농학석사학위논문

Development and Validation of Analytical Method for Pesticide Multi-Residue in Soil and Water Using GC-ECD/NPD

토양 및 농업용수에서 농약 다성분 동시분석법의 확립 및 유효성 검증 (GC-ECD/NPD)

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농생명공학부 응용생명화학 전공

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A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Development and Validation of Analytical Method for Pesticide Multi-Residue in Soil and Water Using GC-ECD/NPD

Advisor : Jeong Han Kim

By

Jinbeum Lee

Major in Applied Life Chemistry

Department of Agricultural Biotechnology

Seoul National University Graduate School

A thesis submitted to the faculty of the Seoul National University Graduate School in partial fulfillment of the requirement for the degree of Master of Science in the School of Agricultural Biotechnology.

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지도교수 김 정 한

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부위원장 김 정 한 (인)

위 원 배 의 영 (인)

ABSTRACT

Development and Validation of Analytical Method for Pesticide Multi-Residue in Soil and Water Using GC-ECD/NPD

Jinbeum Lee

Major in Applied Life Chemistry

Department of Agricultural Biotechnology

The Graduate School

Seoul National University

It needs to safety control for agricultural soil, water that directly effect on producing and safety of crops. This research developed pesticide multiresidue analysis method based on NAQS for 166 pesticides (GC-ECD : group1 26, group2 23, group3 24, group4 24, GC-NPD group 1 18, group2 18, group3 17, group4 16 compounds) and validated method according to KOLAS guideline. On developing method, extraction, wetting and purification steps were optimized. Compared acetonitrile, dichloromethane, ethyl acetate as extraction

solvent and the result of acetonitrile was best. In extraction of soil sample, NaCl 20g was changed to saturated brine 30 mL. Water sample was only filtrated, without purification. Developed method was applied to 3 soil samples (paddy, field, and orchard) and 4 water sample (distilled water, river, underground water and lake) and tested 7 times. From this date, validation parameters on KOLAS guideline (linearity, precision, trueness & bias, method LOD/LOQ, sensitivity, selectivity, working range, ruggedness) were calculated. Measurement uncertainty was estimated from monitoring sample collected in whole country. Endosulfan sulfate was used for GC-ECD and its measurement uncertainty was 1.662 ± 0.121 μg/kg (k=2.042) and chlorpyrifos(GC-NPD) was 0.0076 ± 0.0011 μg/kg (k=2.086)

Keywords: soil, agricultural water, multiresidue analysis, method validation, GC-ECD, GC-NPD, measurement uncertainty

Student number : 2012-21176

CONTENTS

I. Introduction

From 'Farm to Table', It is critical to provide safe and high quality crops for customers. So KFDA is setting MRL (Maximum Residue Level) or heavy metal criteria in crops. In Korea, agricultural product quality control act requires perform risk assessment for residual pesticides in cultural environmental that used in agriculture. It needs to safety control for agricultural soil, water that directly effect on producing and safety of crops. Safety control and quality control is required to systematic connection of crop and cultural environments. Crops that produced on safe soil and water, it will be high quality and safe farm-products.[1]

KFDA sets MRLs for 416 pesticides on crops but there is no regulation for soil. Many researches on residual pesticides performed for only crops. It needs to pesticide safety control on soil, like pesticide penetration or translocation. Pesticides are sprayed on fields repeatedly for increase producing crops. Residuals degraded slowly in the environment and farmers uses that extensively or inappropriate ways so several types of contaminated water, soil, crops can affect to humans indirectly. Contamination of agricultural soil and water is in whole country that may threat agricultural products safety. Residual pesticide can be injected indirect sources in agricultural environments like soil, water, growth regulators. To ensure consumer safety, residue monitoring and recommended/non-recommended chemicals monitoring became challenging task.[4], [5] and [6]

The extraction of residues from soil samples are performed by methods using mechanical shaking extraction with organic solvents like acetone. The

analysis of pesticides can be affected by contaminants existing in the matrix. [3] Pesticides can be absorbed or decomposed on chromatographic instruments by this effect.[2] Soil extraction method is important issue because the interaction between the soil and the pesticides is stronger than that in food. Methods for pesticide monitoring in environmental samples has problems that many countries demand greater sensitivity and inexpensive multiresidue methods at the required detection limit. By the public concern, European Union sets the maximum concentration levels of pesticides residue of 0.1 to 0.5. This research wills establish method of pesticide multiresidue analysis on soil and water on GC-ECD/NPD and validate method according to ISO/IEC 17025 and KOLAS guideline. [7], [8] and [9]

II. Materials & Methods

1. Materials & Reagents

The pesticide standards were obtained from Wako Pure Chemical Industries, Ltd., Dr. Ehrenstorfer GmbH, Chem Service, Inc., Sigma-Aldrich Co. Ltd. Standard stock solutions (1000 μg/mL) of these pesticides were prepared in acetone and stored at -20℃. Acetonitrile, acetone, n-hexane, dichloromethane, and methanol were HPLC grade (Burdick & Jackson). Sodium chloride (Samcheon chemical) was extra-pure grade. SPE cartridge (Florisil, 6 mL) was purchased at Phenomenex.

2. Apparatus and Equipment

SR-2w shaker (Taitec, Japan) was used for homogenization. Hanil Combi 408 centrifuge was from Korea. Purification by SPE cartridge was performed in SPE manifold (Pierce). Samples were concentrated by Hurricane-Lite evaporator. (Korea). Agilent 6890 Series gas chromatography system (Agilent Technologies, Palo Alto, USA) with Agilent 7683 series autosampler was used for performing instrumental analysis. NPD with blos bead and micro-ECD detector was used. Column was DB-5 (30 m × 0.25 mm, 0.25 μm) by Agilent technology. Data acquisition and treatment was performed by Agilent Chemstation software.

3. Sampling and Preparation

Sampling, preparation and preservation method of soil and water samples followed to guidelines of Ministry of Environment, and Rural Development

Administration, EPA(EPA Method 1699: Pesticides in water, soil, sediment, biosolids, and tissue by HRGC/HRMS)

Soil control samples were gathered in farmland that has organic farming certification from NAQS (National Agriculture products Quality management Service). Paddy soil was from Bogye, Ansung. Field soil was from Yangseong, Ansung and orchard soil was from Miyang, Ansung. Soil samples were dried in the shade and passed through 2mm sieve.

Water control samples were gathered in Ansung. River water was from Ansung-river, lake water was from Gosam lake, and underground water was obtained in Yangseong. Deionized water prepared by Simens LaboStar water purifier was used for control sample.

4. Development of pesticide multi-residue analysis for soil and water

Pesticide multiresidue analysis method for soil and water was optimized, based on NAQS multiresidue analysis method. By common order of development of pesticide analysis method, instrumental analysis condition was established first and next, preconditioning process was optimized.

4.1. Development of instrumental condition of pesticide multi-residue analysis for soil and water

Analytes were grouped in 8 groups (4 groups for ECD and 4 groups for NPD) by NAQS method. In each groups, mixture of pesticide standards (2μg/mL) were made in acetone. Pesticides which have low selectivity or low sensitivity were investigated by comparison of detected peaks in chromatograms. For low sensitivity pesticides, it was detected by other

detector, considered in the physico-chemical characteristic of pesticides. For low selectivity pesticides, it was detected by other detector or moved to other groups for maximization of peak resolution.

4.2. Development of preparation condition of pesticide multi-residue analysis for soil and water

Pesticide multi-residue analysis preparation method of NAQS was changed for available in soil and water samples. Purification efficiency of SPE cartridge was confirmed and extraction condition, soil moisture condition was optimized so preparation condition of method for soil and water was established.

4.2.1. Confirming the purification efficiency of SPE cartridge

For GC analysis, purification efficiency of SPE cartridge was confirmed. SPE cartridge (florisil, 1 g) was sequentially conditioned by n-hexane 5 mL and acetone/n-hexane (2/8) 5 mL. GC-NPD4 standard mixture (1 ppm) 1mL was loaded into cartridge and eluted by acetone / n-hexane (2/8) 3 mL three times. Solvent was dried up by nitrogen and resolved in 1 mL acetone.

4.2.2. Comparison of soil wetting condition

Four soil wetting method tested and each extracts analyzed with five times concentration but not with purification.

