. Therefore, the matrix effect of the optimized approach was studied in the two matrices by contrasting standard solutions with matrix-matched standard solutions. It was classified to be a strong matrix effect when the values were below −50% or above +50% (Zheng, Y. Q, 2013).

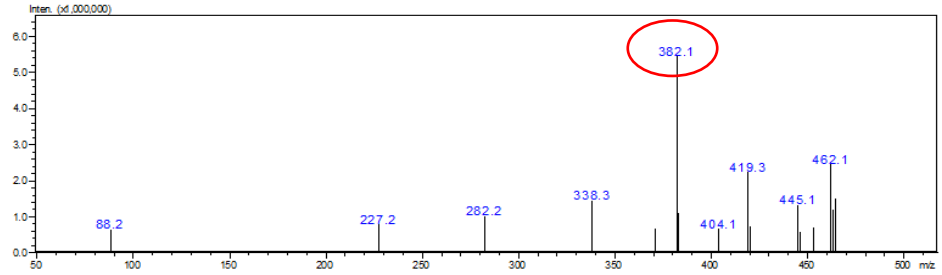
Results and Discussion

LC-MS/MS condition and selected reaction monitoring optimization

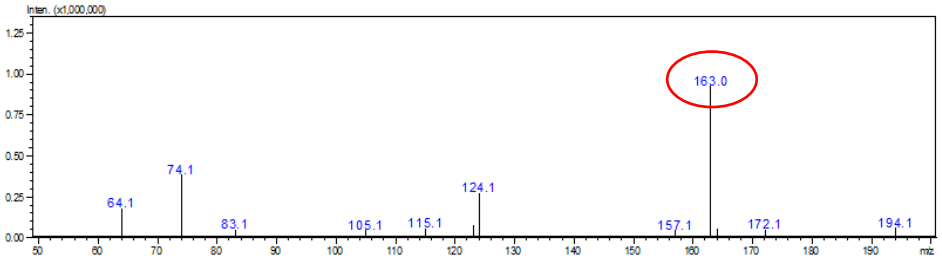
High-performance liquid chromatography (HPLC) has various detectors such as MS/MS, MS, UVD, PAD, FLD and so on. They are widely used analytical tools for pesticide residue analysis (C. Bolognesi, G. Morasso, 2000). Among them, this study was used MS/MS detector. Optimization of the conditions were performed of each compound in full scan mode using quadrupole (Q3) scan with positive ion/negative ion and full scan spectrum mass range was 50-600 *m/z*. On LC-MS/MS, the protonated molecular ion of $[M+H]^+$ at 382.1 m/z, 163.0 m/z, 529.1 m/z were mainly observed in the positive ESI mode (Figure 6). Select the protonated ion for compound as precursor ion for each pesticide. Then, the MRM mode the most selective and sensitive transition was used for quantifier and the second most selective for qualifier. Quantifier ion and qualifier ion of fluxapyroxad was 362.05 *m/z* (-15 eV) and 342.05 *m/z* (-23 eV) and M700F002 was 163.00 *m/z* (-15 eV) and 68.05 *m/z* (-26 eV), M700F048 was 530.00 *m/z* (-15 eV) and 368.00 *m/z* (-26 eV), respectively (Table 5).

Figure 6. Scan spectrum of fluxapyroxad and its metabolites

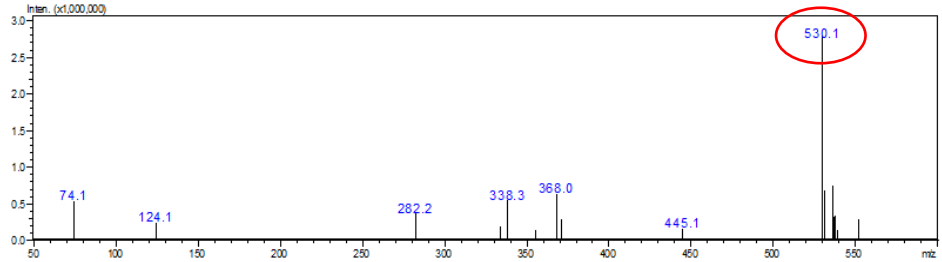
(A)



(B)



(C)



(A) Scan spectrum of fluxapyroxad (*m/z* 382.1)
(B) Scan spectrum of M700F002 (*m/z* 163.0)
(C) Scan spectrum of M700F048 (*m/z* 530.1)

Table 5. The MRM transition parameters of LC-MS/MS for fluxapyroxad and its metabolites

	Monoisotopic Mass	Ionization	Precursor ion (*m/z*)	Product ion (m/z)		Collision energy (eV)		Retention time (min)
				Quantitation	Qualification			
Fluxapyroxad	381.1	$[M+H]^+$	382.1	362.0	342.0	-15	-23	3.1
M700F002	162.0	$[M+H]^+$	163.0	123.0	68.0	-15	-26	3.8
M700F048	529.1	$[M+H]^+$	530.1	368.0	348.0	-15	-26	7.8

ILOQ, MLOQ and calibration curve

ILOD and ILOQ were value for signified a sensitivity of analytical instrument. ILOD is determined as S/N ratio of >3 and ILOQ is determined as S/N ratio of >10 (Fong et al. 1999, Miller 2005). In this study, ILOQ was checked from the results of analysis of several concentration standard solutions, In LC-MS/MS, ILOQ of fluxapyroxad was 0.0025 mg/L and ILOQ of metabolites were 0.005 mg/L (Figure 7). Based on MLOQ calculating equation, MLOQ of fluxapyroxad in samples was 0.01 mg/L. MLOQ of metabolites were 0.02 mg/L. Matrix matched standard curves of fluxapyroxad and its metabolites have a good linearity in sample. The range was between 0.005 to 0.2 mg/kg of fluxapyroxad standard solution (Figure 8). The regression equations were y = 2,932,711x + 19,318. Coefficients of determination (r^2) were over 0.99 and range was between 0.01 to 0.2 mg/kg of M700F002, M700F048 standard solution (Figure 8). The regression equations were y = 195,700x – 2,113.3 and y = 1E+06x + 9,439.8. Coefficients of determination (r^2) were over 0.99 (Figure 8).

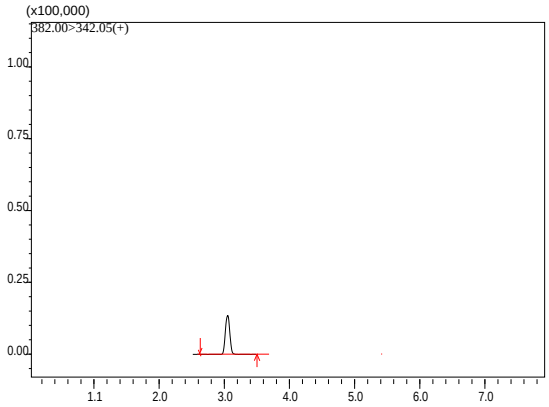
Figure 7. Chromatograms of ILOQ of fluxapyroxad, M700F002 and M700F048 in LC-MS/MS

(A) LOQ - Fluxapyroxad (0.01 mg/kg)

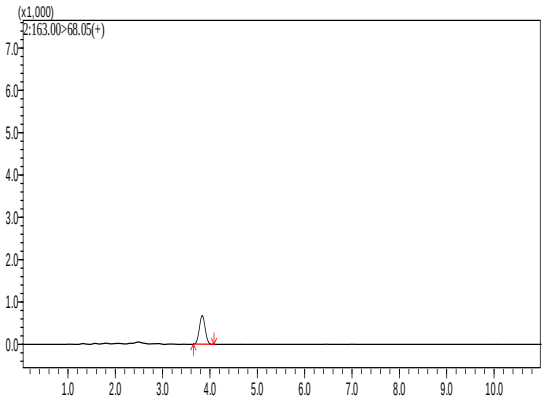
(B) LOQ – M700F002 (0.02 mg/kg)

(B) LOQ – M700F048 (0.02 mg/kg)

(A)


(x100,000)
382.00>342.05(+)
1.00
0.75
0.50
0.25
0.00
1.1
2.0
3.0
4.0
5.0
6.0
7.0

(B)


(x1,000)
2:163.00>68.05(+)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0

(C)

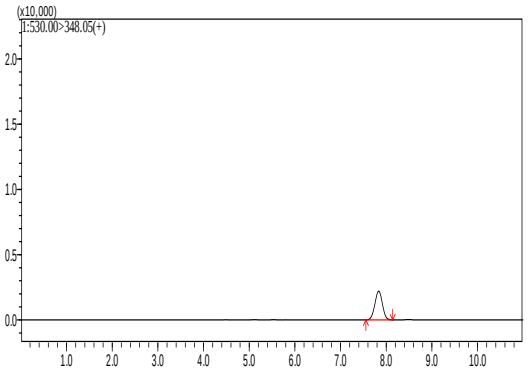

(x10,000)
1:530.00>348.05(+)
2.0
1.5
1.0
0.5
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0

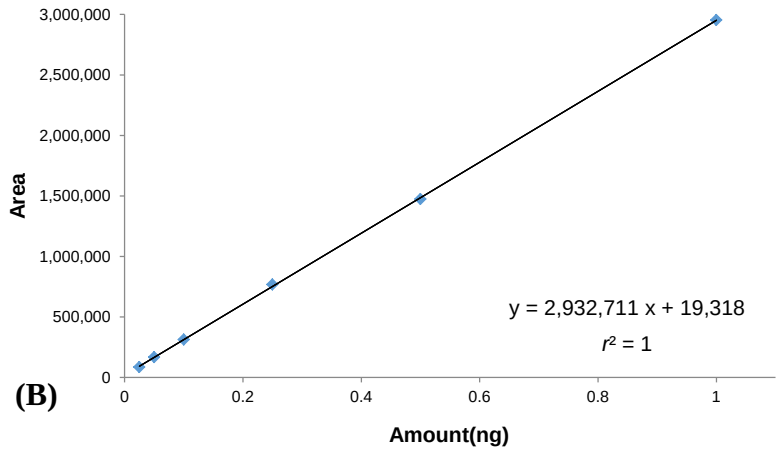
Figure 8. Matrix matched calibration curves of fluxapyroxad and its metabolites

(A) Calibration curve – Fluxapyroxad (Range : 0.005 - 0.2 mg/kg) and

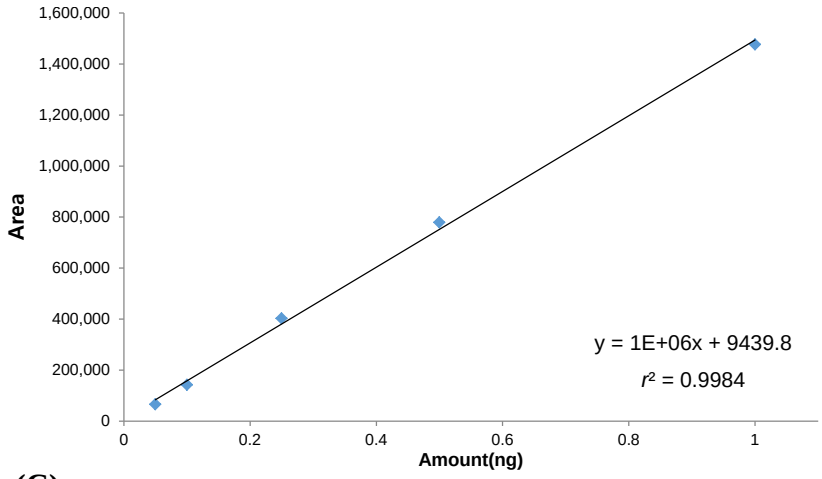
(B) Calibration curve – M700F002 (Range : 0.01 - 0.2 mg/kg)

(C) Calibration curve – M700F048 (Range : 0.01 - 0.2 mg/kg)

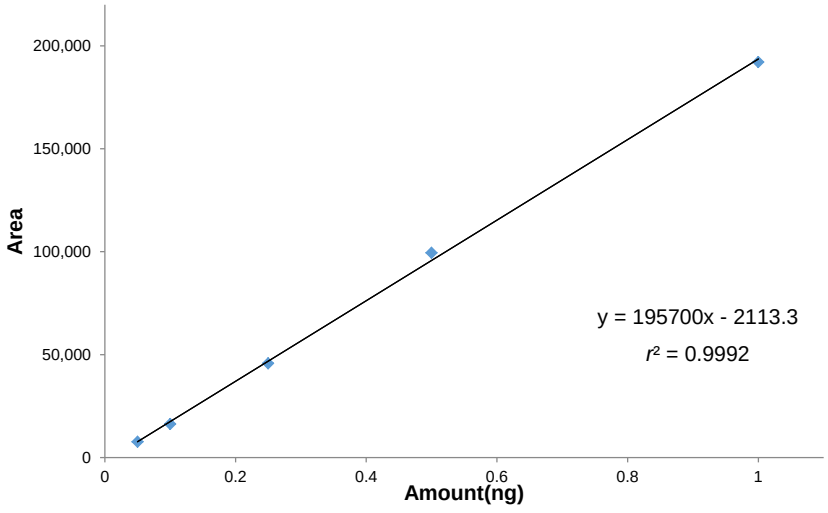
(A)


3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
Area
0
0.2
0.4
0.6
0.8
1
Amount(ng)
y = 2,932,711 x + 19,318
r² = 1

(B)


1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
Area
0
0.2
0.4
0.6
0.8
1
Amount(ng)
y = 1E+06x + 9439.8
r² = 0.9984

(C)


200,000
150,000
100,000
50,000
0
Area
0
0.2
0.4
0.6
0.8
1
Amount(ng)
y = 195700x - 2113.3
r² = 0.9992

Recoveries of fluxapyroxad and its metabolites

The recovery rate test is a test to check the recovered rate (accuracy, %) and the RSD (precision, %) value whether the preprocessing method is sufficiently established. (Fong et al., 1999). Untreated samples were spiked with MLOQ and 10 MLOQ levels of fluxapyroxad and M700F002 M700F048 mixture standard solutions, and the analysis was performed using the established method. **Table 6** shows results of recovery test in ssam cabbage. In case of fluxapyroxad, the range of recoveries were 77.1~90.4% at MLOQ level and 74.2~80.2% at 10 MLOQ level, and RSD was 5.9 and 6.2%, respectively. In case of M700F002 M700F048, the range of recoveries were 96.7~112.3% at MLOQ level and 105.3~107.8% at 10 MLOQ level, and RSD was 7.6 and 1.2%, respectively. In case of M700F048, the range of recoveries were 112.5~119.2% at MLOQ level and 100.1~106.4% at 10 MLOQ level, and RSD was 3.6 and 2.5%, respectively (Table 6) (Figure 9).

Table 6. Recoveries test (MLOQ and 10 MLOQ) of fluxapyroxad, M700F002 and M700F048

Section	Fortified level (mg/kg)	Recovery (%)	RSD (%)
Fluxapyroxad	0.01	83.7	5.9
	0.1	82.0	6.2
M700F002	0.02	106.0	7.6
	0.2	106.8	1.2
M700F048	0.02	116.5	3.6
	0.2	104.5	2.5