1) Soil 50 g loaded with GC-NPD4 standard mixture extracted with 2N NH_4Cl 30 mL and 2 hours waiting, and added acetonitrile 100 mL.

2) Soil 50 g loaded with GC-NPD4 standard mixture extracted with 2N

NH_4Cl 30 mL and immediately added acetonitrile 100 mL.

3) Soil 50 g loaded with GC-NPD4 standard mixture extracted with distilled water 30 mL and immediately added acetonitrile 100 mL.

4) Soil 50 g loaded with GC-NPD4 standard mixture extracted with only acetonitrile 100 mL.

4.2.3. Comparison of soil extraction solvents

Compared three extraction method and analyzed extracts that with and without SPE purification.

1) Soil 50 g loaded with GC-NPD4 standard mixture extracted with acetone 100 mL

2) Soil 50 g loaded with GC-NPD4 standard mixture extracted with acetonitrile 100 mL

3) Soil 50 g loaded with GC-NPD4 standard mixture extracted with acetonitrile 100 mL and distilled water 30 mL added NaCl 20 g.

4.2.4. Comparison of water extraction solvents

Compared three extraction method and each extracts analyzed with five times concentration but not with purification.

1) Water 50 mL loaded with GC-NPD4 standard mixture extracted with dichloromethane 100 mL and NaCl

2) Water 50 mL loaded with GC-NPD4 standard mixture extracted with ethyl acetate 100 mL and NaCl

3) Water 50 mL loaded with GC-NPD4 standard mixture extracted with acetonitrile 100 mL and NaCl

5. Method validation according to KOLAS guideline

Method validation was carried out using KOLAS-G-015 guideline and calculated validation parameters. Linearity, statistic LOD/LOQ, sensitivity, working range was obtained by analysis of pesticide standard solution chromatogram. Selectivity, trueness & bias, precision, method LOD/LOQ was calculated by recovery test. Ruggedness was evaluated by changing inlet liner and GC column. Developed method was applied with monitoring samples and each pesticide was selected in one detector group. Measurement uncertainty was determined with selected pesticides.

5.1. Linearity and sensitivity

Stock solutions of each group were prepared in acetone at concentrations in 5 μg/mL. The solutions were serially diluted in 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.01, 0.005, 0.002 μg/mL and analyzed by GC-ECD/NPD two times. Processing results performed by Chemstation software. The calibration curves - y axis was average area and x axis was concentration - and calculated the correlation coefficient (R^2). The sensitivity was evaluated as the slope of calibration curve.

5.2. Statistical LOD & LOQ

Statistical LOD & LOQ was obtained by using each instrumental measurement of pesticide mixture standards. Statistical LOQ was defined as concentration that signal-to-noise ratio is more than 10. One third of the LOQ was defined as Statistical LOD.

5.3. Working range

The minimum value of working range was calculated as 'statistical LOQ / 5' (By the method, samples concentrated in five times). The maximum value of working range was obtained using the capacity of detector. The most sensitive pesticide selected and found its peak height in 5 ug/mL. Calculated concentration that each pesticides becomes the peak height by extrapolating calibration curves of each pesticides The maximum value of working range was one fifth of the concentration.

5.4. Trueness & Bias, Precision

The 100-LOQ mixtures (in chromatogram, heights of all pesticides in mixture became as 100 LOQ levels) made using statistical LOQ. This mixture were loaded to 3 soil samples (paddy soil, field soil, orchard) and 4 water samples (distilled water, river, lake, underground water) and recovery test was performed. Calculated the recovery of pesticides and CV (Coefficient of Variance in each sample, each pesticides. Trueness & bias determined as average recovery and precision as CV value.

5.5. Selectivity

Compared control soil, water sample with recovery sample chromatogram and confirmed whether pesticide peak is overlaid on matrix peak or other pesticide peaks.

5.6. Method LOD & LOQ

Method LOD was evaluated with the result of seven repeated recovery test. Standard deviation of concentration of recovery test set up into s_0 and average

sample blank called b. Method LOD was defined as b + $3s_0$. Method LOQ was defined as 3 times of Method LOD.

5.7. Robustness

GC inlet liner and column was altered. Ruggedness of column was evaluated by analysis of ECD-3 group mixture with DB-5, ZB-5, DB-17 three columns. Ruggedness of inlet liner was estimated by analysis of ECD-3 group mixture with single taper split liner with glass wool, single taper splitless liner, and single taper splitless liner with glass wool (Agilent technologies), focus liner (Phenomenex). Checked chromatograms and evaluated ruggedness by considering peak shape, height, resolution.

5.8. Measurement uncertainty

Measurement uncertainty was calculated according to KOLAS and EURACHEM guideline (Quantifying Uncertatinty in Analytical Measurement). Measurement uncertainty data is ‘result $\pm$ uncertainty' form so it was calculated by monitoring sample results. Monitoring samples for the whole country land and water was analyzed by developed method and choosed one pesticides that detected in high concentration. In ECD, endosulfan sulfate was selected and NPD pesticide was chlorpyrifos. Based on NAQS measurement uncertainty estimation method, making standard solution, purity of pesticide standard, linearity was considered for determining uncertainty.

III. Result & Discussion

1. Establishment of instrumental parameters for simultaneous multiple pesticide residue analysis method in soil and water

NAQS (National agricultural products quality management service) analyze 172 pesticides by using GC-ECD and GC-NPD as shown in the Table 1.

	μ-ECD (Agilent 6890)	NPD, blos bead (Agilent 6890)
Column	DB-5 (30 m × 0.25 mm, 0.25 μm)	
Inlet	Temperature : 250℃ 1 μL split ratio 50 : 1	Temperature : 250 ℃ 1 μL splitless
Detector	Temperature : 320 ℃ Make up(N2) : 60 mL/min	Temperature : 320 ℃, H2 : 3.5 mL/min Air : 120 mL/min, Make up(N2) : 5 mL/min
Oven	80℃(2 min hold) → 10℃/min → 200℃ → 2℃/min → 220℃(4 min hold) → 10℃/min → 300℃(9 min hold) ※ Total run time: 45min	

Table 1. Instrumental parameter for NAQS's simultaneous multiple pesticide analysis

If these pesticides are analyzed at once, there will be many pesticedes which have similar retention time. NAQS separate 172 pesticides into 4 groups of NPD and 4 groups of ECD, in total 8groups, to analyze more efficiently for pesticide's peaks. (Table. 2)

Group	Pesticides	No.
GC-ECD1	Anilofos, Bromopropylate, Carbophenothion, Chlorfenvinphos, Chlorfluazuron, Chlorobenzilate, Cyflufenamid, Cyhalothrin(lambda), Deltamethrin, Dicloran, Dicofol, Dimethenamid, Disulfoton, Etrimfos, Fenpropathrin, Fenvalerate, Flutolanil, Folpet, Halfenprox, Heptachlor(Heptachlor epoxide), Lufenuron, Oxadiazon, Oxyfluorfen, Paclobutrazole, Parathion-methyl, Permethrin, Propiconazole, Triflumuron, Trifluralin	29
GC-ECD2	Aldrin, Azoxystrobin, Bifenthrin, Captan, Chlorfenapyr, Clofentezine, Dieldrin, Difenoconazole, Endosulfan(α,β and sulfate), Flucythrinate, Imazalil, Indanofan, Metobromuron, Metribuzin, Mevinphos, Penconazole, Probenazole, Prochloraz, Procymidone, Prometryn, Simazine, Simeconazole, Tefluthrin, Tetraconazole, Tetradifon, Thifluzamide, Zoxamide	27
GC-ECD3	Acrinathrin, BHC(α, β, γ and δ), Bromobutide, Butachlor, Cyfluthrin, Dichlofluanid, Dithiopyr, Ethion, Fenamidone, Fenoxanil, Fipronil, Fthalide, Indoxacarb, Iprodione, Isoprothiolane, Kresoxim-methyl, Mefenacet, Metolachlor, Nuarimol, Piperophos, Pyridalyl, Quintozene(Pentachloroaniline), Tolylfluanid, Triadimenol	24
GC-ECD4	Alachlor, Bifenox, Chinomethionat, Chlordane(cis, trans), Chlorothalonil, Cypermethrin, DDT, Diclofop-methyl, Endrin, Ethalfluralin, Etridiazole, Fenarimol, Flufenoxuron, Mecarbam, Methoxychlor, Methyl-pentachlorophenyl sulfide, Ofurace, Pirimiphos-ethyl, Propanil, Pyridaben, Pyridaphenthion, Pyrimidifen, Thiobencarb, Tralomethrin, Triadimefon, Vinclozolin	25
GC-NPD1	Dichlorvos(DDVP), Methabenzthiazuron, Terbuthylazine, Iprobenfos, Simetryn, Metalaxyl, Terbutryn, Malathion, Parathion, Pendimethalin, Triflumizole, Hexaconazole, Myclobutanil, Cyproconazole, Edifenphos, Etoxazole, Pyraclofos, Furathiocarb	18
GC-NPD2	Azinphos-methyl, Bitertanol, Buprofezin, Cadusafos, Chlorpropham, Diniconazole, Diphenamid, Fenamiphos, Fenitrothion(MEP), Iprovalicarb, Isofenphos, Methidathion, Molinate, Phosphamidon, Prothiofos, Tebufenpyrad, Terbufos, Thiazopyr	18
GC-NPD3	Chlorpyrifos-methyl, Diazinon, Dimepiperate, Diphenylamine, Fenbuconazole, Fenothiocarb, Fenthion(MPP), Flusilazole, Fosthiazate, Metconazole, Phorate, Phosalone, Pirimiphos-methyl, Profenofos, Pyriminobac-methyl(E, Z), Tebuconazole	16
GC-NPD4	Chlorpyrifos, Cyprodinil, Dimethoate, EPN, Esprocarb, Ethoprophos, Fenazaquin, Fludioxonil, Mepronil, Napropamide, Phenthoate, Pyrazophos, Tebupirimfos, Tolclofos-methyl, Triazophos	15
Total		172