Figure 9. Chromatogram of recovery test of fluxapyroxad, M700F002 and M700F048

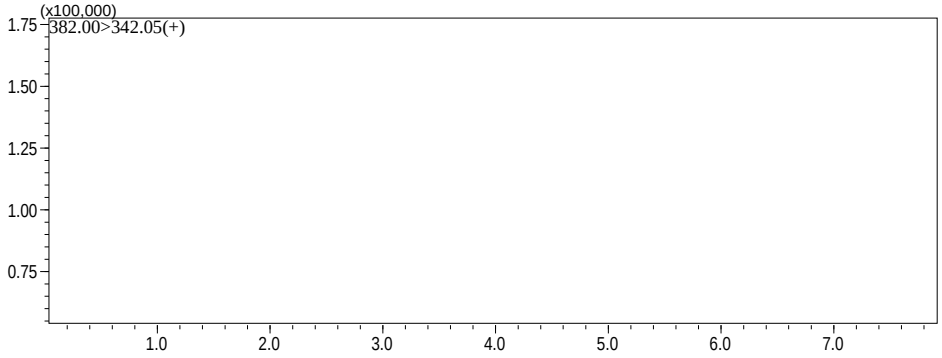
(A) Fluxapyroxad

(B) M700F002

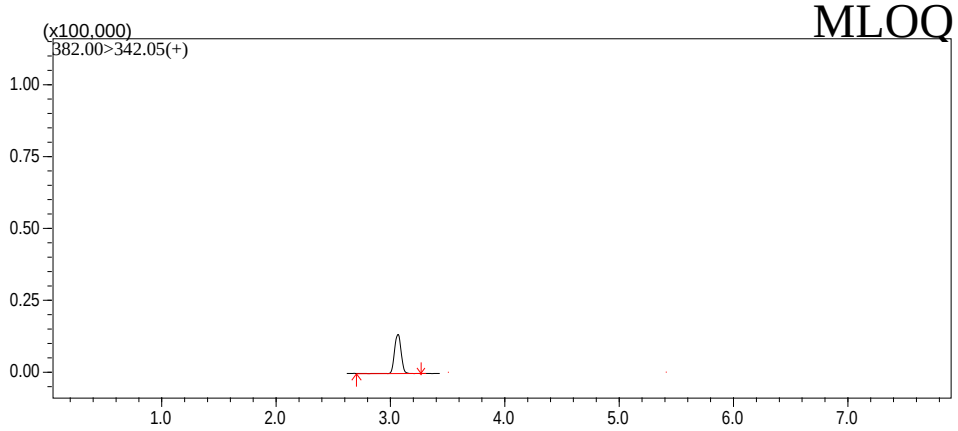
(B) M700F048

(A) Fluxapyroxad

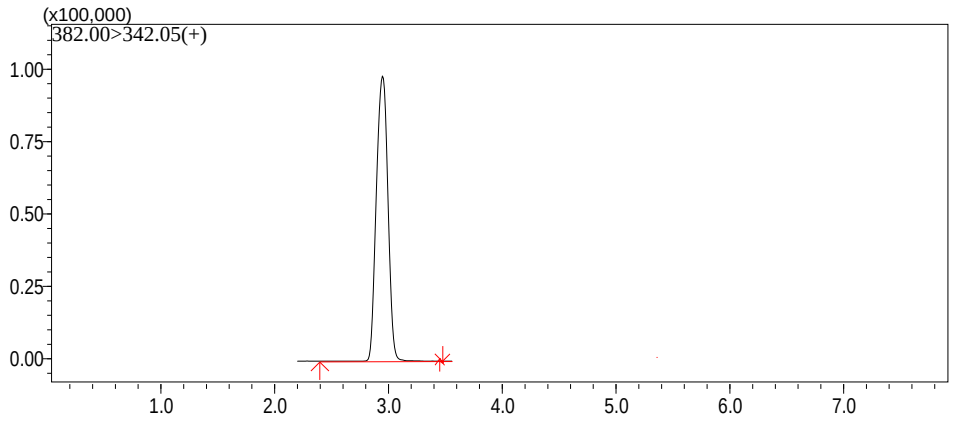
Control



MLOQ

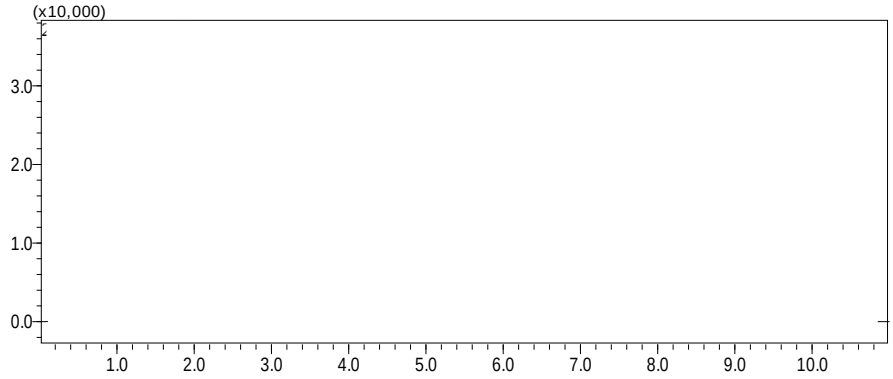


10MLOQ

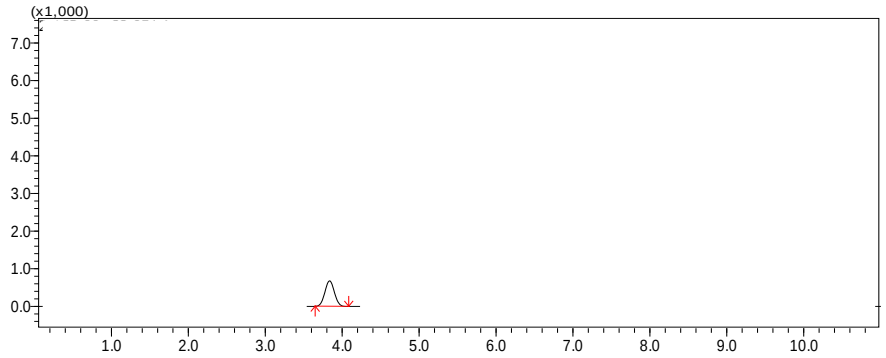


(B) M700F002

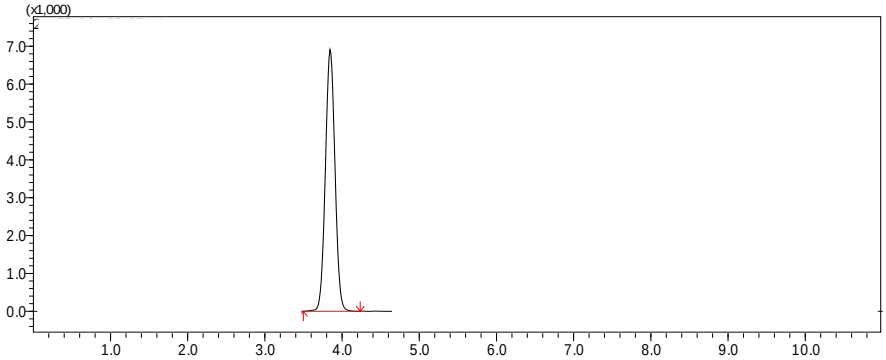
Control



MLOQ

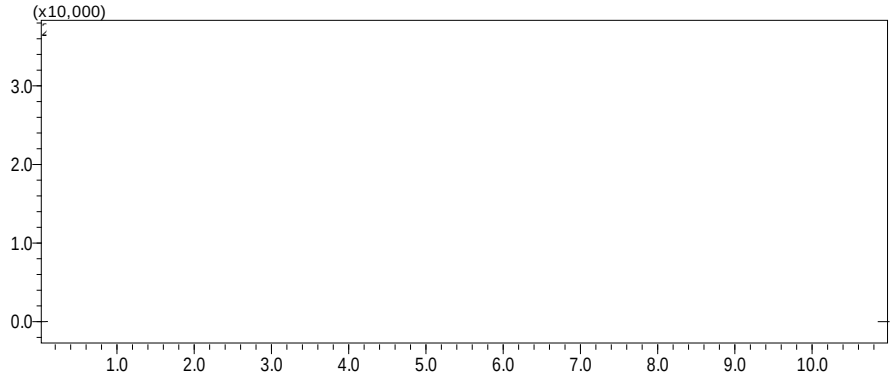


10MLOQ

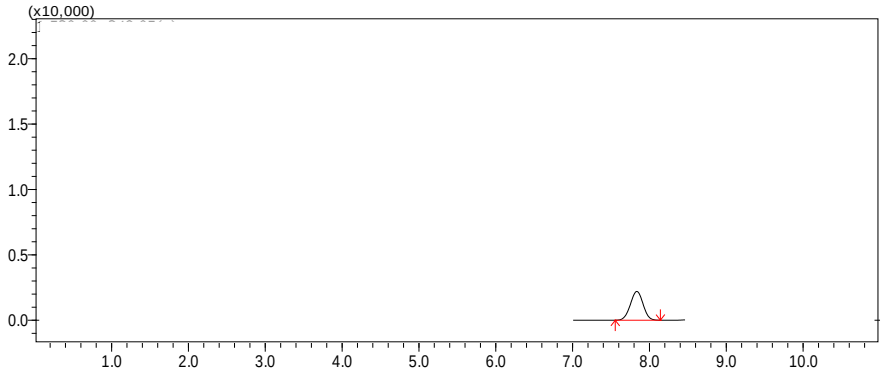


(C) M700F048

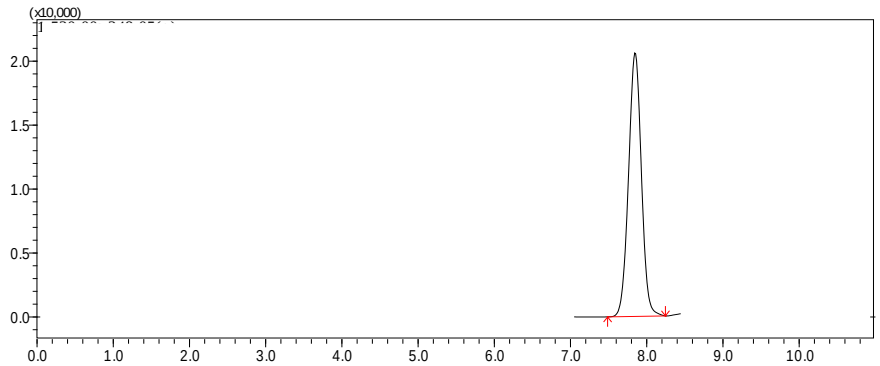
Control



MLOQ



10MLOQ

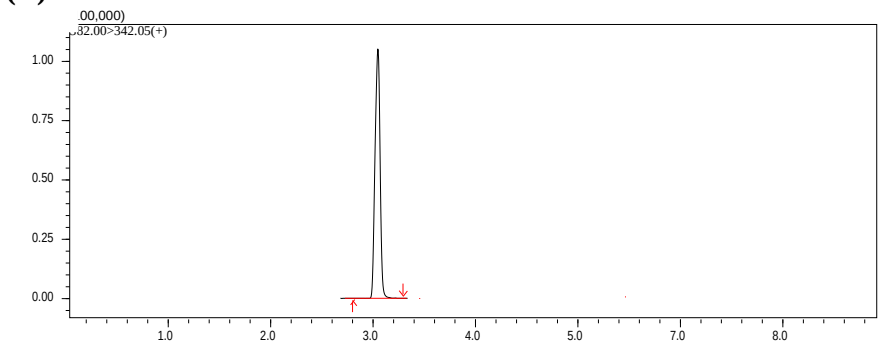


Storage stability test of fluxapyroxad and its metabolites

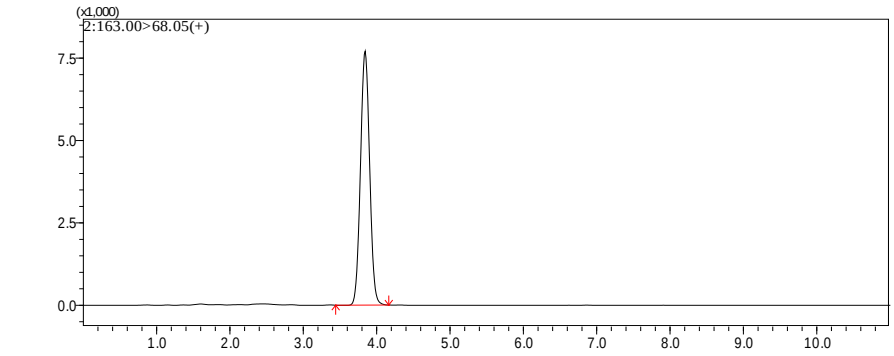
The storage stability test is a test to confirm that decomposition should not occur while the target compound is preserved. In pesticide residual analysis, it is generally difficult to carry out sample preparation immediately after sampling (Fu et al., 2016; He et al., 2016). Although samples usually are deep frozen, the question arises whether residues are degraded during storage. In this experiment, the fortified each samples of ssam cabbage were analyzed using the optimized method. The results showed that recovery of fluxapyroxad ranged from 86.2 to 102.0%, RSD 8.9%, recovery of M700F002 ranged from 109.8 to 115.7 %, RSD 2.7 % and M700F048 ranged from 92.2 to 97.5 %, RSD 3.1 % (Table 7) (Figure 10). These accuracy and precision tests indicated that the target compounds were not degraded during the storage period.

Figure 10. Representative chromatograms of storage stability test (A) Fluxapyroxad, (B) M700F002 and (C) M700F048

(A)


00,000)
32.00>342.05(+)
1.00
0.75
0.50
0.25
0.00
1.0
2.0
3.0
4.0
5.0
7.0
8.0

(B)


(x1,000)
2:163.00>68.05(+)
7.5
5.0
2.5
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0

(C)

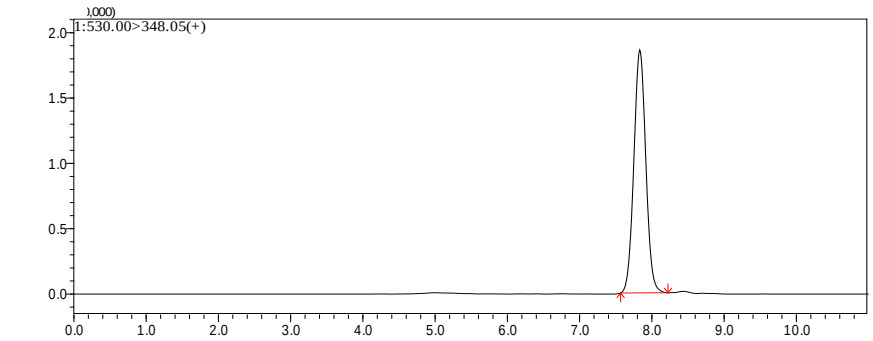

,000)
1:530.00>348.05(+)
2.0
1.5
1.0
0.5
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0

Table 7. Storage stability of fluxapyroxad and its metabolites

Section	Fortified level (mg/kg)	Recovery (%)	RSD (%)
Fluxapyroxad	0.1	96.1	8.9
M700F002	0.2	113.2	2.7
M700F048	0.2	95.6	3.1

Dissipation of fluxapyroxad and its metabolites in ssam cabbage

Each plot samples of ssam cabbage were analyzed using the optimized method. No residue in ssam cabbage control sample and the results of fluxapyroxad and its metabolites residue in field trials were presented in Table 9. In the ssam cabbage, fluxapyroxad residue in plot 1 (35/28 before harvest) and plot 2 (35/28/21 before harvest) were less than 0.01 mg/kg in both of sample. In plot 3 (35/28/21/14 before harvest) was residue 0.28 mg/kg. In plot 4 (28/21/14/7 before harvest) was 1.05 mg/kg. In plot 5 (21/14/7/0 before harvest) was 1.15 mg/kg. Overall, highest residual amounts was found in plot 5 and residual amount of fluxapyroxad in ssam cabbge and when analyzing pesticide residue, dilute plot 3, plot 4, plot 5 in a calibration curve by 10 times. M700F002 and M700F048 residue in plot 1 (35/28 before harvest), plot 2 (35/28/21 before harvest), plot 3 (35/28/21/14 before harvest), plot 4 (28/21/14/7 before harvest) and plot 5 (21/14/7/0 before harvest) were less than 0.02 mg/kg in sample (Table 8) (Figure 11) (Figure 12).

Table 8. Maximum residue of fluxapyroxad and its metabolites

Plot (before harvest)	Residual maximum amount (mg/kg)		
	Fluxapyroxad	M700F002	M700F048
1 (35/28)	<0.01	<0.02	<0.02
2 (35/28/21)	<0.01	<0.02	<0.02
3 (35/28/21/14)	0.28	<0.02	<0.02
4 (28/21/14/7)	1.05	<0.02	<0.02
5 (21/14/7/0)	1.15	<0.02	<0.02

Figure11. Dissipation of fluxapyroxad and its metabolites in ssam cabbage

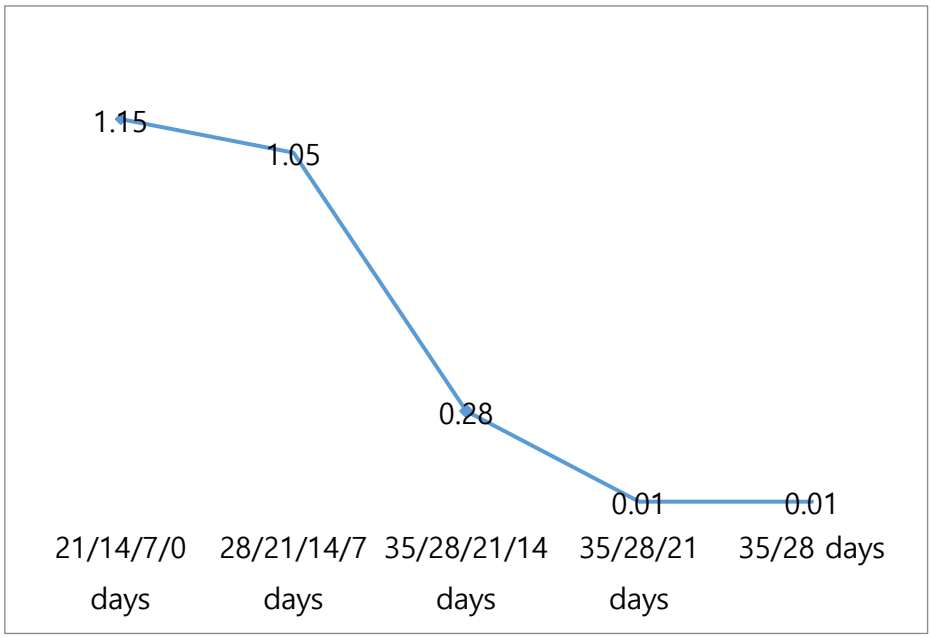
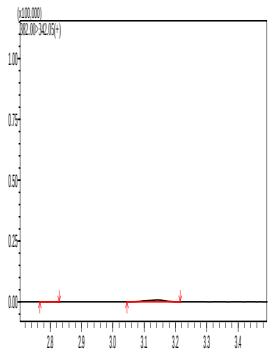

1.15
1.05
0.28
0.01
0.01
21/14/7/0 days
28/21/14/7 days
35/28/21/14 days
35/28/21 days
35/28 days

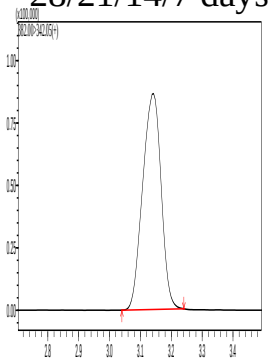
Figure 12. Chromatograms of residue analysis of (A) Fluxapyroxad and (B) M700F002, M700F048

(A) Representative chromatogram of ssam cabbage (Fluxapyroxad)

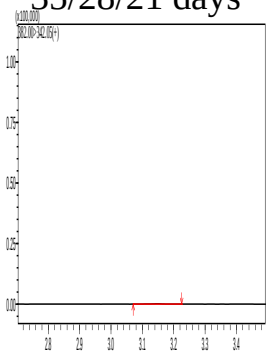
35/28 days



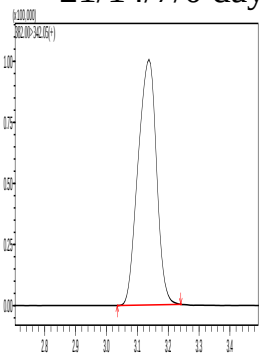
28/21/14/7 days



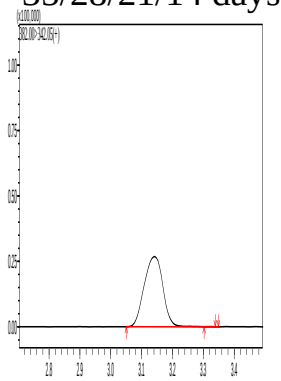
35/28/21 days



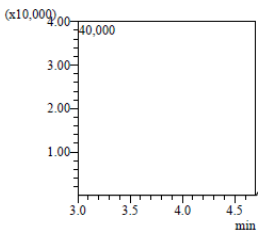
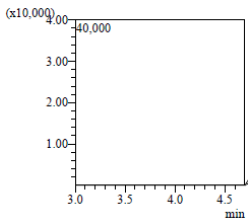
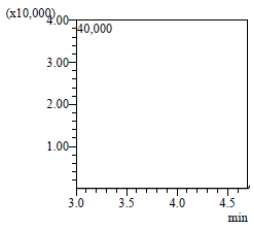
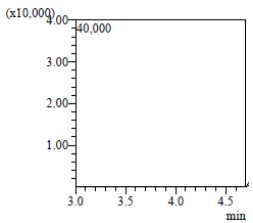
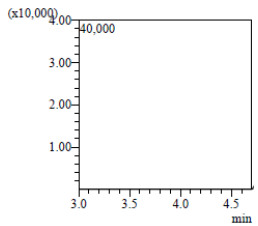
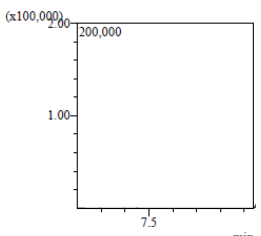
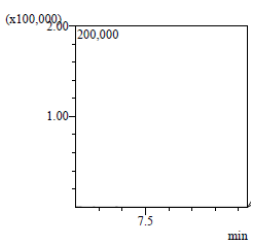
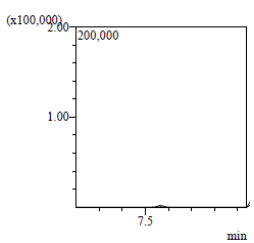
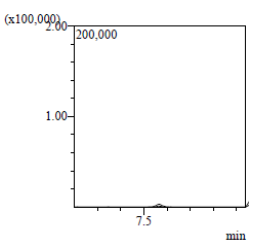
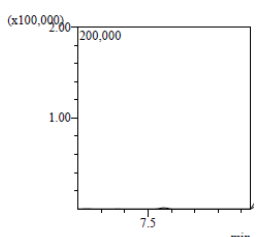
21/14/7/0 days



35/28/21/14 days



(B) Representative chromatogram of ssam cabbage (M700F002, M700F048)

	35/28 days	35/28/21 days	35/28/21/14 days	28/21/14/7 days	21/14/7/0 days
M700F002					
M700F048					

Part 2

Simultaneous Determination of PAHs in Water/Soil samples by GC-MS/MS

Introduction

Polycyclic aromatic hydrocarbons (PAH)

The term polycyclic aromatic hydrocarbons (PAHs) commonly refers to a large class of organic compounds that contain only carbon and hydrogen and are comprised of two or more fused aromatic rings (IRAC., 2010). People are usually exposed to mixtures of PAHs. This compounds have environmental concern because of the documented carcinogenicity in experimental human (IARC, 1991; USEPA, 1992; EPA. CDC., 2009) (Table 9). Due to their toxicity, The United States Environmental Protection Agency (EPA) monitors 16 priority PAHs due to health concerns: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd]pyrene, benzo(g,h,i)-perylene, and dibenz(a,h)anthracene. Those sixteen, are known carcinogens, raising concerns regarding human-health risks and several of the PAHs were classified by IRAC according to carcinogenicity (WHO, 2018; IRAC, 2018) (Table 10). In this study, 25 priority PAHs were analyzed including EPA monitors 16 priority PAHs. The added PAHs were benzo(e)pyrene, 5 - methylchrysene, 1 - methylnaphthalene, 2 - methylnaphthalene, 2,6 - dimethylnaphthalene, retene, 1-acenaphthenone, 1-naphthol, 2-hydroxy-1-nphthoic acid (Table 9) (Table10)

Table 9. IARC Monographs on the evaluation of carcinogenic risks to humans

Group	Definition
Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable as to its carcinogenicity to humans
Group 4	Probably not carcinogenic to humans

Table 10. Agents classified by the IARC monographs

NO.	Compound	Group
1*	Benzo(a)pyrene	1
2*	Benz(a)anthracene	2B
3*	Benzo(b)fluoranthene	2B
4*	Benzo(k)fluoranthene	2B
5*	Benzo(ghi)perylene	3
6*	Chrysene	2B
7*	Dibenz(ah)anthracene	2A
8*	Indeno(123cd)pyrene	2B
9*	Acenaphthene	3
10*	Acenaphthylene	
11*	Anthracene	3
12*	Fluoranthene	3

13*	Fluorene	3
14*	Naphthalene	2B
15*	Phenanthrene	3
16*	Pyrene	3
17	Benzo(e)pyrene	3
18	5-Methylchrysene	3
19	1-Methylnaphthalene	
20	2-Methylnaphthalene	
21	2,6-Dimethylnaphthalene	
22	Retene	
23	1-Acenaphthenone	
24	1-Naphthol	
25	2-Hydroxy-1-Naphthoic acid	

* EPA list PAHs

Benzo(e)pyrene was an isomer of benzo(a)pyrene, which is less carcinogenic than benzo(a)pyrene. 5-Methylchrysene is listed in the European Union (EU) list of PAHs. Benzo(e)pyrene and 5-Methylchrysene belong to IARC Group 3. Retene is the most abundant PAHs composition. The metabolites of Acenaphthene 1-acenaphthenone and the metabolites of naphthalene 1-naphthol and the metabolites of phenanthrene 2-Hydroxy-1-Naphthoic acid were also analyzed in this study. (JAIRAJ V. POTHULURI et al., 1992; ATSDR., 2005; Young-Soo Keum,. 2006; D. Wang et al,. 2009)

Circulation of PAH in environmental

Number of aromatic rings increases, the rate of biodegradation decreases sharply. The calculated half-life for selected compounds in real experiments varies from about 100 days to 2 years. For this reason, PAHs is very stable. These compounds were produced from incomplete combustion and mainly released into the atmosphere. PAHs have been detected long distance from their source (World Health Organization, 2003; Bjørseth, Sortland et al, 1983; McVeety, Hites et al, 1988; Bossert and Bartha, 1986; Coover and Sims, 1987; Park et al., 1990; Wild et al., 1991). Rapid development of industrial society is causing environmental pollution by releasing many pollutants into the air, water quality and soil. There are a lot of pollutants that we do not know about in the fine dust and yellow dust coming from China, and many of them are now being created by the increased production of synthetic chemicals and fossil fuels (Jong-Hyang Kim et al., 2011; Tan et al., 2011; S. B. Hawthorne et al., 2007; IRAC., 1987; EPA. CDC., 2009; Magdalena Surma et al., 2014).

Analysis of PAH to date

PAHs have been analyzed for a long time. In general, PAHs were the two most frequently used techniques to determine PAHs are high performance liquid chromatography (HPLC) with fluorescence detector (FID), ultra violet detector (UVD), or diode array detection (DAD) (Method 1654, U.S. Environmental Protection Agency. 1992; Method 8310 U.S. Environmental Protection Agency. 1986) and gas chromatography (GC) with electron captured detector (ECD), nitrogen phosphorous detector (NPD), flame ionization detector (FID) or mass spectrometry (MS) detection in electron impact mode (EI+) with selected ion monitored (SIM) (M. Olson et al,. 2004; Z. Wang,. 2008; Z. Khan et al,. 2005). In the current method, PAHs were analyzed by GC-MS using selected ion monitored (SIM) and GC/MS/MS using multiple reaction monitoring (MRM) pseudo-MRM monitoring (PMRM) PMRM transitions have proven to be advantageous for PAHs analysis (Dr. Stephan Schroeder, shimadzu).

SIM & MRM & PMRM

For a long time, many compounds have long been analyzed as using selected ion monitored (SIM). But, Despite numerous improvements to single quadrupole MS cannot keep up with the performance, sensitivity and specificity of triple quadrupole MS. However, due to the unique structure and stability of PAHs, the traditional Multiple Reaction Monitoring (MRM) methods are fragile to the compounds in this group and are difficult to use (V. Varlet et al, 2007; N. Barco-Bonilla et al, 2011; B. Veyrand et al, 2007; Dayue Shang et al, 2014). So, complementing this MRM mode, there is a new monitoring method for analyzing PAHs, which is called PMRM mode. This monitoring is a technique to monitor the same molecular ion *m/z* used in quantitative analysis, both precursor and product ions are the same in fist quadrupole (Q1) and third quadrupole Q3 (D. Shang et al., 2014) show the various monitoring references (Table 11) used for PAHs analysis. Looking at the table, SIM mode has been used for a long time, and MRM mode and PMRM mode have become more and more popular in recent years. (Table 11)

Definitions of terms relating to mass spectrometry

(IUPAC Recommendations, 2013; Kermit K. Murray et al., 2013)

Selected ion monitoring (SIM)

Operation of a mass spectrometer in which the abundances of ions of one or more specific *m/z* values are recorded rather than the entire mass spectrum. (IUPAC Gold Book, 2005-2017) (Figure 13)

Selected reaction monitoring (SRM)

Data acquired from one or more specific product ions corresponding to m/z selected precursor ions recorded via two or more stages of mass spectrometry.

- Note 1: Selected reaction monitoring in multiple-stage mass spectrometry is known as consecutive reaction monitoring.
- Note 2: Selected reaction monitoring applied to multiple product ions from one or more precursor ions is known as multiple reaction monitoring (de Hoffmann. J., 1996);

Multiple reaction monitoring (MRM)

Application of selected reaction monitoring to multiple product ions from one or more precursor ions.

- Note: This term should not be confused with consecutive reaction monitoring, which involves the serial application of three or more stages of selected reaction monitoring (Roepstorff and Fohlman,1984) (Figure 14).

Pseudo multiple reaction monitoring (PMRM)

This monitoring looks similar to MRM mode, but PMRM captures both qualitative ion and quantitative ions at the same molecular ion m/z in Q1 and Q2, unlike MRM, which differentiates precursor ion and product ions in Q1 and Q2 (D. Shang et al., 2014; W. Lian et al., 2016;) (Figure 15)

Precursor ion:

Deprecated: parent ion.

Ion that reacts to form particular product ions or undergoes specified neutral losses. The reaction can be of different types including unimolecular dissociation, ion/molecule reaction, change in charge state, possibly preceded by isomerization.

Product ion:

Deprecated: daughter ion.

Ion formed as the product of a reaction involving a particular recursor ion.

Electron ionization (EI)

Deprecated: electron impact ionization. Ionization that removes one or more electrons from an atom or molecule through interactions with electrons that are typically accelerated to energies between 10 and 150 eV.

Table 11. Various monitoring references used for PAHs analysis

Detection	No.	Reference
MS (SIM)	1	Magdalena Surma et al, (2014) The application of d-SPE in the QuEChERS method for the determination of PAHs in food of animal origin with GC–MS
	2	Anna Sadowska-Rociek et al., (2014) Comparison of different modifications on QuEChERS sample preparation method for PAHs determination in black, green, red and white tea
	3	Consuelo S´anchez-Brunete et al., (2007) Analysis of 27 polycyclic aromatic hydrocarbons by matrix solid-phase dispersion and isotope dilution gas chromatography-mass spectrometry in sewage sludge from the Spanish area of Madrid
	4	Eun-Jeong Park et al. (2012) Levels and Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in the Taehwa River, Ulsan, Korea
	5	Jiping Ma et al. (2010) Determination of 16 polycyclic aromatic hydrocarbons in environmental water samples by solid-phase extraction using multi-walled carbon nanotubes as adsorbent coupled with gas chromatography–mass spectrometry

	6	YueZhao,BoHong et al., (2014)Accurateanalysisofpolycyclicaromatichydrocarbons(PAHs) and alkylatedPAHshomologsincrudeoilforimproving the gaschromatography/massspectrometryperformance EcotoxicologyandEnvironmentalSafety100 242–250
MS/MS (MRM)	**7**	YizhenWang et al., (2018) Polycyclic aromatic hydrocarbons and organochlorine pesticides in surface water from the Yongding River basin, China: Seasonal distribution, source apportionment, and potential risk assessment
	8	Leesun Kim et al., (2016) Polycyclic Aromatic Hydrocarbons in Agricultural Waterways in Gyeonggi and Gangwon Provinces, Korea
	9	Abou-Arab et al., (2014) Levels of polycyclic aromatic hydrocarbons (PAHS) in some Egyptian vegetables and fruits and their influences by some treatments. Int. J. Curr. Microbiol. Appl. Sci. 3, 277e293.
	10	Nagalakshmi Haleyur, (2016)Comparison of rapid solvent extraction systems for the GC–MS/MS characterization of polycyclic aromatic hydrocarbons in aged, contaminated soil MethodsX 3 364–370

	11	Jutta Lintelmann et al., (2006) particulate matter using high-performance liquid chromatography–tandem mass spectrometry Journal of Chromatography A, 1133, 241–247
PMRM	**12**	Dayue Shang et al, (2014) Rapid and sensitive method for the determination of polycyclicaromatic hydrocarbons in soils using pseudo multiple reactionmonitoring gas chromatography/tandem mass spectrometry
	13	Jeffrey Yan et al, (2008) Determination of polycyclic aromatic hydrocarbons in surface water using simplified liquid–liquid micro-extraction and pseudo-MRM GC/MS/MS†
	14	Wenliu Lian et al., (2016) Analysis of polycyclic aromatic hydrocarbons in cigarette samples using gel permeation chromatography clean-up by gas chromatography–tandem mass spectrometryb Microchemical Journal 129, 194–199
	15	Hongping Chen et al., (2016) Determination of 16 Polycyclic Aromatic Hydrocarbons in Tea by Simultaneous Dispersive Solid-Phase Extraction and Liquid–Liquid Extraction Coupled with gas Chromatography–Tandem Mass Spectrometry 9:2374–2384 DOI 10.1007/s12161-016-0427-4

Figure 13. Selected ion monitoring (SIM)

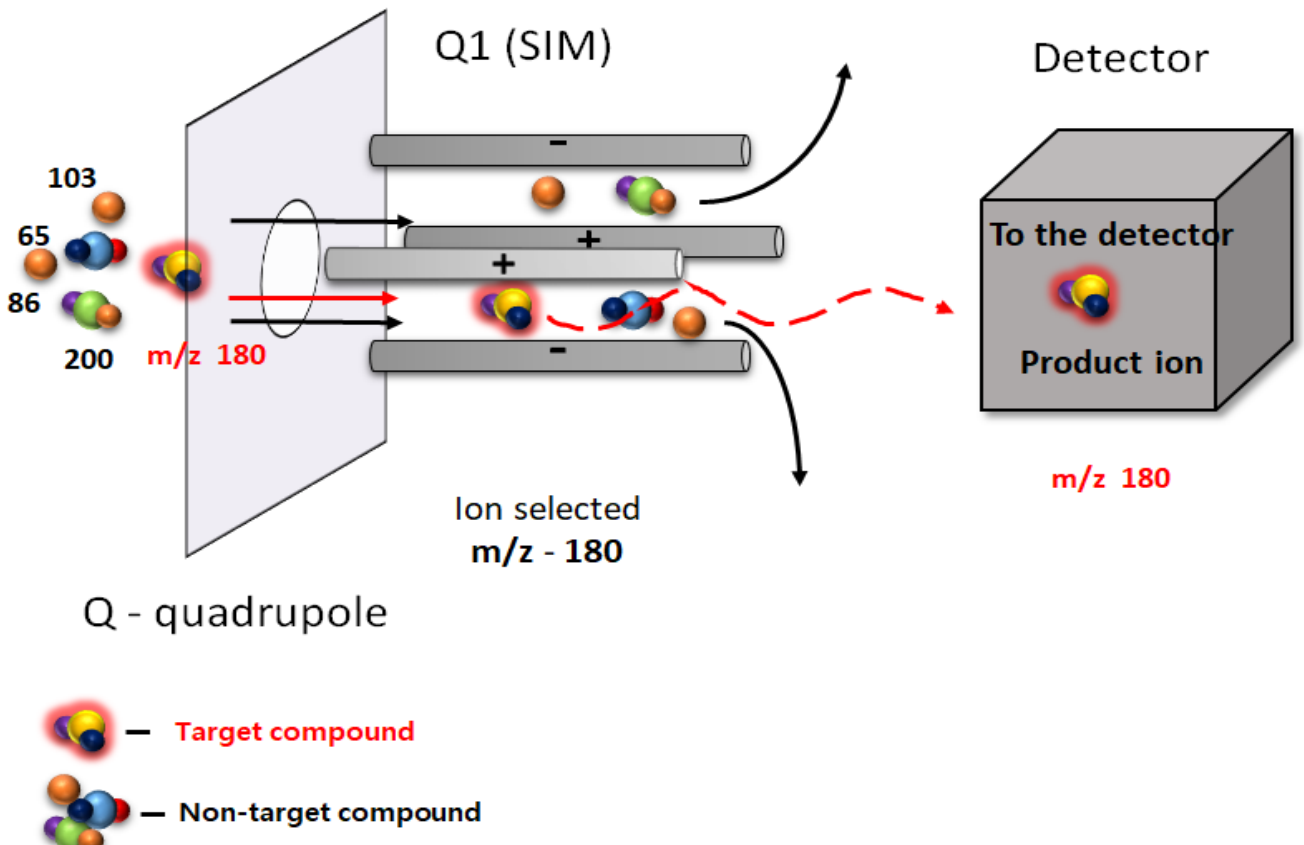

Q1 (SIM)
Detector
103
65
86
200
m/z 180
To the detector
Product ion
m/z 180
Ion selected
m/z - 180
Q - quadrupole
Target compound
Non-target compound