Table 2. List of the NAQS's GC analysis pesticides (172pesticides)

Add three more pesticides (pyrifluquinazon, sulfoxaflor, penthiopyrad) on method mentioned above and rearranged pesticides group to improve selectivity and sensitivity.

Disulfoton($C_8H_{19}O_2PS_3$) is a organophosphorous insecticide and classified in ECD-1 group in existing analytical method. But in ECD-1 group standard chromatogram, disulfoton showed much smaller peak compare to the others and it also overlaid with etrimfos peak. This pesticide has P on its structure(Figure 1.), it is assumed that NPD detector can detect this pesticide. Experiment showed that disulfoton showed much higher sensitivity when it moved to the NPD-4 group. (Figure 2.)

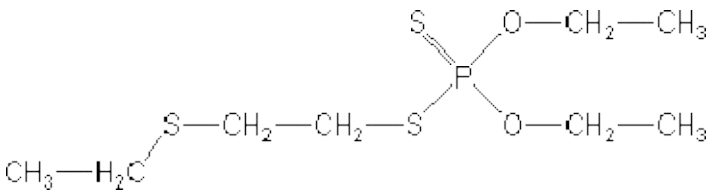
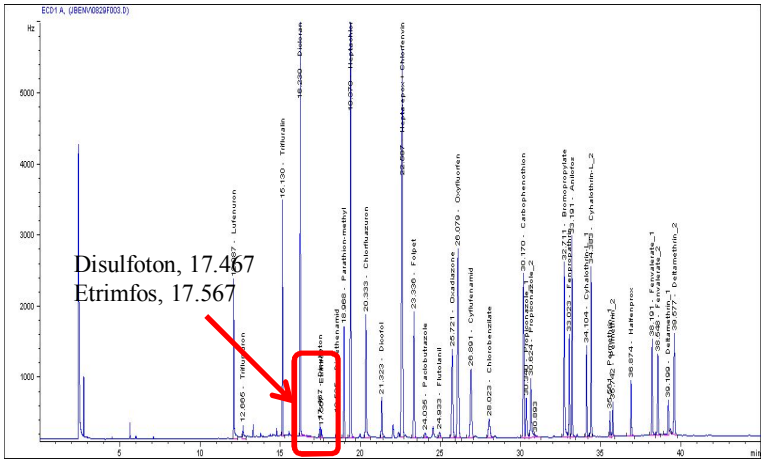
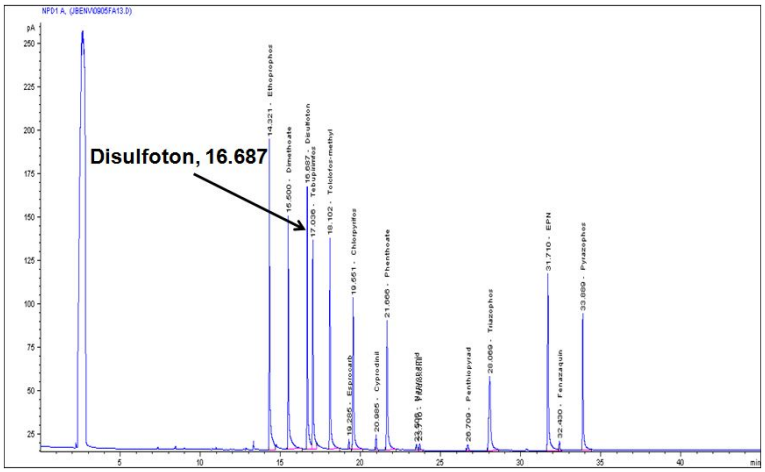


Figure 1. Chemical Formulation of disulfoton



(a)

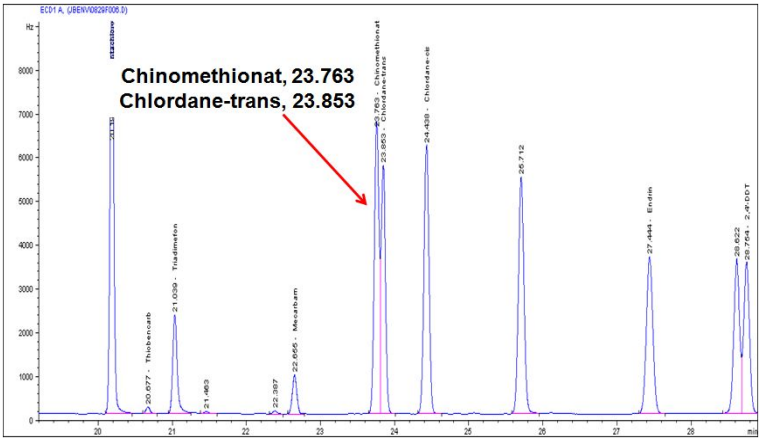


(b)

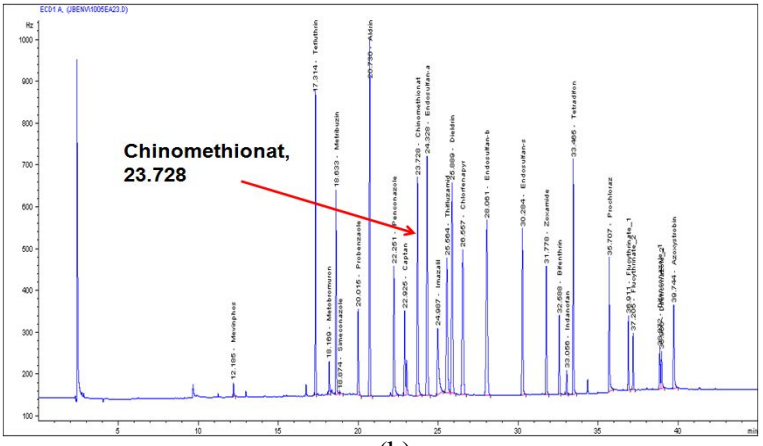
Figure 2. Chromatogram of Disulfoton in (a) ECD-1

(b) NPD-4

Chinomethionat ($C_{10}H_6N_2OS_2$) is a quinoxaline fungicide and is belong to the ECD-4 group. But in ECD-4 standard chromatogram, it's hard to analyze accurately because it doesn't separate perfectly with trans-chlordane peak. By moving to ECD-2 group, solved the overlay with other peak and improved selectivity. (Figure 3.)



(a)



(b)

Figure 3. Chromatogram of Chinomathionat in (a) ECD-4

(b) ECD-2 Chromatogram

The similar way as mentioned above, 12 pesticides are rearranged to new group based on improving sensitivity and selectivity or addition of new analytic pesticide and it's described in Table 3. Instrumental parameter of final established analytical method is described on Table 4. Some pesticides moved to the LC-UVD because they showed better sensitivity and selectivity when it's analyzed in LC-UVD than GC.

Compound	Group before	Group after	Remarks
Chinomethionat	ECD4	ECD2	Peak overlap
Diphenamid	NPD2	NPD1	Peak overlap
Disulfoton	ECD1	NPD4	Sensitivity
Etrimfos	ECD1	NPD1	Sensitivity
Mepronil	NPD4	NPD3	Peak overlap
Penthiopyrad	Newly added	NPD4	
Pirimiphos-ethyl	ECD4	NPD2	Sensitivity
Procymidone	ECD2	ECD4	Peak overlap
Prometryn	ECD2	NPD2	Sensitivity
Pyrifluquinazon	Newly added	ECD1	
Sulfoxaflor	Newly added	ECD4	
Tetraconazole	ECD2	ECD3	Peak overlap

Table 3. List of compounds which changed the group

Group	Pesticides	No.
GC-ECD1	Anilofos, Bromopropylate, Carbophenothion, Chlorfluazuron, Chlorobenzilate, Cyflufenamid, Cyhalothrin(lambda), Deltamethrin, Dicloran, Dicofol, Dimethenamid, Fenpropathrin, Fenvalerate, Flutolanil, Folpet, Halfenprox, Heptachlor(Hepatchlor epoxide), Lufenuron, Oxadiazone, Oxyfluorfen, Parathion-methyl, Permethrin, Propiconazole, Pyrifluquinazon, Triflumuron, Trifluralin	26
GC-ECD2	Aldrin, Azoxystrobin, Bifenthrin, Captan, Chinomethionat, Chlorfenapyr, Dieldrin, Difenconazole, Endosulfan(α,β and sulfate), Flucythrinate, Imazalil, Indanofan, Metobromuron, Metribuzin, Mevinphos, Penconazole, Probenzole, Prochloraz, Simeconazole, Tefluthrin, Tetradifon, Thifluzamid, Zoxamide	23
GC-ECD3	Acrinathrin, BHC(α,β,γ and δ), Bromobutide, Butachlor, Cyfluthrin, Dichlofluanid, Dithiopyr, Ethion, Fenamidone, Fenoxanil, Fipronil, Fthalide, Indoxacarb, Iprodione, Isoprothiolane, Kresoxim-methyl, Mefenacet, Nuarimol, Piperophos, Pyridalyl, Quintozene(Pentachloroaniline), Tetraconazole, Tolyfluanid, Triadimenol	24
GC-ECD4	Alachlor, Bifenox, Chlordane(cis, trans), Chlorothalonil, Cypermethrin, DDT, Diclofop-methyl, Endrin, Ethalflualin, Etridiazole, Fenarimol, Mecarbam, Methoxychlor, Methyl-pentachlorophenyl sulfite, Ofurace, Procymidone, Propanil, Pyridaben, Pyridaphenthion, Pyrimidifen, Sulfoxaflor, Thiobencarb, Tralomethrin, Triadimefon, Vinclozolin	24
GC-NPD1	Cyproconazole, Dichlorvos(DDVP), Diphenamid, Edifenphos, Etoxazole, Etrimfos, Hexaconazole, Iprobenfos, Malathion, Methabenzthiazuron, Myclobutanil, Parathion, Pendimethalin, Pyraclofos, Simetryn, Terbuthylazine, Terbutryn, Triflumizole	18
GC-NPD2	Azinphos-methyl, Bitertanol, Buprofezin, Cadusafos, Chlorpropham, Fenamiphos, Fenitrothion(MEP), Iprovalicarb, Isofenphos, Methidathion, Molinate, Phosphamidon, Pirimiphos-ethyl, Prometryn, Prothiofos, Tebufenpyrad, Terbufos, Thiazopyr	18
GC-NPD3	Chloropyrifos-methyl, Diazinon, Dimepiperate, Diphenylamine, Fenbuconazole, Fenothiocarb, Fenthion(MPP), Flusilazole, Fosthiazate, Mepronil, Metconazole, Phorate, Phosalone, Pirimiphos-methyl, Profenofos, Pyriminobac-methyl(E), Tebuconazole	17
GC-NPD4	Chlorpyrifos, Cyprodinil, Dimethoate, Disulfoton, EPN, Esprocarb, Ethoprophos, Fenazaquin, Fludioxonil, Napropamide, Penthiopyrad, Phenthoate, Pyrazophos, Tebupirimfos, Tolclofos-methyl, Triazophos	16
Total		166

Table 4. Established groups and its components

2. Establishment of sample preparation for simultaneous multiple pesticide residue analysis method in soil and water

Sample preparation procedure for multiple pesticide residue analysis among agriculture product is as follows ; weigh 50g of sample, extract by acetonitrile, homogenize for 3minutes and add 20-30g of NaCl. Centrifuge for 30minutes and concentrate 10ml of the supernatant. Dissolved to 1ml and clean up by using florisil SPE cartridge and then analyze by instrument. All mentioned above is diagramed in Figure 4.

. The development of analysis method runs opposite order to the real analysis order. Confirm the clean-up efficiency of SPE cartridge and establish extraction and soil wetting.