Figure 2. Multiple reaction monitoring (MRM)

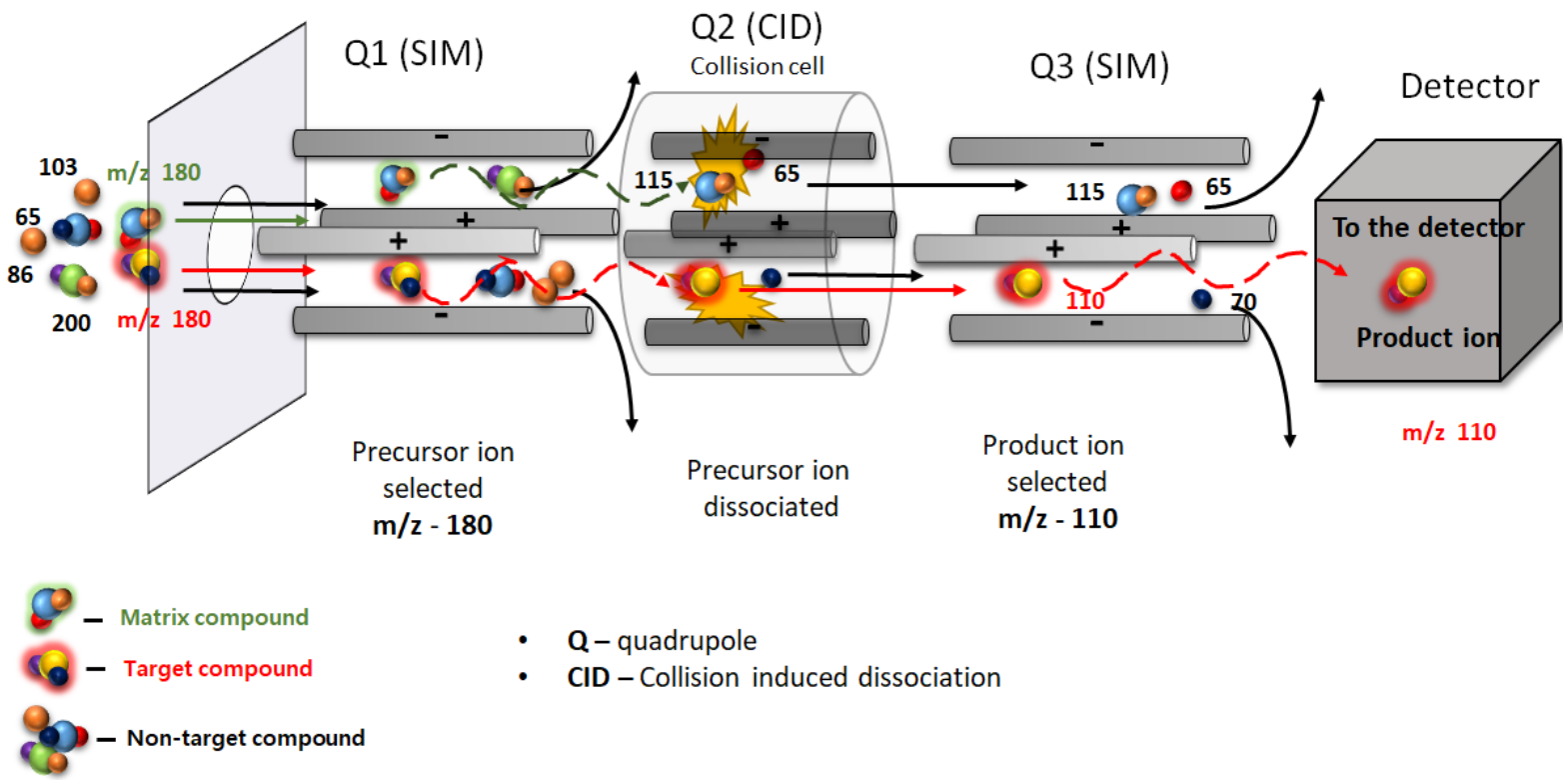
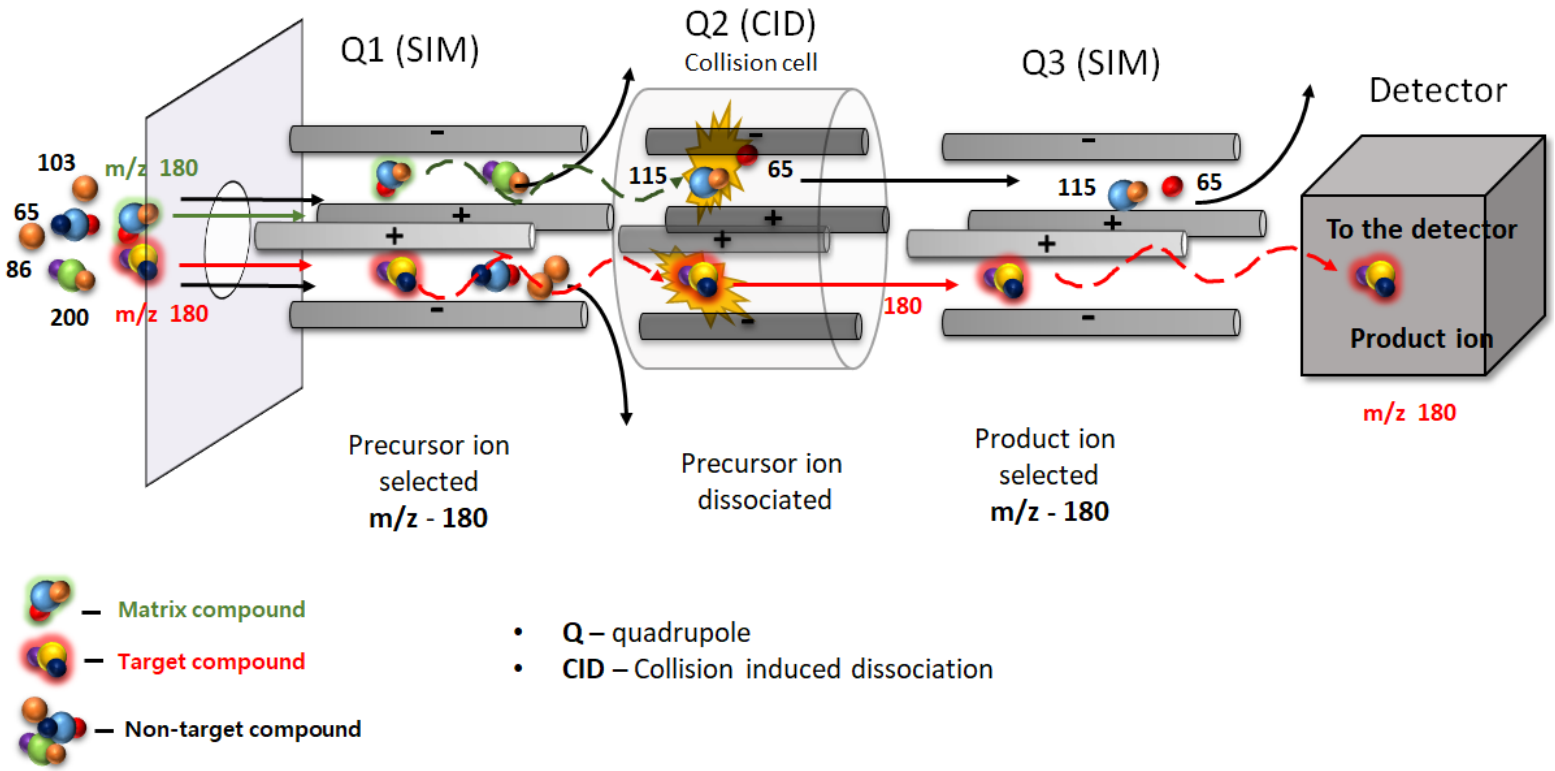

Q1 (SIM)
Q2 (CID)
Collision cell
Q3 (SIM)
Detector
103
m/z 180
65
86
200
m/z 180
115
65
115
65
110
70
To the detector
Product ion
m/z 110
Precursor ion selected
m/z - 180
Precursor ion dissociated
Product ion selected
m/z - 110
Matrix compound
Target compound
Non-target compound
Q – quadrupole
CID – Collision induced dissociation

Figure 15. Pseudo multiple reaction monitoring (PMRM)



- **Q** – quadrupole
- **CID** – Collision induced dissociation

The purpose of studies

PAHs was existed in the atmosphere and flows with the rain into the river or soil and it is causing environmental pollution. PAHs have many carcinogenic compounds (Oleszczuk P et al. 2006) continuous research is needed to understand the monitoring and behavior. In this study, established a higher sensitivity analysis method for 25 types of PAH and monitored samples of sewage treatment plant and rice paddy soil sample that may be contaminated by PAH.

Materials and Methods

Chemicals and consumable

HPLC grade acetonitrile (ACN), acetone (ACT) and dichloromethane (DCM) was purchased from Fisher Scientific (Seoul, South korea), sodium sulfate ($NaSO_4$) from wako (Japan), sodium chloride (NaCl), monopotassium phosphate (KH_2PO_4), Diethylene glycol was purchased from Sigma-Aldrich (St. Louis, PA, USA) and Polyethylene glycol. The QuEChERS materials were extract kit original unbuffered, EN kit 15662, AOAC kit (Resteck, USA).

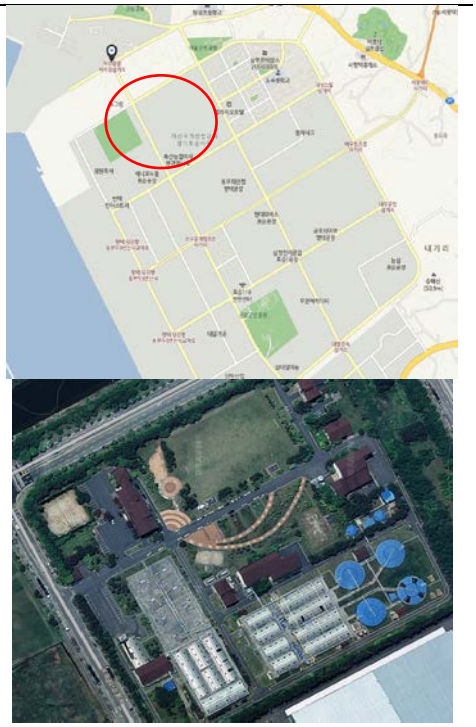
Standard solutions

Each analytical standard stock was dissolved in acetonitrile, acetone and methanol to make concentrated stock solution at concentration of 1,000 mg/L. The working solutions were prepared by serial dilution of stock solution with acetone

Sampling for soil and water

Soil control samples were brought from farms in hwa-sung. Hwa-sung paddy soil was collected 14.6 kg. All soils were dried and homogenized and analyzed. Water samples were brought from river. 2 L samples were collected from two sewage treatment plants (Seongnam, Poseung) at each sampling period. Also distilled water was used as control sample as well. Samples transferred to the laboratory were stored at 4 ℃ or lower in the dark before extraction. As shown in the picture (Figure 16),

Figure 16. Sampling site: Water - (a) Seongnam Sewage Treatment Plant, Seongnam City Water Quality Restoration Center, (b) Poseung Sewage Treatment Plant, Soil - (C) Hwa-seong paddy soil

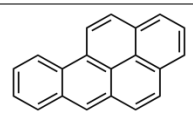
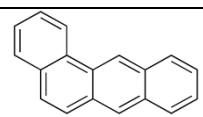
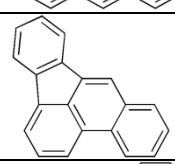
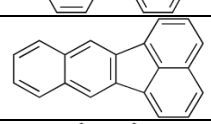
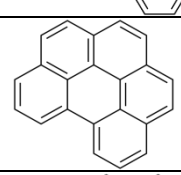
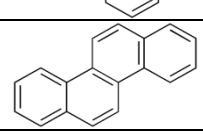
(a) Seong-nam	(b) Po-seung
	

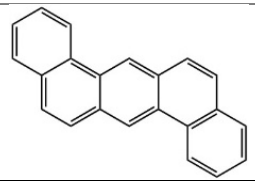
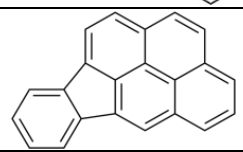
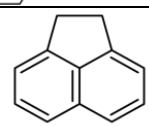
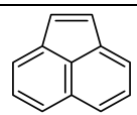
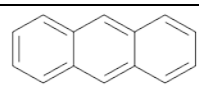
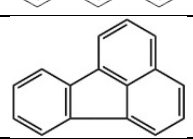
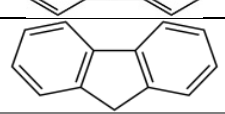
(C) Hwa-seong

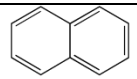
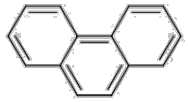
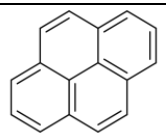
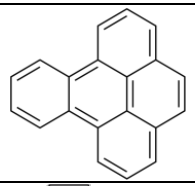
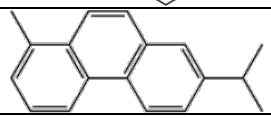
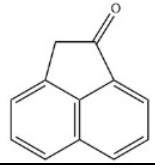
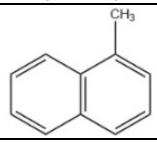
Analytical standard

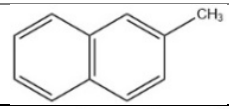
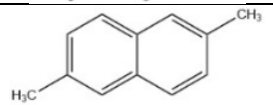
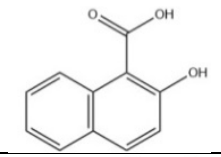
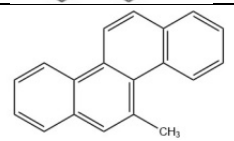
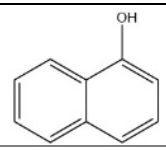
Analysis of 25 PAH compounds: Naphthalene, phenanthrene, acenaphthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, 1-Methylnaphthalene, 2-Methylnaphthalene, fluoranthene, 2,6-dimethylnaphthalene, 1-naphthol, pyrene, fluorene, benzo(g,h,i)perylene were purchased form Sigma-Aldrich (St. Louis, PA, USA) and chrysene, anthracene, acenaphthylene, benzo(a)anthracene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(b)fluoranthene were purchased from Supelco (Oakville, Ontario) 2-Hydroxy-1-Naphthoic acid, 1-Acenaphthenone TIC (Tokyo, Japan), Retene was purchased Chemservice (West Chester, PA, USA), 5-Methylchrysene TRC (North York, ON, Canada) (Table 9).

Table 9. Structure and characteristics of PAHs

NO.	Compound	Molecular Formula	M.W (g/mol)	Exact Mass (g/mol)	CAS. NO	Structure
***1**	**Benzo(a)pyrene**	C20H12	252.32	252.09	50-32-8	
***2**	**Benz(a)anthracene**	C18H12	228.29	228.09	56-55-3	
***3**	**Benzo(b)fluoranthene**	C20H12	252.32	252.09	205-99-2	
***4**	**Benzo(k)fluoranthene**	C20H12	252.32	252.09	207-08-9	
***5**	**Benzo(ghi)perylene**	C22H12	276.34	276.09	191-24-2	
***6**	**Chrysene**	C18H12	228.29	228.09	218-01-9	

***7**	**Dibenz(ah)anthracene**	C22H14	278.35	278.11	53-70-3	
***8**	**Indeno(123cd)pyrene**	C22H12	276.34	276.09	193-39-5	
***9**	**Acenaphthene**	C12H10	154.21	154.08	83-32-9	
***10**	**Acenaphthylene**	C12H8	152.20	152.06	208-96-8	
***11**	**Anthracene**	C14H10	178.23	178.08	120-12-7	
***12**	**Fluoranthene**	C16H10	202.26	202.08	206-44-0	
***13**	**Fluorene**	C13H10	166.22	166.08	86-73-7	

***14**	**Naphthalene**	C10H8	128.17	128.06	91-20-3	
***15**	**Phenanthrene**	C14H10	178.23	178.08	85-01-8	
***16**	**Pyrene**	C16H10	202.26	202.08	129-00-0	
17	**Benzo(e)pyrene**	C20H12	252.32	252.09	192-97-2	
18	**Retene**	C18H18	234.34	234.14	483-65-8	
19	**1-Acenaphthenone**	C12H8O	168.20	168.06	2235-15-6	
20	**1-Methylnaphthalene**	C11H10	142.20	142.08	90-12-0	

21	**2-Methylnaphthalene**	C11H10	142.20	142.08	91-57-6	
22	**2,6-Dimethylnaphthalene**	C12H12	156.23	156.09	581-42-0	
23	**2-Hydroxy-1-Naphthoic acid**	C11H8O3	188.18	188.05	2283-08-1	
24	**5-Methylchrysene**	C19H14	242.32	242.11	3697-24-3	
25	**1-Naphthol**	C10H8O	144.17	144.06	90-15-3	

***** EPA 16 (NCBI – pubchem, Wikipedia, NIST database)

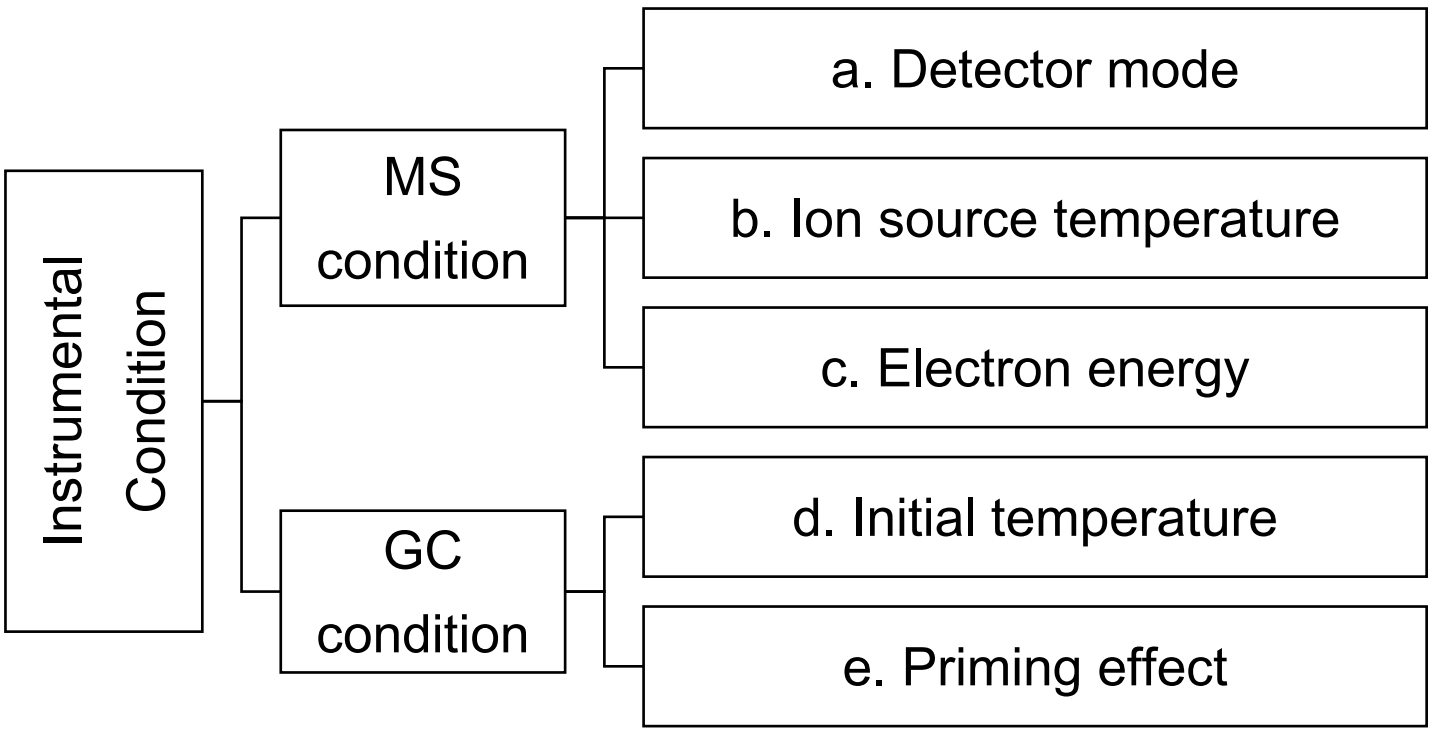
Establishment of instrument and condition

GC-MS/MS analysis for the water was performed on GC-MS SCION TQ triple-quadrupole system (Bruker, USA) equipped with an Restek Rxi-5Sil MS column (30 m × 0.25 mm i.d., 0.25 μm). Standard mixture injection volumnes were 2 μL in the splitless mode. The oven temperature program was as follows: (a) 50 ℃ was held for 2 min and raised at a rate of 10 °/min to 310 ℃ which was finally held for 5 min. (b) 60 ℃ was held for 2 min and raised at a rate of 10 ℃/min to 310 ℃ which was finally held for 5 min. (a) 70 ℃ was held for 2 min and raised at a rate of 10 ℃/min to 310 ℃ which was finally held for 5 min. The GC/MS transfer line and inlet temperatures were set at 280 and 280 ◦C, respectively. Helium (≥99.999%) was used as carrier gas and argon was used as collision gas. For MS/MS analysis, Compared to the four mode, Multiple Reaction Monitoring (MRM), Pseudo-MRM (PMRM) mode, Selected Ion Monitoring (SIM) and Mixed PMRM & MRM. Ion source temperature was compared from 200◦C to 300◦C degrees. The electron ionization energy was -80eV. GC and MS instruments and conditions are summary on **Figure 17**

Priming effect

Untreated spinach (10 g) sample in a 50 mL propylene tube. And ACN (10 mL) was added to each tube and shaken (1500 rpm) using a Geno Grinder (1600 MiniG^{TM}, SPEX$^{®}$ SamplePrep, New Jersey, USA) for 1 min. Then, added the sodium chloride (NaCl) 1 g and $MgSO_4$ 4 g. After the mixture was shaken vigorously for another 1 min, the tube was centrifuged at 3,500 rpm for 5 minutes (Combi 408, Hanil Science industrial, Korea). The supernatant (1 mL) was transferred into dSPE tube ($MgSO_4$ 150 mg, PSA25 mg) and vortexed (1 min) on a Multi Speed Vortex (MSV-3500, Biosan, Riga, Latvia) before centrifugation at 15000 rpm for 5 min. Final sample was injected into GC-MS/MS. Then, spinach extracts were injected with the the same GC conditions for priming treatment.

Figure 17. Establishment of instrumental Condition


Instrumental Condition
MS condition
GC condition
a. Detector mode
b. Ion source temperature
c. Electron energy
d. Initial temperature
e. Priming effect

Method validation

1) ILOQ (Instrumental Limit of Quantitation)

After matrix matched standard solutions PAHs working solution were analyzed by GC-MS/MS. The ILOQ was settled as the concentration where the signal-to-noise ratio was higher than 10.

2) MLOQ (Method Limit of Quantitation)

MLOQ was calculated by equation below

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

3) Calibration curve and linearity

(A) Water

Matrix matched standard solution	MSTD 1 (0.002 mg/L)	MSTD 2 (0.005 mg/L)	MSTD 3 (0.010 mg/L)	MSTD 4 (0.020 mg/L)	MSTD 5 (0.100 mg/L)	MSTD 6 (0.200 mg/L)
Standard solution	0.002 mg/L 100 µL	0.005 mg/L 100 µL	0.01 mg/L 100 µL	0.02 mg/L 100 µL	0.1 mg/L 100 µL	0.2 mg/L 100 µL

A series of matrix-matched PAHs working solutions with concentration of 0.002, 0.005, 0.01, 0.02, 0.1, and 0.2 mg/L were prepared with a blank extract. The relative standard deviation (RSD) was calculated at the calibration curve.

(A) Soil

Matrix matched standard solution	MSTD 1 (0.005 mg/L)	MSTD 2 (0.010 mg/L)	MSTD 3 (0.020 mg/L)	MSTD 4 (0.050 mg/L)	MSTD 5 (0.100 mg/L)
Standard solution	0.01 mg/L 200 µL	0.02 mg/L 200 µL	0.04 mg/L 200 µL	0.1 mg/L 200 µL	0.2 mg/L 200 µL

A series of matrix-matched PAHs working solutions with concentration of 0.01, 0.02, 0.04, 0.1 and 0.2 mg/L were prepared with a blank extract. The relative standard deviation (RSD) was calculated at the calibration curve.

4) Establish sample pretreatment conditions

(A) Water

The modified liquid-liquid extraction (LLE) method (EPA method 610, 1984; EPA method 625, 1984; Ministry of environment). sample 40 mL was into 50 mL of propylene tube. 0.2 g of KH_2PO_4 added and adjusted to pH 4.5, followed by 2 g of sodium chloride. After mixing, Extracted with different combinations of extraction solvents method as follows: (A) original method (2 mL DCM), (B) 2 mL DCM + 2 mL ACN, (C) 1 mL DCM+1.5 mL DCM and (D) 1 mL DCM+1.5 mL DCM + 2 mL ACN. A were added and shaken for 1 min at room temperature using a shaker (Mini-G, JAPAN). Centrifugation at 3500rpm for 5 min. Pick the DCM Layer. The supernatants were through the sodium sulfate ($NaSO_4$) and transferred 2 mL viral. Add the protect the volatile components, (A) DEG 2 uL (B) PEG 2 uL were added and used (A) Nitrogen concentrator (Windy–v, CHONGMIN, Korea). Re-dissolved (A) ACT 100 uL, (B) ACN 100 uL. Finally, sample was injected into GC-MS/MS.

(B) Soil

Sample preparation was modification of the QuEChERS method based on the evaluation of the applicability for analysis of 23 compounds in PAHs were proposed, including the choice of extraction solvent and optimization of the purification effect with different adsorbers. Homogenized samples (5 g) in a 50 mL propylene tube were fortified with PAHs at 10 mg/kg and the samples left for 30 minutes. Next, 5 mL of water and 10 mL of (A) acetonitrile (B) acetonitrile contain 0.1% formic acid (v/v) were added to each tube and shaken (1500 rpm) using a Geno Grinder (1600 MiniG^{TM}, SPEX$^{®}$ SamplePrep, New Jersey, USA) for 1 min. Then, added the QuEChERS extraction kit (A) original unbuffered with sodium chloride (NaCl) 1 g and $MgSO_4$ 4 g (B) European EN 15662 with NaCl 1 g $MgSO_4$ 4 g and trisodium citrate dehydrate (TSCD) 1 g, disodium hydrogen citrate sequihydrate (DHS) 0.5 g (C) AOAC 2007.01 with $MgSO_4$ 6 g, sodium acetate (NaOAc) 1.5 g. After the mixture was shaken vigorously for another 1 min, the tube was centrifuged at 3,500 rpm for 5 minutes (Combi 408, Hanil Science industrial, Korea). The supernatant 200 μL, ACN 200μL. 2 μL of final sample was injected into GC-MS/MS.

5) Final sample preparation method

(A) Water

Sample 40 mL was into 50 mL of propylene tube. 0.2 g of KH_2PO_4 added and adjusted to pH 4.5, followed by 2 g of sodium chloride. After mixing add the 2 mL DCM + 2 mL ACN. A were added and shaken for 1 min at room temperature using a shaker (Mini-G, JAPAN). Centrifugation at 3500rpm for 5 min. Pick the DCM Layer. The supernatants were through the sodium sulfate ($NaSO_4$) and transferred 2 mL microtube with 10 % PEG 4 uL. Concentrated Nitrogen concentrator (Windy–v, CHONGMIN, Korea). Redissolved in ACT 100 uL. Finally, sample was injected into GC-MS/MS.

(B) Soil

Homogenized samples (5 g) in a 50 mL propylene tube were fortified with PAHs at 10 mg/kg and the samples left for 30 minutes. Next, 5 mL of water and 10 mL of acetonitrile was added to each tube and shaken (1500 rpm) using a Geno Grinder (1600 MiniG™, SPEX® SamplePrep, New Jersey, USA) for 1 min. Then, added the QuEChERS AOAC extraction kit with $MgSO_4$ 6 g, sodium acetate (NaOAc) 1.5 g. After the mixture was shaken vigorously for another 1 min, the tube was centrifuged at 3,500 rpm for 5 minutes (Combi 408, Hanil Science industrial, Korea). The supernatant 200 μL, ACN 200μL. 2 μL of final sample was injected into GC-MS/MS.

Analyte protectant(AP) effects

AP effects method as follows: (A) DEG 2 *u*L, (B) PEG 2 *u*L and picked one more effective. Then, add the (A) 100% PEG, (B) 50% PEG, (C) 20% PEG, (D) 10% PEG, (E) 2% PEG, (F) 1% PEG, 4 *u*L respectively.

Recovery test of PAHs in water and Soil samples

(A) water

Sample 40 mL was into 50 mL of propylene tube were fortified with PAHs working solution at three levels of 0.01, 0.05 and 0.1 mg/L (MLOQ, 5 MLOQ and 10 MLOQ) of each compound and the analysis was performed using the established method.

(A) soil

Sample 10 g was into 50 mL of propylene tube were fortified with PAHs working at three levels of 0.01, 0.02 and 0.1 mg/kg (MLOQ, 2 MLOQ and 10 MLOQ) of each compound and the analysis was performed using the established method.

Matrix effect

The most common way to avoid matrix effects in instrument is to use matrix-matched calibration standards. The Matrix effects (ME, %) was calculated using the following equation: (Lehotay et al., 2010; Rajski, Lozano, Uclés, Ferrer, & Fernández-Alba, 2013).

$$\mathrm{ME},\% = \left(\frac{\text{slope of matrix matched calibration curve}}{\text{slope of solvent standard calibration curve}} - 1\right) \times 100$$

Results and Discussion

Water, Soil sampling date

The water samples were collected from September 2017 to April 28, 2018 and soil samples sampling date and dry date shown in the **Table 10, Table 11**.

Table 10 . Water sampling date

Date					
2017			2018		
Date	**Seongnam**	**Poseung**	**Date**	**Seongnam**	**Poseung**
9/11	O (2 L)	O (2 L)	**1/8**	O (2 L)	O (2 L)
9/25	O (2 L)	O (2 L)	**1/22**	O (2 L)	O (2 L)
10/10	O (2 L)	O (2 L)	**2/5**	O (2 L)	O (2 L)
10/23	O (2 L)	O (2 L)	**2/19**	O (2 L)	O (2 L)
11/6	O (2 L)	O (2 L)	**3/5**	O (2 L)	O (2 L)
11/20	O (2 L)	O (2 L)	**3/19**	O (2 L)	O (2 L)
12/4	O (2 L)	O (2 L)	**4/2**	O (2 L)	O (2 L)
12/19	O (2 L)	O (2 L)	**4/24**	O (2 L)	O (2 L)

Table 11. Soil sampling date and dry date

	Hwaseong-
Sample Weight	14.6 kg
Sample date	March 11
Dry date	March 13

GC Instrumental condition

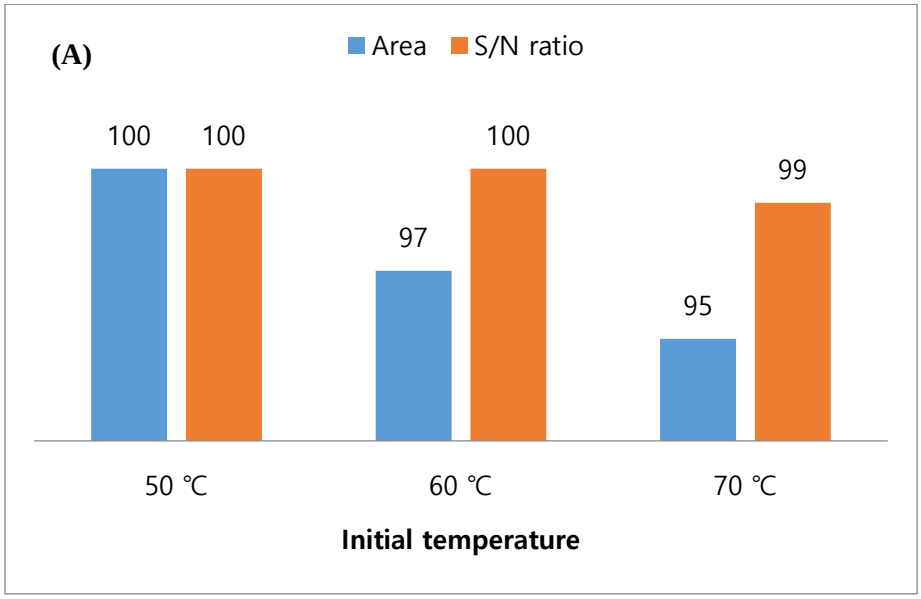
GC-MS/MS analysis for the water was performed on GC-MS SCION TQ triple-quadrupole system (Bruker, USA) equipped with an Restek Rxi-5Sil MS column (30 m X 0.25 mm id, 0.25 μm df 0.25 mm). Standard mixture injection volum were 2 μL in the splitless mode. The oven temperature, started at 50 ℃, was held for 2 min and raised at a rate of 10 °/min to 310 ℃ which was finally held for 5 min. Total run time was 33.0 min. Transfer line and injector temperature set at 280 ℃ and 280 ℃, respectively. The MS was operated in electron impact (EI) mode with ion source temperature was set at 300 ℃ and electron ionization energy was -80 eV. High purity helium gas (>99.999%) was used as the carrier gas with a constant flow rate of 1 mL min. For better sensitivity (quantifier and qualifier), PMRM mode was employed to the analytes.

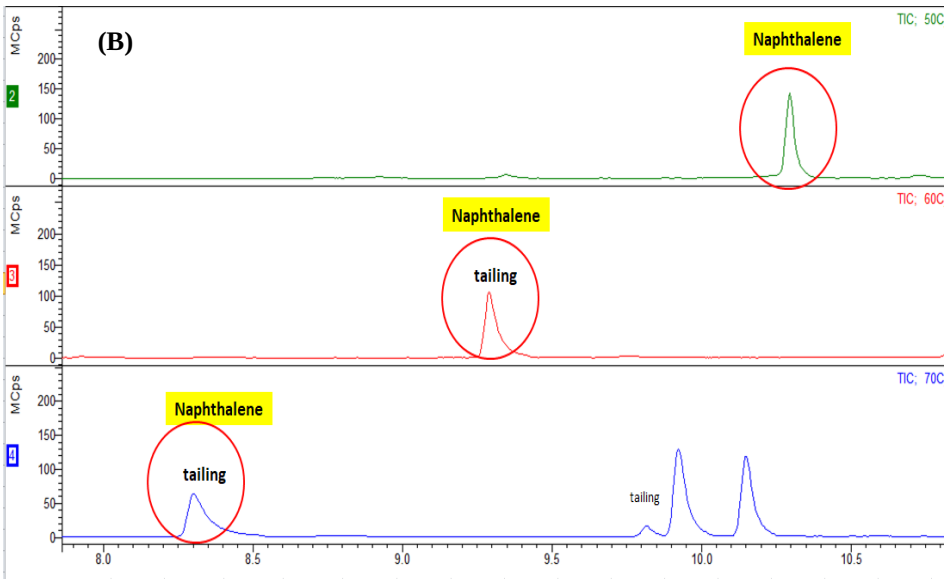
GC condition

(A) Initial temperature condition

In basic temperature programming conditions, we tried to raise the initial temperature from 50 ℃ to 70 ℃. As the initial temperature condition goes to 70 ℃, the peak area and S/N ratio decrease and tailing occurs. Thus, the initial temperature was finally set at 50 degrees (Figure 18).