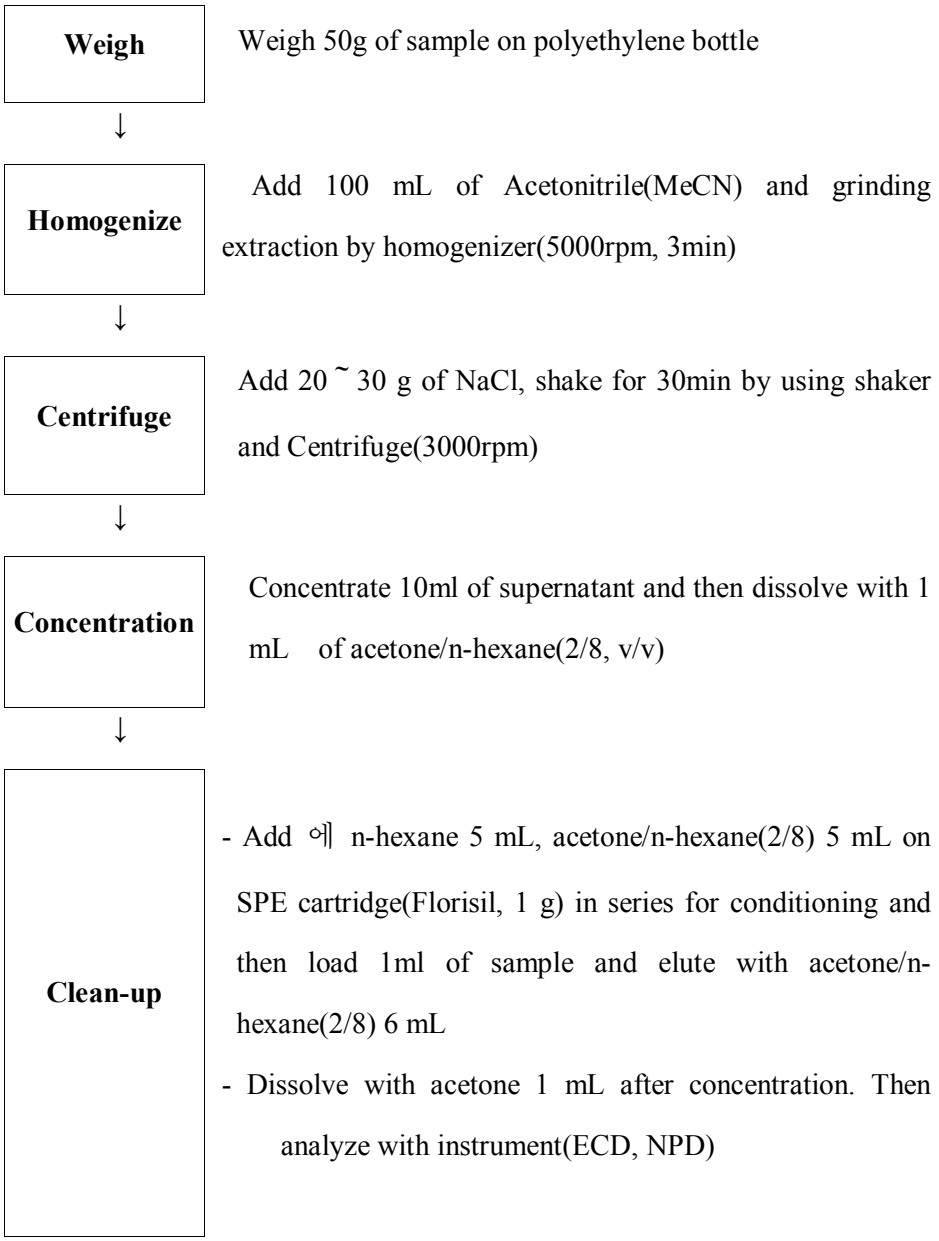


Figure 4. NAQS multiple residue pesticide analysis method

2.1 Confirmation on clean-up efficiency of SPE cartridge

For the GC analysis, confirm the clean-up efficiency of florisil SPE cartridge. Add n-hexane 5 mL, acetone/n-hexane(2/8) 5 mL on SPE cartridge(Florisil, 1 g) in series for conditioning, after that load 1mL of GC-NPD4 standard solution(1 ppm). Elute 3times with acetone/ n-hexane(2/8), 3 mL for each time. Elute samples were concentrated with nitrogen and dissolved with 1mL of acetone. The result of recovery is described on Table 5. Except dimethoate, result showed that 6mL elution is enough for the method.

Recovery (%)	0-3 mL	3-6 mL	6-9 mL	Recovery
Ethoprophos	38.1			38.1
Dimethoate	34.0	26.7	25.3	86.0
Disulfoton	54.9			54.9
Tebupirimphos	78.0			78.0
Tolclofos-methyl	78.9			78.9
Esprocarb	94.8			94.8
Chlorpyrifos	97.6			97.6
Cyprodinil	111.7			111.7
Phenthoate	102.4			102.4
Napropamid	115.8			115.8
Fludioxonil	114.8			114.8
Penthiopyrad	114.3			114.3
Triazophos	117.6			117.6
EPN	117.5			117.5
Fenazaquin	115.2			115.2
Pyrazophos	116.0			116.0

Table 5. Recovery by elution step of pesticide standard(GC-NPD4)

2.2 Optimization of soil wetting

To apply agricultural product analysis method to the soil, the experiment called ‘wetting’ has to be added on the method. Soil particle has ions on itself, so it stick substance together based on electrostatic attraction. In case of polarized pesticide, efficiency of extraction would be very low without any process because it will be taken hold for the soil particle. By adding high concentration of ionized solution like NH_4Cl, it reduces the electrostatic attraction and it leads to the higher efficiency on extraction. This is called ‘soil wetting’..

To compare the extraction method, few experimental held based on experiment using 2N NH_4Cl. Recovery test is proceeded with paddy soil 50g spike by GC-NPD4 stock solution. In this experiment, 4 different extraction method is used ; Extract by adding 2N NH_4Cl 30 mL and swelling for 2 hours. Extract by adding 2N NH_4Cl 30 mL and without swelling. Instead of NH_4Cl, extract by adding diluted water 30 mL and NaCl 20 g without swelling. Extract without add nothing. The Recovery of each extraction is arranged on Table 6.

There’s no big difference in recovery between swelling with NH_4Cl and diluted water. Shortening the analysis time is more efficient method, so without water and without swelling method is chosen for the optimized parameter.

Recovery (%)	①	②	③	④
Ethoprophos	114.9	117.3	95.3	116.2
Dimethoate	94.3	110.3	95.1	115.4
Disulfoton	32.7	66.8	57.2	76.1
Tebupirimphos	73.6	91.3	81.5	105.7
Tolclofos-methyl	79.7	91.7	83.6	104.8
Esprocarb	85.4	97.8	91.5	111.4
Chlorpyrifos	89.5	106.0	98.8	122.8
Cyprodinil	101.4	111.5	107.4	121.7
Phenthoate	86.3	110.7	104.0	133.1
Napropamid	117.6	126.9	121.2	141.5
Fludioxonil	107.3	114.0	111.1	127.4
Penthiopyrad	102.2	115.3	111.0	136.8
Triazophos	114.8	136.0	128.5	162.7
EPN	125.7	148.0	140.1	172.8
Fenazaquin	103.8	116.7	110.0	133.3
Pyrazophos	110.6	131.5	123.5	156.3

Table 6. Comparing soil wetting on extraction(GC-NPD4)

2.3 Optimization for Soil extraction method

Selection of extraction solvent is referred on soil residue analysis method for herbicide (2009), soil residue analysis method for insecticide (2010), soil residue analysis method for fungicide (2011) published by NAQS and other many paper. All these materials showed that pesticide analyze by GC is generally extracted by acetone and acetonitrile. However by adding saturated NaCl 30mL, it will eliminate polarized matrix due to the salting out effect. And this will lead to a better peak shape..

To find out suitable extraction condition, 3 different experiment were held ; Extract by 100mL of acetone. Extract by 100mL of acetonitrile. Extract by 100mL of acetonitrile and 30mL of saturated NaCl. Also it analyzed before and after clean-up of SPE cartridge and compared efficiency of extraction. In result, acetonitrile showed better recovery than acetone and in case of adding saturated NaCl showed higher recovery. (Table 7.)

Recovery(%)	Before purification			After purification		
	①	②	③	①	②	③
Ethoprophos	78.2	135.3	144.0	62.4	85.0	90.0
Dimethoate	147.8	164.8	155.4	37.4	41.2	42.8
Disulfoton	53.2	76.3	99.9	44.3	43.4	55.8
Tebupirimphos	76.3	110.8	115.1	69.4	81.8	91.9
Tolclofos-methyl	86.2	107.6	112.2	77.8	81.4	92.4
Esprocarb	95.6	116.4	113.1	91.6	95.9	103.8
Chlorpyrifos	104.8	125.5	123.1	96.4	100.6	111.1
Cyprodinil	114.8	119.9	119.8	111.2	107.1	122.4
Phenthoate	111.2	132.2	127.9	92.7	103.7	116.1
Napropamid	130.3	136.3	137.4	113.4	117.1	132.1
Fludioxonil	118.6	119.8	125.6	98.9	110.4	121.1
Penthiopyrad	123.6	129.0	129.0	102.2	112.8	125.1
Triazophos	137.8	145.2	149.2	106.7	122.5	140.9
EPN	134.4	135.9	143.2	109.6	128.6	142.1
Fenazaquin	119.0	119.2	124.6	100.7	114.6	126.7
Pyrazophos	129.1	129.4	138.7	96.6	124.4	135.9

Table 7. Recovery of extraction solvent before and after soil clean-up (GC-NPD4)

2.4 Optimization for water extraction method

In case of water, experiment proceeded without swelling. By adding 30mL of saturated NaCl can lead to better separation effect but it give big difference on volume of the sample. So add 20g of NaCl instead of 30mL of saturated NaCl.

Extracting pesticide on water by using organic solvent is same meaning as liquid-liquid extraction. Thus dichloromethane (DCM), ethyl acetate (EA), acetonitrile is used for comparing efficiency of extraction which is well used in liquid-liquid extraction. On 50mL of water, NPD-4 standard solution was spiked and three different organic solvent is used. Extracted by adding 100mL of organic solvent and 20g of NaCl. To prevent interference on clean-up step and to achieve pure efficiency of extraction solvent, concentrated 5 fold without clean-up. (On the procedure of clean-up, 5 fold concentrations are followed). The result is showed on Table 8.

Pesticides in case of early retention time showed low extraction efficiency. This is because polarized pesticides which run through column fast combine with water and it is hard to be extracted. Ethyl acetate which is much higher in polarity compared to acetonitrile showed higher recovery than acetonitrile but it wasn't meaningful. Beside in the screening analysis method which recovery standard is 50~150%, acetonitrile has much higher amount of pesticides suitable for the 50~150%. This research is based on combination with analysis of agricultural product, so if there's no big difference acetonitrile extraction method is suitable.