Figure 18. Scan TIC of 25 PAHs at 1.0 μg/mL of solvent standard mixture. (A) Influence of initial temperature on average peak area & S/N ratio of 25 PAHs, (B) Shape of peak according to temperature of naphthalene.

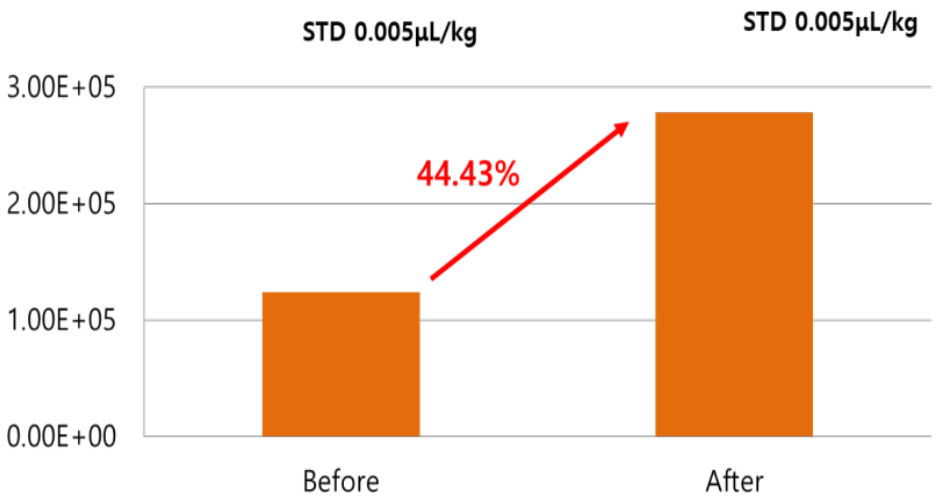

(A)
Area
S/N ratio
100
100
97
100
95
99
50 ℃
60 ℃
70 ℃
Initial temperature


(B)
MCps
TIC; 50C
Naphthalene
TIC; 60C
Naphthalene
tailing
TIC; 70C
Naphthalene
tailing
tailing
8.0
8.5
9.0
9.5
10.0
10.5

(B) Priming effects

New column or a new inlet liner is installed, a several matrix-matched blanks should be injected before injecting standards because the matrix-matched STD blocking the active sites of a new column, liner or GC instrument. So target material can reach the detector as much as possible. This phenomenon was called priming effects (Schenck & Lehotay, 2000;, patel et al., 2005). In this study, standards and spinach extracts were injected into the GC system after a new liner was installed to evaluate the practical priming effect. The results show when the PAHs mixture was injected after the spinach extract injections, the peak area of all the target compounds increased by 44.43% compared with that from the first injections, peak area increased compared with that from the first injection after a new liner was installed (Figure 19).

Figure 19. Priming effects

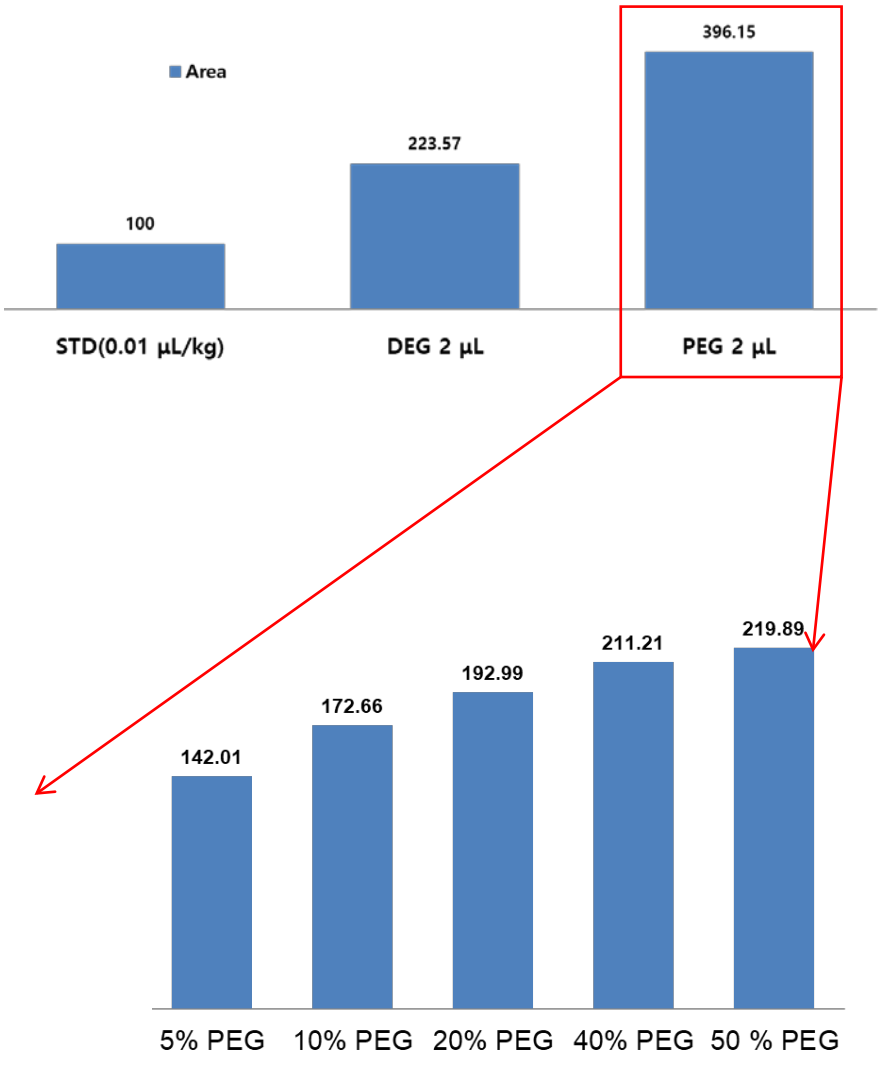

STD 0.005µL/kg
STD 0.005µL/kg
3.00E+05
2.00E+05
1.00E+05
0.00E+00
44.43%
Before
After

Analyte protectant (AP) effects in GC-MS/MS

In GC analysis, AP acts instead of the target analyte in the active site present in the analytical instrument or analytical column. A kind of AP were polyglycol glycol (PEG) 300, diecoylglycol (DEG), sugar, fatty acid etc (M. Anastassiades et al., 2003; K. Maštovska., 2005). DEG and PEG were compared by AP effect test. It also protected volatile components. As a result of comparing PEG and DEG in STD (0.01 *u*L/kg), DEG was 223.57% and PEG was 369.15% when the area ratio of untreated STD (0.01 *u*L/kg) was 100%, indicating that PEG had a higher AP effect than DEG (Figure 20).

If you use a lot of AP, it will cause the column problem such as the peak broad, fronting, tailing phenomenon. Therefore, when injected with 3 μL of PEG by concentration (5, 10, 20, 40, 50%) as expected, the higher the concentration of PEG, the greater the AP effect. However, from 30%, fronting occurred in 5-methylnaphthalene. Finally, selected 20% PEG (Figure 20)..

Figure 20. AP effect of diethyleneglycol (DEG) & polyethyleneglycol (PEG)


Area
100
223.57
396.15
STD(0.01 µL/kg)
DEG 2 µL
PEG 2 µL
142.01
172.66
192.99
211.21
219.89
5% PEG
10% PEG
20% PEG
40% PEG
50 % PEG

/

MS/MS condition

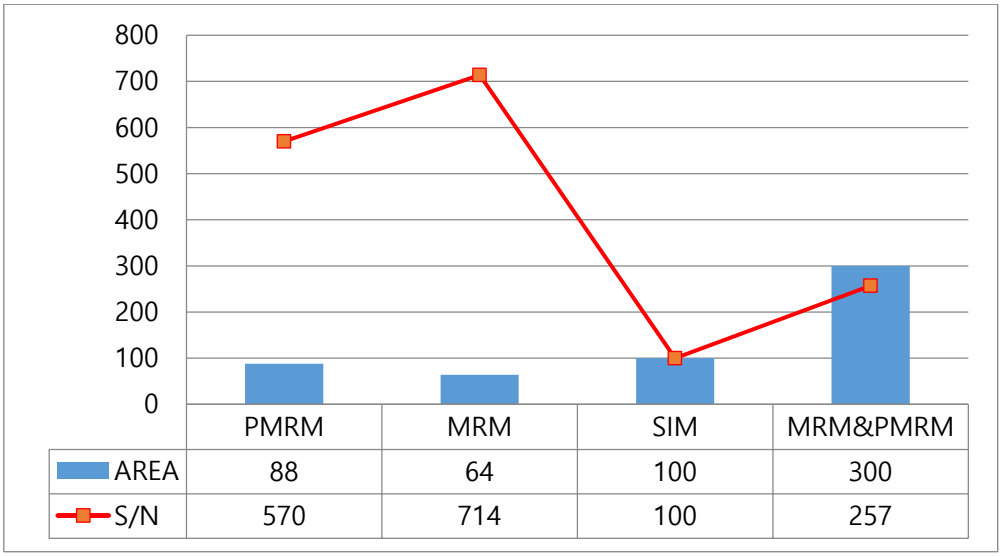
(A) MRM, SIM, PMRM and MRM & PMRM optimization

Using the PAHs mixture (0.01 uL / kg) was used in the most common SIM mode, MRM mode, recently introduced PMRM mode, and MRM & PMRM mode. MRM&PMRM was the most suitable for the comparison of S/N ratio and Area value (Figure 21). **Figure 22** shows total ion chromatogram (TIC) of 25 PAHs. First of all, a full scan spectrums of each compounds were obtained in the mass range of 50 to 350 m/z using quadrupole 1 (Q1). On the basis of selectivity, specific precursor ions were selected. The most selective and sensitive transition was used for quantifier and the second most selective for qualifier. The details of MRM & PMRM transitions and collision energies and retention times for 25 PAHs are presented in **Appendix Table S1.**

(B) Electron ionization energy & Ion source temperature

Generally, GC/MS has electron ionization energy of -70eV and ion source temp of 230℃ or 200℃. However, for the improved peak shape or peak area increased the ion source temperature and electron ionization energy Ion source temperature as higher as it will go and I will have awesome peak shape (Aviv Amirav et al., 2013). The ion source temperature was raised from 200 ° C to 300 ° C by 10 ° C and electron ionization energy was raised from -70eV to -90eV by -5eV. As a result of raising the ion source temperature from 200 ℃ to 300 ℃, ion source temperature as high as it can be seen that the peak area and sensitivity were improved (Figure 23) and electron ionization energy was able to obtain the highest sensitivity at -80 eV rather than -70 eV which is generally used much (Figure 24).

Figure 21. Comparison of area and S/N ratio of MRM, SIM, PMRM and MRM & PMRM



	PMRM	MRM	SIM	MRM&PMRM
AREA	88	64	100	300
S/N	570	714	100	257

Figure 22. PAHs 25 species of total ion chromatograms (TIC)

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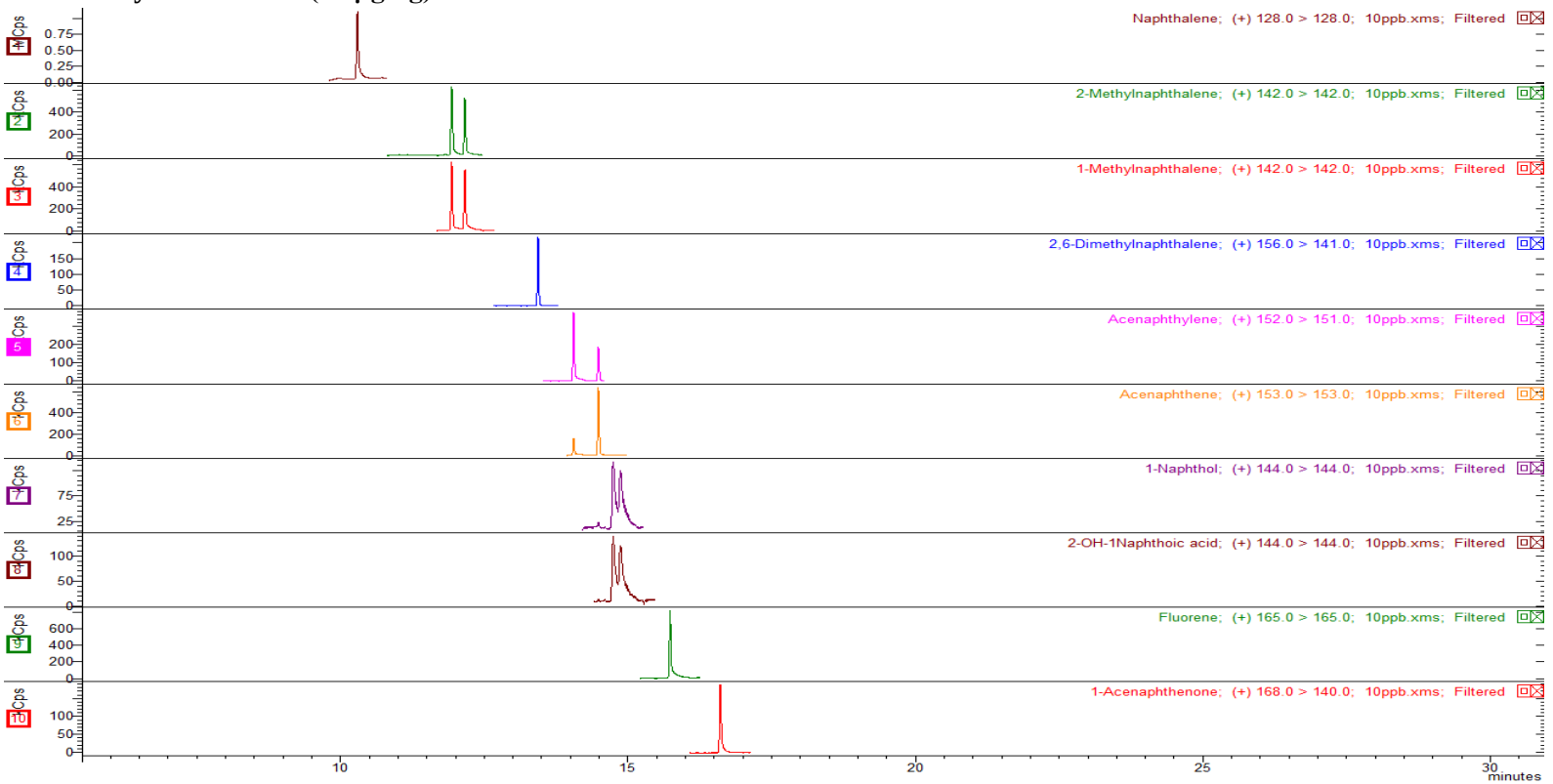
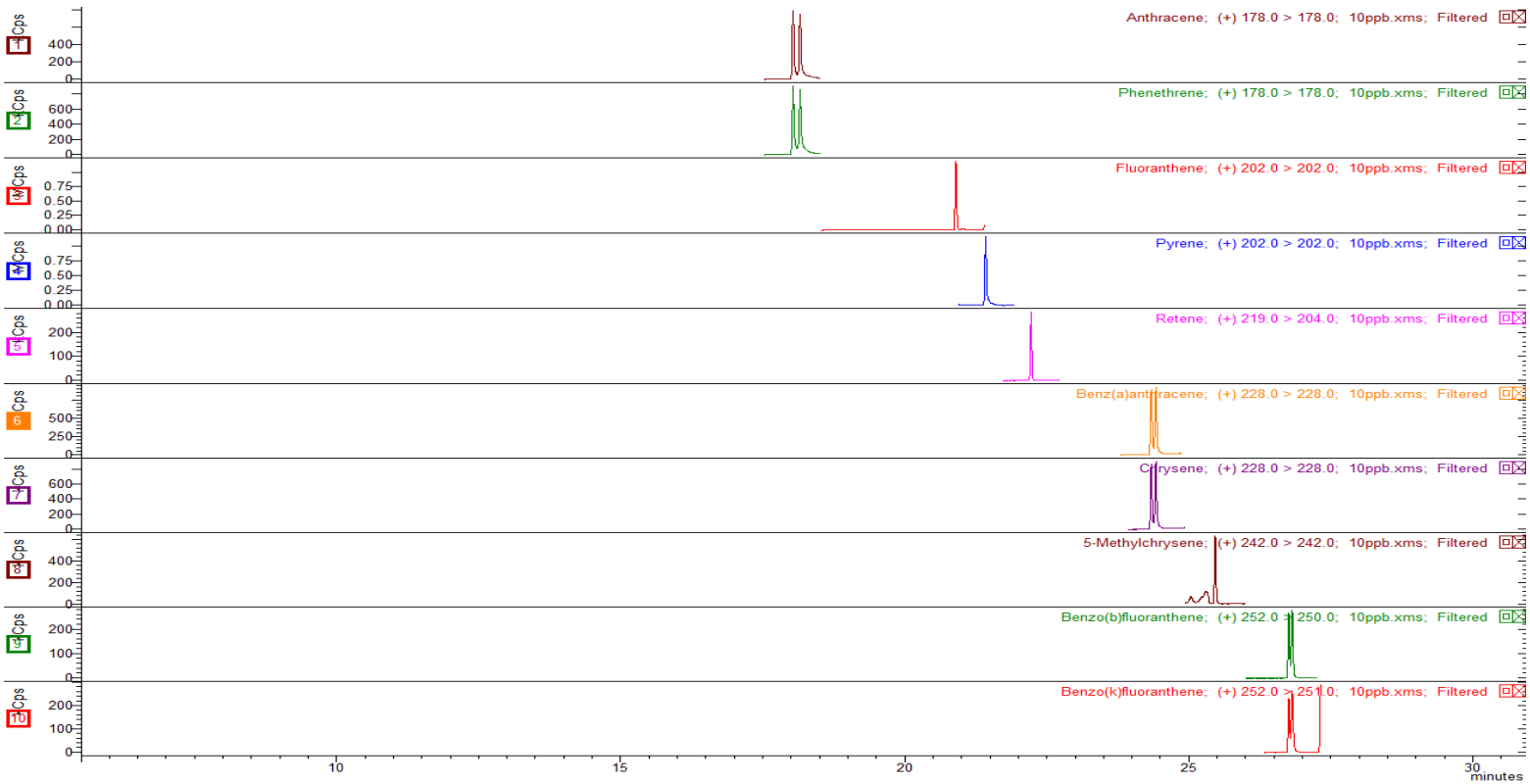
(A) - (1) naphthalene, (2) 1 – methylnaphthalene, (3) 2 – methylnaphthalene, (4) 2,6 – dimethylnaphthalene, (5) Acenaphthylene, (6) acenaphthene, (7) 1-naphthol, (8) 2-hydroxy-1-naphthoic acid, (9) fluorene and (10)1-acenaphthenone obtained by GC-MS/MS (10 µg/kg)



(B) - (1) anthracene, (2) phenanthrene, (3) fluoranthene, (4) pyrene, (5) retene, (6) benzo(a)pyrene, (7) chrysene, (8) 5 - methylchrysene, (9) benzo(b)fluoranthene and (10) benzo(k)fluoranthene obtained by GC-MS/MS (10 µg/kg).