To find out if there's a difference for highly polarized pesticides which show low recovery on detector, 3 repetition recovery test was proceeded by

using GC-NPD4 and GC-ECD3 standard solution spiked on water. In result, pesticides analyze by ECD also showed similar result. But in the additional experiment, pesticides analyze by LC didn't show the same result. (Data not shown)

Recovery(%)	Acetonitrile	Dichloromethane	Ethyl acetate
Ethoprophos	52.3	5.4	41.2
Dimethoate	106.2	73.5	73.4
Disulfoton	17.2	12.9	21.0
Tebupirimphos	51.2	37.9	61.2
Tolclofos-methyl	40.9	39.0	53.4
Esprocarb	66.0	73.5	78.1
Chlorpyrifos	58.0	80.1	79.3
Cyprodinil	88.7	121.0	106.4
Phenthoate	85.9	106.7	105.1
Napropamid	99.2	138.9	122.7
Fludioxonil	91.6	127.7	113.3
Penthiopyrad	95.2	126.4	116.2
Triazophos	105.9	151.5	133.8
EPN	109.3	146.7	128.5
Fenazaquin	94.3	129.3	112.4
Pyrazophos	100.7	142.8	117.0

Table 8. Recovery in distilled water by extraction solvent (GC-NPD4)

3. Confirmation of method validation based on KOLAS-G-015

Adjustment can't be followed only with development of the method. Method validation has to be follow to validate it is suitable for the purpose of the experiment so customer can use this method with no doubt. (KOLAS) Method validation is proving that they are able to provide sufficient data for accomplishing object of analysis when method is applied. (ICH, AOAC)

In detail, confirming method validation means calculating performance parameters of the analysis method. ISO/IEC 17025 presents brief standardization of these performance parameters. (IUPAC) At this moment, evaluation has to be followed by not only calculating figures but also comparing other qualification required by other organization. (EURACHEM)

There're several guidelines for the confirmation of method validation. For example CODEX is the international food standard. In USA FDA is guideline for the medicine. In our nation, it is well organized in KOLAS-G-015. Method validation has to choose one guideline (or more) and perform.

In this experiment, confirmed the validation by following KOLAS guide line under the consultation with NAQS, also review related guideline like CODEX, SACO which has connection to the agricultural product.

3.1 Linearity, Sensitivity

As R-squared is closed to the 1, linearity is high. Generally in screening analysis method, it is recommended that R^2 has higher than 0.99 and in component analysis it has to be at least 0.997. The mixture of standard was analyzed in each concentration and calculate calibration curve. By using this cline of calibration curve and R-squared(R^2) is identified. (Table 9.) In most

of the pesticides show linearity which is higher than 0.99 R-squared but phosphamidon, iprovalicarb, fosthiazate showed lower than 0.99 in R-squared.

Coefficient of Determination (R^2)	Number of Pesticides	Ratio (%)
< 0.99	3	1.8
0.99 ~ 0.997	11	6.6
> 0.997	152	91.6
Total	166	100.0

Table 9. Linearity range of pesticides

Sensitivity	Ratio of Pesticide ECD (%)	Ratio of Pesticide NPD (%)
0 - 25	0.8	29.2
25 - 50	1.6	16.7
50 - 100	4.1	8.3
100 - 500	26.2	19.4
500 - 1000	21.3	26.4
1000 - 2000	27.0	0.0
2000 - 3000	14.8	0.0
3000 -	4.1	0.0
Total	100	100

Table 10. Sensitivity range of pesticides

Sensitivity is ratio of the changed response based on change of the concentration of the analytic substance. Very little substance in the sample can be detected if sensitivity is very high. High sensitivity stands for better analysis method. Sensitivity can be defined by cline of the calibration curve. Sensitivity of each pesticides are organized in Table 10.

3.2 Statistical LOD/LOQ

LOD (Limit of Detection) means concentration which can be detected but not be possible to quantify. (ICH) Lower than LOD is marked as 'not detected' if it is detected on the chromatogram. LOQ (Limit of Quantitation) is the concentration which is the lowest level that can be quantified. Therefore lower than LOQ only can be marked as 'detected' and not be able to report the certain concentration.

LOD and LOQ are the important parameter which can show how accurate they can analyze. So it is very needed in analysis of trace analysis. For example, if MRL is 0.05 μg/mL, this method has to analyze lower than 0.05 μg/mL.

KOLAS give differ between instrumental LOD/LOQ and MDL (Method detection limit). In instrumental LOD/LOQ, it depends on the ratio of S/N (signal to noise). If S/N ratios is 10 than it is defined as statistical LOQ (3 in LOD). Pesticides are analyzed in each different concentration and statistical LOD/LOQ is calculated. (Table 11.)

(ppm)	ECD1	ECD2	ECD3	ECD4	NPD1	NPD2	NPD3	NPD4
<0.01	-	-	5	-	2	2	5	5
0.01-0.05	16	12	8	13	4	5	2	5
0.05-0.1	6	6	13	8	5	4	2	
0.1-1	10	8	5	10	7	6	9	6
>1	1	1	-	-	1	2	-	-

Table 11. Number of pesticides that its statistical LOQ contained in each concentration range

3.3 Working Range

In analysis method quantification proceeded in the assumption that calibration curve is linear. But calibration curve can't maintain linear endlessly. There's a limitation on detector to detect. When it reached to certain concentration, detector is saturated and can't increase the signal as the usual. (Figure 5.)

So certain range which is applicable for the analysis method has to elucidate while on the process of validation. Sample has to be concentrated or diluted if they are not fit to the range. Working range can be defined as the concentration's range which can achieve certain typical degree of the uncertainty of measurement in the result of experiment. Based on KOLA and other guide lines, and agreement with NAQS, minimum and maximum values are determined.

Minimum value of the Working range is set to the 1/5 of the statistical. In the analysis method, concentration factor is 5 so at the last, it is divided by 5. LOQ is the minimum concentration which can quantify so it' s reasonable to use it as the minimum value of the working range. Maximum value of the working range is related to the saturated detector' s signal value. And it is assumed that it does not affect to the pesticide. For example, if calibration curve can' t maintain the linearity in the typical concentration, that concentration becomes the maximum value of the working range for that pesticide. Working range for the each pesticide are arranged in Table 12.

GC-ECD1	Working range (mg/kg)	
	Lower limit	Upper limit
Lufenuron	0.002	3.019
Triflumuron	0.100	29.461
Trifluralin	0.002	2.798
Dicloran	0.002	1.488
Dimethenamid	0.020	26.796
Parathion-methyl	0.004	5.368
Heptachlor	0.002	1.678
Chlorfluazuron	0.004	4.092
Dicofol	0.010	16.993
Heptachlor epoxide	0.002	2.022
Folpet	0.020	4.349
Flutolanil	0.100	140.804
Oxadiazone	0.005	7.015
Oxyfluorfen	0.004	3.530
Cyflufenamid	0.010	9.138

Table 12. Working ranges of some selected pesticides

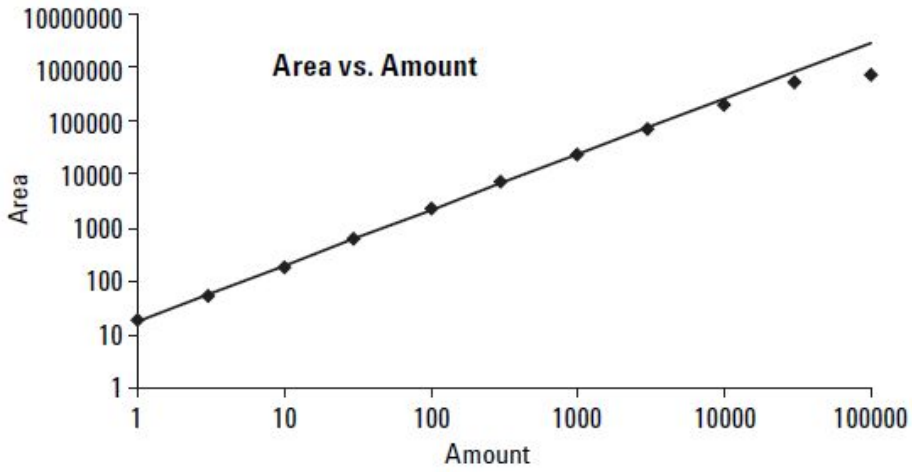


Figure 5. Plot of area versus amount with both axes scaled logarithmically

(This figure is from Agilent technical note 5989-3423EN)

3.4 Trueness & Bias, Precision

Trueness is the how close measured quantity is compared to the accepted reference value. Bias is the Quantification of the Trueness. If bias decreases, trueness increases. Analyzing CRM is the best way to measure the Trueness. But if there's no CRM, it can be measured by recovery test.