(C)- Figure 12. MRM chromatograms of (1) benzo(a)pyrene, (2) benzo(e)pyrene, (3) indeno(1,2,3-cd)pyrene, (4) dibenz(a,h)anthracene and (5) benzo(g,h,i)-perylene obtained by GC-MS/MS (10 µg/kg).

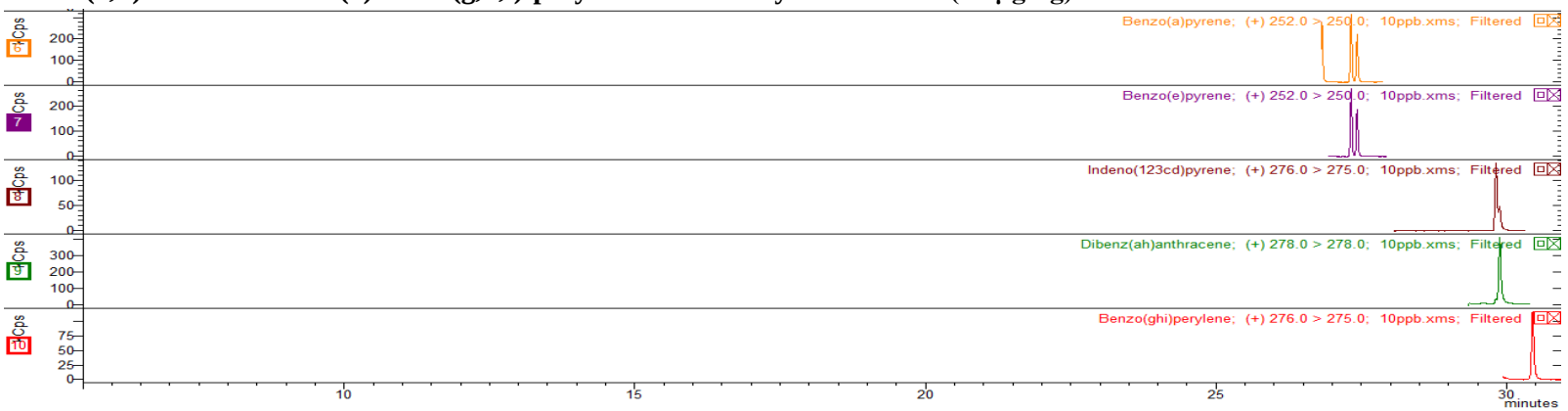
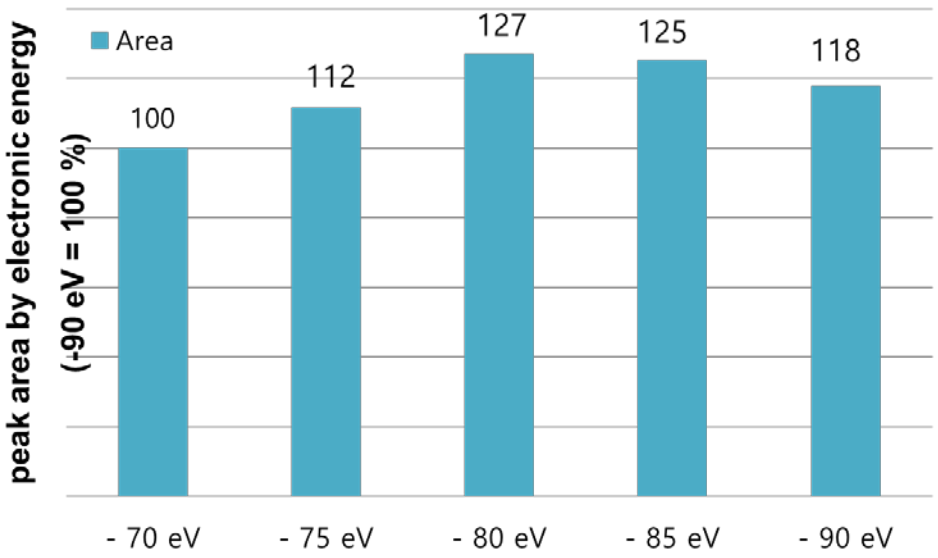


Figure 23. Electron ionization energy

(A)


peak area by electronic energy
(-90 eV = 100 %)
Area
100
112
127
125
118
- 70 eV
- 75 eV
- 80 eV
- 85 eV
- 90 eV

(B)

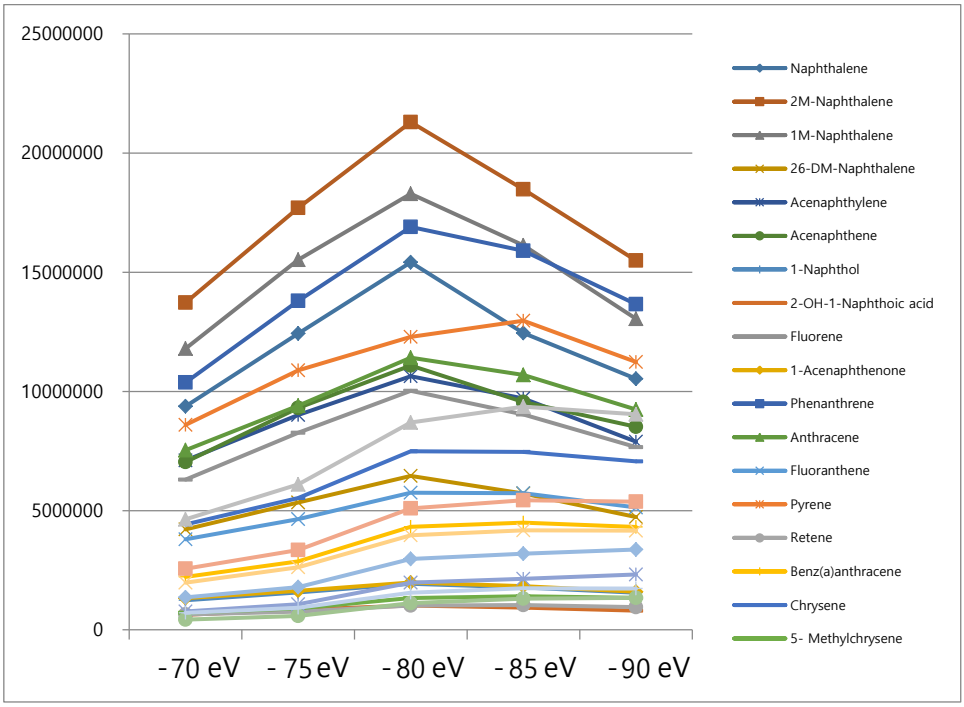
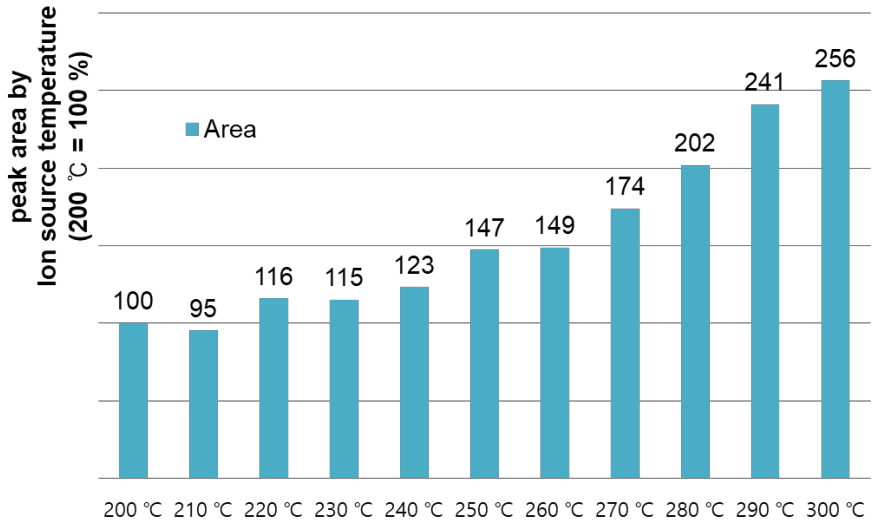
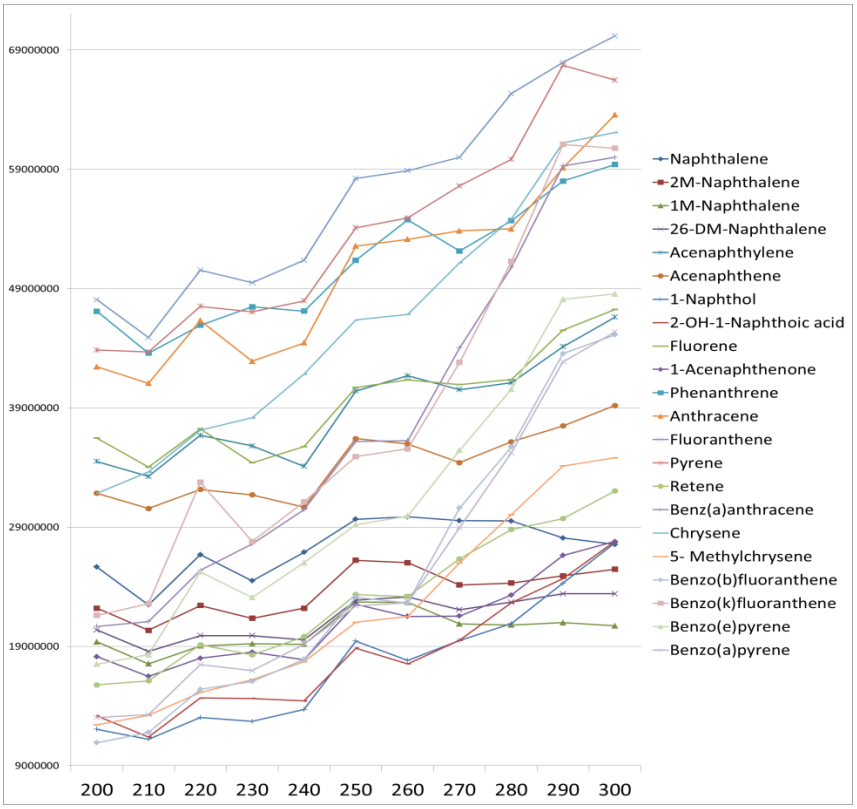

25000000
20000000
15000000
10000000
5000000
0
-70 eV
-75 eV
-80 eV
-85 eV
-90 eV
Naphthalene
2M-Naphthalene
1M-Naphthalene
26-DM-Naphthalene
Acenaphthylene
Acenaphthene
1-Naphthol
2-OH-1-Naphthoic acid
Fluorene
1-Acenaphthenone
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Retene
Benz(a)anthracene
Chrysene
5- Methylchrysene

Figure 24. Ion source temperature

(A)


peak area by
Ion source temperature
(200 ℃ = 100 %)
Area
100
95
116
115
123
147
149
174
202
241
256
200 ℃
210 ℃
220 ℃
230 ℃
240 ℃
250 ℃
260 ℃
270 ℃
280 ℃
290 ℃
300 ℃

(B)


69000000
59000000
49000000
39000000
29000000
19000000
9000000
200
210
220
230
240
250
260
270
280
290
300
Naphthalene
2M-Naphthalene
1M-Naphthalene
26-DM-Naphthalene
Acenaphthylene
Acenaphthene
1-Naphthol
2-OH-1-Naphthoic acid
Fluorene
1-Acenaphthenone
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Retene
Benz(a)anthracene
Chrysene
5- Methylchrysene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(e)pyrene
Benzo(a)pyrene

Establish sample pretreatment conditions

(A) Water

Extraction solvent test

In this study, addition of acetonitrile extraction solvent was evaluated to increase the recovery efficiency of the target compound (Jeffrey Yan et al., 2008). **Figure 25** shows the ratio of PAHs that satisfied the recovery ranges percentage in the applied extraction procedures. From method A to D Area percentage were 75, 117, 69 and 52 % (PAHs mixture (1 mg/kg) percentage is based on 100%)). Method B using 2 ml of DCM and 2 ml of ACN for extraction. The results of this method B show that the extraction efficiency of PAHs of 25 species is better than other methods.

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Re-dissolved ACN? or ACT?

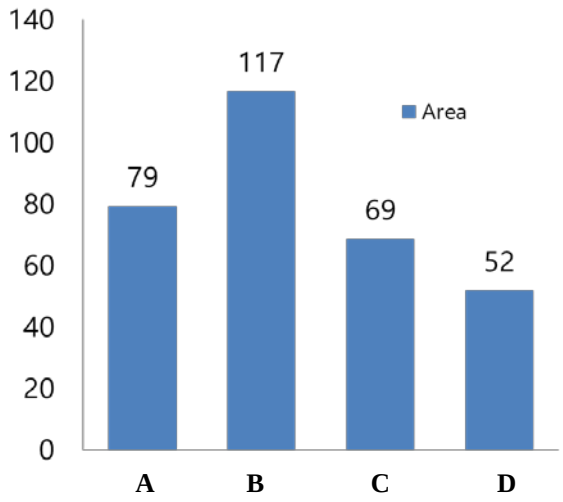
20% PEG was added to the PAHs mixture (1 μL/kg) and softly concentrated with nitrogen concentrator for 20 min and re-dissolved acetone (ACT) or is acetonitrile (ACN). **Figure 25** show that recovery rate of the samples reused by based on ACT (100%) is better than ACN (68.62%). In this study PAHs are more soluble in ACT solution than ACN solution.

Figure 25. Establish sample pretreatment conditions

(A) Extraction solvent test (standard 1 mg/kg based on 100%, n=2)

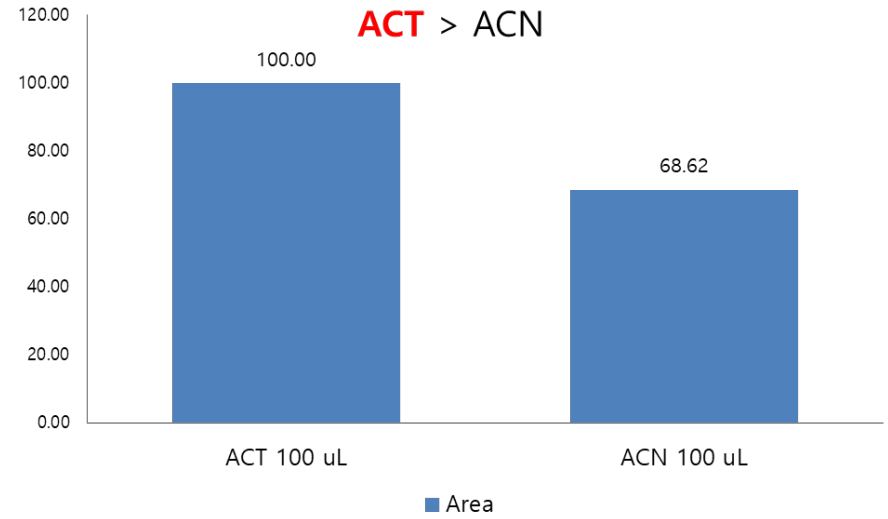
(B) Re-dissolved ACN? or ACT?

(A)



Method A	2 mL DCM
Method B	2mL DCM + 2 mL ACN
Method C	1. 1.5 mL DCM 2. 0.5 mL DCM
Method D	1. 1.5 mL DCM 2. 0.5 mL DCM + 2 mL ACN

(B)



(B) Soil

Modification of sample extraction.

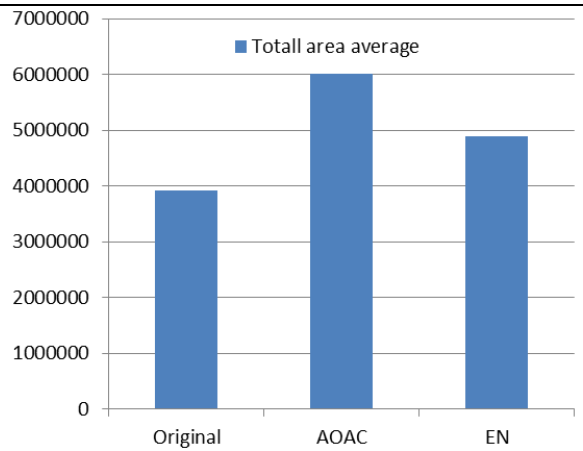
Compared the recovery rates used AOAC, EN, original unbuffered QuEChERS Extraction Kit and using ACN and ACN with 0.1% formic acid as extraction solvent. As a result of comparing the QuEChERS extraction kits, the highest extraction rate was AOAC kit. In addition, the recovery rate of ACN was twice as high as that used 0.1% formic acid in ACN (Table 12).

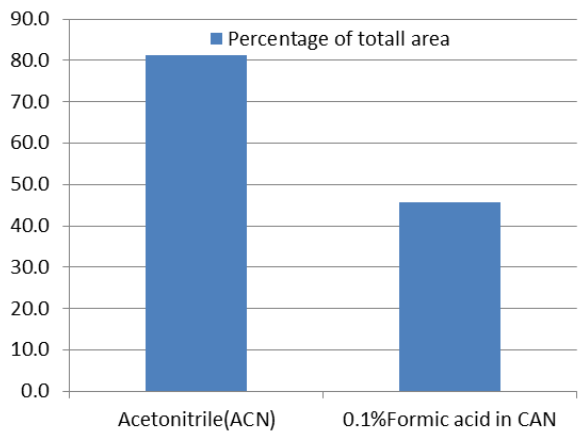
ILOQ, MLOQ and calibration curve

In this study, ILOQ was checked from the results of analysis of several concentration standard solutions, In GC-MS/MS, ILOQ of water and soil was 0.002 mg/kg and 0.005 mg/kg. Based on MLOQ calculating equation, MLOQ of PAHs in water sample was 0.002 mg/kg and soil sample was 0.01 mg/kg. Matrix matched standard curves of PAHs has a good linearity in samples of water. The range of water and soil were between 0.002 a 0.1 mg/kg and 0.005 to 0.1 mg/kg of PAHs standard solution. The regression equations were **Table S2.** Coefficients of determination (r^2) were over 0.99 in samples (Table S2).

Table 12. PAHs percentages with recoveries (70 and 120%) and RSD (≤20%) in recovery test results from different QuEChERS extraction kit and extraction solvents for soil sample (n=3)

QuEChERS extraction kit	Extraction solvents
(A) sodium chloride (NaCl) 1 g and $MgSO_4$ 4 g	(A) ACN
(B) NaCl 1 g $MgSO_4$ 4 g, trisodium citrate dehydrate (TSCD) 1 g disodium hydrogen citrate sequihydrate (DHS) 0.5 g	
(C) $MgSO_4$ 6 g and sodium acetate (NaOAc) 1.5 g	(A) ACN
	(B) 0.1% formic acid in ACN





Recoveries of PAHs

Water and soil recovery test provide precision and accuracy of sample preparation method by recovered rate (accuracy, %) and RSD (precision, %) (Fong et al., 1999). Untreated water samples were spiked with MLOQ 5 MLOQ and 10 MLOQ levels of PAHs mixture standard solutions, and soil samples were spiked with MLOQ 2 MLOQ and 10 MLOQ levels. The analysis was performed using the established method. **Table S3** shows results of recovery test. In case of PAHs the range of recovered rate (accuracy, %) and RSD (precision, %) data in water and soil. Except for some naphthalene related substances, the recovered rate was 70-120%, RSD≤20% was shown (Table S4). Compounds related to naphthalene are considered to have low recovery rates because they are highly volatile and tend to be contaminated during the pretreatment process.

Analyze of PAHs in water soil samples

Analyzed the water samples taken from Sengnam, Poseung through the established pretreatment process, but no PAHs were detected in all samples. (Table S4) In this study, PAHs not detected from the sewage treatment plant discharge water. These results show that the sewage treatment system is operating well.

Conclusion

In this study, we investigated the residual of fungicide fluxapyroxad and its metabolites M700F002 and M700F048 used in ssam cabbage cultivation and established an optimal analytical method of PAHs in water and soil samples. Analysis of fluxapyroxad and metabolites ensured reliability through QuEChERS pretreatment method and analytical method validation of the sample. In the ssam cabbage the residual amount of fluxapyroxad in terms of total treatment in each treatment group was 1.15-0.28 mg/kg, which showed a tendency to decrease over time. PAHs were analysis for about 25 species as environmental pollutants. Compounds related to naphthalene are considered to have low recovery rates because they are highly volatile and tend to be contaminated during the pretreatment process. So except for naphthalene and its related substances, the recovered rate was 70-120%, RSD≤20% was shown in water and soil samples. Analyzed the water samples taken from Sengnam, Poseung through the established pretreatment process, but no PAHs were detected in all samples. Based on the conditions of GC and MS established in this research and the pretreatment method established in experiments of water quality and soil, it can be applied to various actual water quality and soil experiments. Furthermore, it will also help to improve human health and protect the environment.

Appendix

Table S1. The optimized GC-MS/MS parameters including retention time of each PAHs, MRM & PMRM transitions, and collision energy

Compound	Retention time	Nominal mass *(m/z)*	Ionization	Precursor ion>Product ion (CE,eV)	
				Quantifier ion *(m/z)*	Qualifier ion *(m/z)*
Naphthalene	10.28	128.2	$[M+H]^+$	128.0>128.0 (-5)	128.0>102.0 (-15)
Acenaphthylene	14.04	152.2	$[M+H]^+$	152.0>151.0 (-15)	151.0>151.0 (-15)
Acenaphthene	14.46	154.2	$[M+H]^+$	153.0>153.0 (-5)	153.0>152.0 (-5)
Fluorene	15.72	166.2	$[M+H]^+$	165.0>165.0 (-10)	165.0>166.0 (-10)
Anthracene	18.15	178.2	$[M+H]^+$	178.0>178.0 (-5)	178.0>177.0 (-10)
Phenethrene	18.02	178.2	$[M+H]^+$	178.0>178.0 (-5)	178.0>152.0 (-15)
Fluoranthene	20.89	202.2	$[M+H]^+$	202.0>202.0 (-5)	201.0>201.0 (-15)
Pyrene	21.41	202.2	$[M+H]^+$	202.0>202.0 (-5)	201.0>201.0 (-20)
Chrysene	24.42	228.3	$[M+H]^+$	228.0>228.0 (-5)	228.0>226.0 (-30)
Benz(a)anthracene	24.34	228.3	$[M+H]^+$	228.0>228.0 (-5)	228.0>226.0 (-30)

Benzo(b)fluoranthene	26.77	252.3	$[M+H]^+$	252.0>250.0 (-30)	250.0>250.0 (-30)
Benzo(k)fluoranthene	26.83	252.3	$[M+H]^+$	252.0>250.0 (-30)	250.0>250.0 (-30)
Benzo(a)pyrene	27.44	252.3	$[M+H]^+$	252.0>250.0 (-30)	250.0>250.0 (-30)
Indeno(123cd)pyrene	29.88	276.3	$[M+H]^+$	276.0>275.0 (-30)	274.0>274.0 (-30)
Dibenz(ah)anthracene	29.93	278.4	$[M+H]^+$	278.0>278.0 (-5)	278.0>252.0 (-30)
Benzo(ghi)perylene	30.46	276.3	$[M+H]^+$	276.0>275.0 (-30)	276.0>274.0 (-30)
5-Methylchrysene	25.46	242.3	$[M+H]^+$	242.0>242.0 (-5)	242.0>241.0 (-10)
Benzo(e)pyrene	27.32	252.3	$[M+H]^+$	252.0>250.0 (-30)	250.0>250.0 (-30)
2-Methylnaphthalene	119.92	142.2	$[M+H]^+$	142.0>142.0 (-5)	141.0>141.0 (-10)
1-Methylnaphthalene	12.14	142.2	$[M+H]^+$	142.0>142.0 (-5)	141.0>141.0 (-10)
2,6-Dimethylnaphthalene	13.42	156.2	$[M+H]^+$	156.0>156.0 (-5)	141.0>141.0 (-10)
1-Naphthol	14.78	144.2	$[M+H]^+$	144.0>144.0 (-5)	144.0>115.0 (-5)
2-OH-1Naphthoic acid	15.43	188.2	$[M+H]^+$	144.0>144.0 (-5)	144.0>115.0 (-5)
1-Acenaphthenone	16.60	168.2	$[M+H]^+$	168.0>140.0 (-10)	168.0>139.0 (-10)
Retene	22.21	234.3	$[M+H]^+$	219.0>204.0 (-10)	234.0>219.0 (-10)

Table S2. Matrix matched calibration curves of PAHs

Compound	Water (Range : 0.002 - 0.05 mg/kg)		Soil	
	regression equations	Coefficients of determination (r^2)	regression equations	Coefficients of determination (r^2)
Naphthalene	y = 182266x + 769053	0.9977	y = 268346x - 152388	0.9991
Acenaphthylene	y = 55029x - 27712	0.9976	y = 42575x - 107301	0.9988
Acenaphthene	y = 96467x + 1204	0.9998	y = 97135x - 278442	0.9994
Fluorene	y = 128252x + 100711	0.9991	y = 123850x - 463747	0.9996
Anthracene	y = 39467x - 18322	0.9985	y = 98534x - 478635	0.9986
Phenethrene	y = 136804x + 304291	0.9982	y = 164886x - 660456	0.9986
Fluoranthene	y = 149122x - 85972	0.9999	y = 132179x - 715891	0.9957
Pyrene	y = 139046x - 46207	0.9989	y = 120670x - 627802	0.9968
Chrysene	y = 91104x - 27843	0.9995	y = 26058x - 125125	0.9972
Benz(a)anthracene	y = 102370x - 23249	0.9998	y = 10552x - 52576	0.9980
Benzo(b)fluoranthene	y = 25352x - 899.07	0.9999	y = 7021.2x - 37807	0.9976
Benzo(k)fluoranthene	y = 28875x - 3260	0.9992	y = 16306x - 78227	0.9982
Benzo(a)pyrene	y = 27269x + 17184	0.9994	y = 8152.5x - 33023	0.9988
Indeno(123cd)pyrene	y = 182266x + 769053	0.9998	y = 4375.5x - 22265	0.9983
Dibenz(ah)anthracene	y = 81015x + 7039.2	0.9999	y = 16202x - 71077	0.9980
Benzo(ghi)perylene	y = 19889x + 6109.2	0.9997	y = 2999.1x - 16912	0.9976
5-Methylchrysene	y = 23156x + 5445.2	0.9999	y = 44421x - 186605	0.9981
Benzo(e)pyrene	y = 27190x + 1875.2	1.0000	y = 11237x - 54023	0.9980
2-Methylnaphthalene	y = 110742x + 391844	0.9956	y = 126390x - 412344	0.9988
1-Methylnaphthalene	y = 84431x + 108936	0.9991	y = 109103x - 278578	0.9988
2,6-Dimethylnaphthalene	y = 41050x + 40585	0.9994	y = 42031x - 94640	0.9996
1-Acenaphthenone	y = 27694x - 935.06	0.9997	y = 14686x - 16658	0.9968
Retene	y = 26903x + 3052.8	0.9998	y = 17677x - 50990	0.9995

Table S3. Recoveries of PAHs (n=3)

	water recovery (%)						soil recovery (%)					
	MLOQ(0.002 mg/kg)		5 MLOQ(0.01 mg/kg)		10 MLOQ (0.02 mg/kg)		MLOQ (0.01 mg/kg)		5 MLOQ (0.02 mg/kg)		10 MLOQ (0.1 mg/kg)	
Compound	Average	RSD (%)	Average	RSD (%)	Average	RSD (%)	Average	RSD (%)	Average	RSD (%)	Average	RSD (%)
Acenaphthylene	102.0	3.6	119.6	9.9	87.2	0.5	90.9	8.6	98.2	5.6	100.6	3.4
Acenaphthene	109.7	3.7	108.3	3.8	91.4	0.6	90.3	6.7	103.6	2.4	98.5	6.1
Fluorene	116.5	2.4	112.9	5.7	92.7	3.5	94.4	5.3	98.0	5.3	102.7	2.5
Anthracene	114.8	4.8	111.5	2.8	88.9	3.1	88.7	3.6	98.1	3.0	103.7	5.6
Phenethrene	99.2	5.1	121.7	29.2	79.3	4.0	98.7	7.5	99.5	3.2	104.5	5.2
Fluoranthene	95.7	9.2	118.4	2.8	95.3	5.3	93.6	4.6	97.0	2.5	99.9	3.3
Pyrene	101.7	3.7	121.2	3.2	99.0	3.4	92.5	4.3	95.9	2.4	92.2	3.8
Chrysene	103.0	6.3	120.6	2.3	99.5	3.1	80.7	2.7	70.5	4.2	70.9	3.7
Benz(a)anthracene	106.1	1.2	117.3	3.8	99.6	3.1	91.5	2.3	86.0	3.1	94.3	2.0
Benzo(b)fluoranthene	104.2	5.7	113.5	4.7	100.4	3.2	87.8	2.3	77.4	5.4	75.1	6.3
Benzo(k)fluoranthene	104.5	5.1	113.0	5.7	107.3	3.5	82.7	5.8	80.8	4.5	77.9	4.2
Benzo(a)pyrene	108.1	2.6	112.4	1.8	98.0	3.2	76.6	4.9	79.6	4.6	70.9	2.8
Indeno(123cd)pyrene	102.7	4.5	112.3	2.3	95.8	3.4	80.8	1.4	72.7	4.1	75.6	6.8
Dibenz(ah)anthracene	117.2	7.1	122.5	1.6	102.4	4.8	78.5	2.7	76.0	2.3	82.4	1.6
Benzo(ghi)perylene	107.8	4.3	113.7	3.3	95.4	4.7	89.1	5.0	91.1	2.8	84.6	0.5
5-Methylchrysene	105.1	1.7	110.9	6.5	100.7	2.0	82.4	5.8	82.0	0.2	90.5	1.8
Benzo(e)pyrene	95.7	2.6	112.4	6.2	97.1	4.2	79.5	8.2	80.9	8.1	77.8	2.7
1-Acenaphthenone	111.3	2.8	114.8	2.7	103.4	1.8	70.6	3.7	101.7	9.7	103.9	3.9
Retene	101.0	4.0	115.5	0.7	95.2	2.3	86.6	7.9	89.8	3.2	97.5	2.8
Naphthalene	-	-	-	-	-	-	93.5	8.2	101.3	1.9	97.1	3.9
2-Methylnaphthalene	-	-	-	-	-	-	98.7	8.0	101.8	3.2	93.6	3.2
1-Methylnaphthalene	-	-	-	-	-	-	97.2	8.7	96.7	1.9	97.5	5.8
2,6-Dimethylnaphthalene	-	-	-	-	-	-	93.1	7.0	101.5	3.2	101.2	3.5

Table S4. Contents of 19 PAHs in Real samples in water (LOQ -0 .002 mg/kg)

Sengnam, Poseung (Real sample)																
Date / Compound	9/11	9/25	10/10	10/23	11/6	11/20	12/4	12/19	1/8	1/22	2/5	2/19	3/5	3/19	4/2	4/24
Acenaphthylene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Acenaphthene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Fluorene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Anthracene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Phenethrene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Fluoranthene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Pyrene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Chrysene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Benz(a)anthracene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Benzo(b)fluoranthene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Benzo(k)fluoranthene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Benzo(a)pyrene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Indeno(123cd)pyrene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Dibenz(ah)anthracene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Benzo(ghi)perylene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
5-Methylchrysene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Benzo(e)pyrene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
1-Acenaphthenone	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Retene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

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Abstract in Korean

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전다래

본 연구는 엇갈이배추 재배 중 사용한 살균제 플룩사피록사드와 그 대사물 M700F002와 M700F048의 잔류성을 규명하고, 물과 토양 시료 중 PAHs의 최적 분석법을 확립하고 실제 시료에 대한 응용 모니터링을 확인하기 위해 수행되었다. 플룩사피록사드와 대사물의 분석은 시료의 QuEChERS 전처리 방법과 분석법 검증을 통하여 신뢰도를 확보하였다. 플룩사피록사드+메탈락실-M 8(4+4)% 액상수화제를 계획된 수확 전 살포날짜에 각 처리구에 살포하고 수확 후 전처리 과정을 거쳐 LC-MS/MS을 이용하여 분석하였다. 엇갈이배추 에서 각 처리구의 플룩사피록사드 전체 환산 잔류량은 1.15-0.28 mg/kg으로 시간에 지남에 따라 감소하는 경향을 보였다. PAHs는 환경오염물질로서 약 25종에 대해서 MS/MS분석에서의 최적의 감도와 선택성을 확보하기 위하여 Ion source temperature, Detection mode, Electron energy등을 최적화하여 기존 PAHs의 분석에 사용했던 분석조건보다 GC-MS/MS를 이용하여 높은 감도와 향상된 신호대 잡음비를 얻을 수 있었다. 액-액 분배 및 농축 등 전처리법을 개선하고, 각 성분에 대해 분석정량한계, 검량선의 직선성, 회수율 등을 검증하였으며 간단하고 신속한 동시 분석법을 확립하였다. 또한 실제 물 및 토양 시료에 대한 응용성을 확인하였다.

주요어: 플룩사피록사드, M700F002, M700F048, 엇갈이배추, 다환방향족탄화수소, GC-MS/MS, MRM, PMRM, 물, 토양

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