Guide line for general single pesticide analysis method in NAQS and KFDA(Ministry of food and drug safety) is 70~120%. In screening method, it have to cover 50~150%.

Precision proximity among experiment's result's which is repeatedly performed under the designated condition. Precision is generally present as the Relative standard deviation(CV). In this experiment, under the agreement with NAQS, CV of the 7 repeated recovery tests is used as the precision. Acceptable CV is 20% in the single pesticide analysis method, but in the screening analysis method it is up to 30%. The number of the pesticide which satisfied trueness, bias and precision surveyed in each sample is as followed.

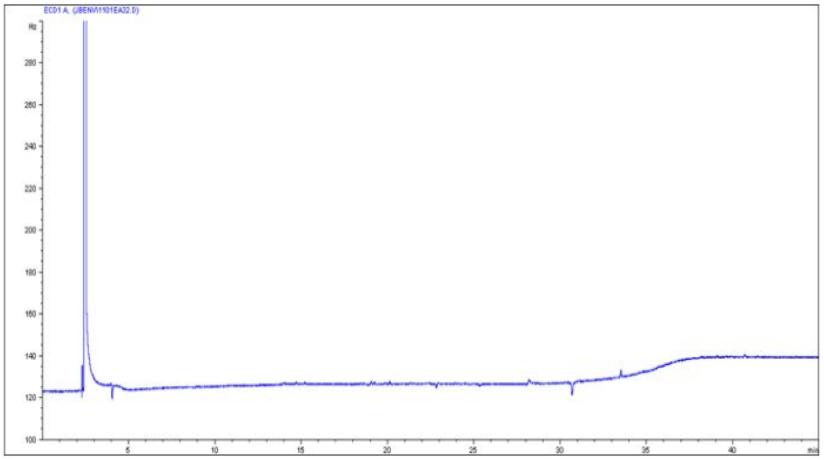
Most of the pesticides were satisfied but some polarized pesticide didn't fit into the standard. With pesticide with low recovery showed low repeatability.

	Soil			Water			
(%)	Paddy	Field	Orchard	DW	River	UG	Lake
ECD-1	29	30	25	29	29	31	28
ECD-2	24	18	22	20	24	19	24
ECD-3	23	26	28	22	26	27	15
ECD-4	26	26	25	18	25	22	23
NPD-1	4	16	16	18	17	12	16
NPD-2	11	10	14	7	8	6	14
NPD-3	15	15	15	14	14	13	16
NPD-4	13	14	13	8	12	8	10
Ratio	74.7	79.9	81.4	70.1	79.9	71.1	75.3

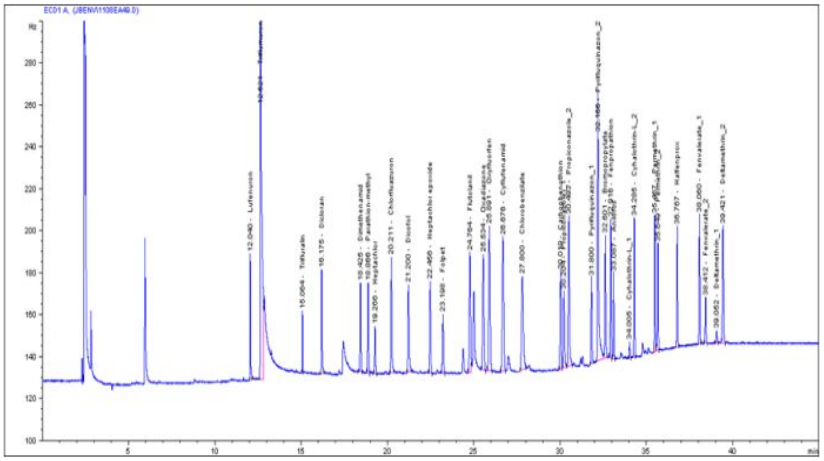
Table 13. Ratio of pesticides satisfying both recovery and precision condition. (DW : Distilled Water. UG : Underground Water)

3.5 Selectivity

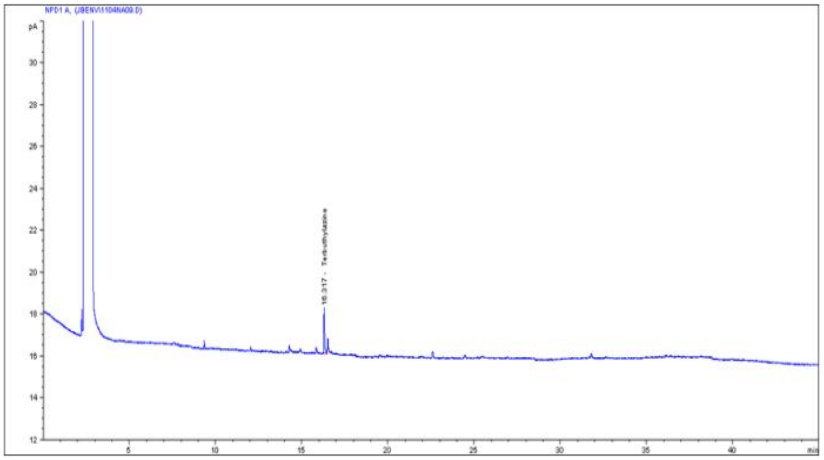
Selectivity is the accuracy of the measurement when there's interference. It is the parameter that how well target peak is separated from the other peak. If there's overlay between other peaks, it is impossible to quantify or qualify accurately. Before the instrumental analysis, some pesticides move the group to increase the selectivity. Each blank chromatogram is shown as followed. There's almost no peak on water. In soil, there' some peaks but didn't overlaid with pesticide. (Figure 6.)



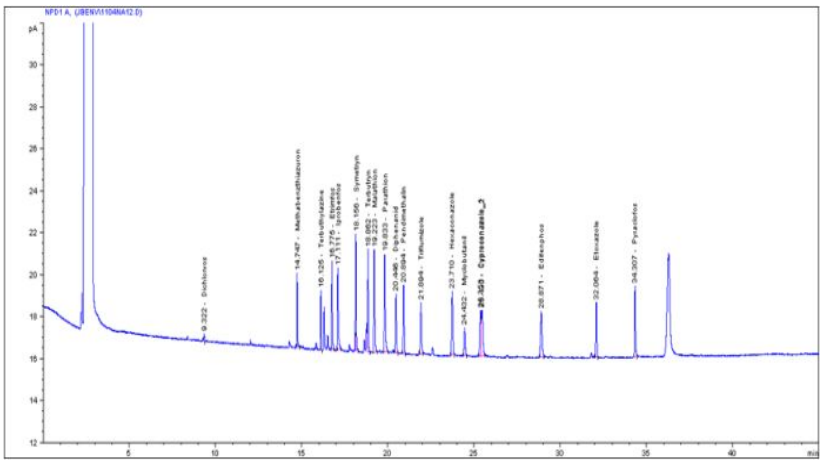
(a) Chromatogram of river control



(b) Chromatogram of river control with spiked ECD-1



(c) Chromatogram of field soil control



(d) Chromatogram of field soil control with spiked NPD-1

Figure 6. Some chromatograms for estimate selectivity

3.6 Method LOD & LOQ

In KOLAS, Method detection limit is the minimum value or the minimum concentration which give differ from 0. So LOD is the lowest value among MDL and uncertainty of measurement when method detection limit is bigger than uncertainty of measurement. (Taylor, 1989) Also LOQ is defined as minimum concentration which can be decided under suitable degree of uncertainty of the measurement. And it is noticed that LOQ is defined a s 3 times of the LOD.

There's some difference between guide lines but commonly statistical principle using RSD of the repeatable experiment is conducted for assumption of LOD.

Under KOLAS standard, 7repeat was performed on the concentration of 2LOQ and 3 times of s_0 is calculated as the MDL. And MQL is 3 times of the MDL. Concentration factor is the 5, so MDL, MQL divided by 5 is used as the result. (Table 14.)

But there's a different between method or meaning from this experiment and experiment suggested from NAQS or MFDS. Therefore it is hard to use it as the validation factor. Value of LOD/LOQ obtained from statistical principle is fit to the definition but not intuitive. Thus guide line like SANCO suggest more realistic standard. It defines LOQ as minimum concentration which satisfied precision and repeatability and exclude LOD from the performance character. If certification institution like KOLAS and regulatory agency which deal with real actual affair KOLAS make settlement and conduct proper method, than it will be possible to validate effectively and practically.

	Soil			Water			
mg/kg	Paddy	Field	Orchard	DW	River	UG	Lake
ND	3	4	3	9	5	5	2
<0.01	41	44	54	32	37	31	17
0.01-0.05	69	74	69	70	57	68	71
0.05-0.1	26	21	24	22	28	35	32
0.1-1	49	45	39	55	60	46	69
>1	6	6	5	6	7	9	3

Table 14. Number of pesticides that its method LOQ is contained in each concentration and each sample

3.7 Measurement uncertainty

Uncertainty of the measurement is defined as parameters that specialized distribution of the value which can affect to the measured quantity under acceptable reason. Unlike other method validation parameters uncertainty of the measurement is the factor of the experimental result, not the experimental method. But uncertainty occurred from experimental method is considered as the important parameter which present for the effect of the experimental method.

From the detected pesticide in the monitoring samples which is collected from 9 provinces is calculated for the uncertainty of the measurement. Detailed calculating method is referred from NAQS and Quantifying Uncertainty in Analytical Measurement of EURACHEM. By analyzing factors like making of stock solution, sample preparation, linearity, repeatability influence of these factors to the measured value is calculated. Under the agreement with NAQS, uncertainty of the measurement is calculated for one pesticide for each detector. (endosulfan sulfate(GC-ECD), chlorpyrifos(GC-NPD)) Results are shown as followed.

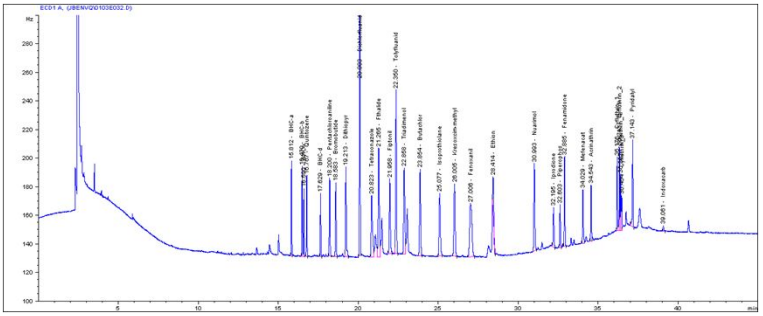
	Measure	Standard uncertainty	RSD	Degree of Freedom
Preparation	0.200	0.001836	0.009179	5952.98
Standard	1.000	0.010141	0.010143	471.27
Repeatability	1.000	0.031742	0.029171	18
Calibration curve	166.21	2.532336	0.015236	4
LOQ	0.05	0	0	∞
Combined	1.662	0.059229	0.03564	30.03
K value of 95% confidential range				2.042
Expanded standard uncertainty				0.121

Table 15. Measurement uncertainty of endosulfan-sulfate

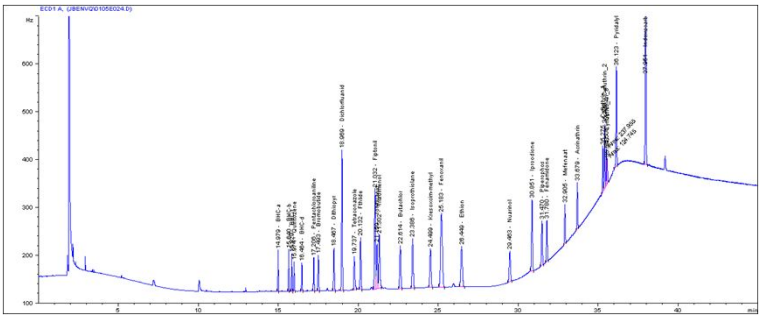
3.8 Ruggedness

3.8.1. Ruggedness from GC column

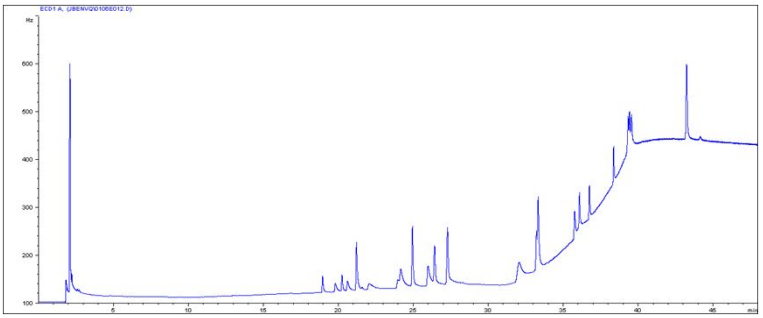
For evaluation of the GC column, 2 columns are used to analyze GC-ECD3. ZB-5 which has same as the DB-5((5%-Phenyl)-methylpolysiloxane) which is now using for the experiment and DB-17((5%-Phenyl)-methylpolysiloxane) which is more polarized compare to the DB-5 are compared for the ruggedness. Results showed that ZB-5 presented similar separation with DB-5 but DB-5 (30 m × 0.25 mm, 0.25 μm) showed much better sensitivity. But DB-17 showed way much different pattern compare to the others.



(a) ECD3 chromatogram with DB-5 column



(b) ECD3 chromatogram with ZB-5 column

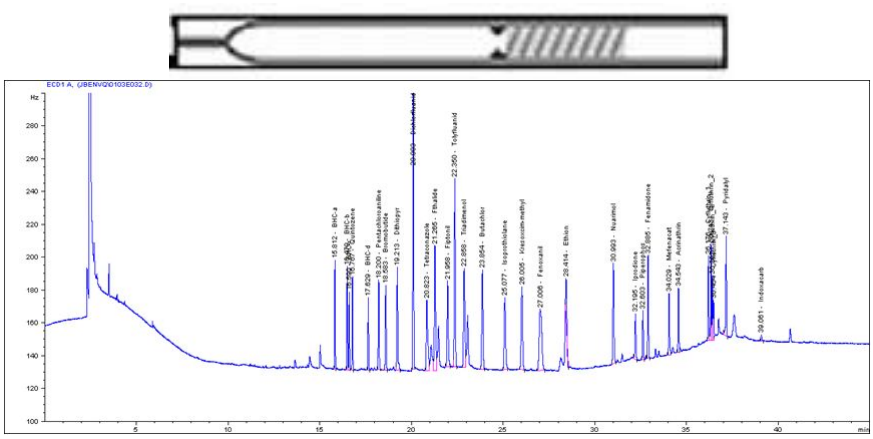


(c) ECD3 chromatogram with DB-17 column

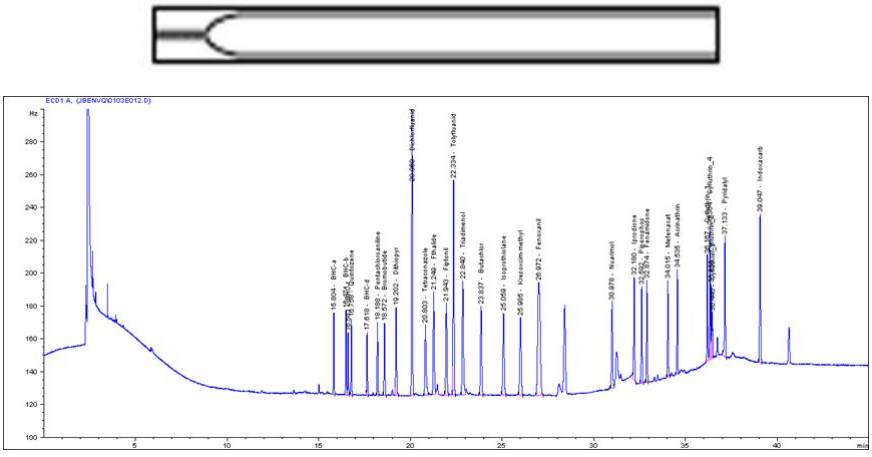
Figure 7. Ruggedness by column

3.8.2. Ruggedness of the inlet liner of GC

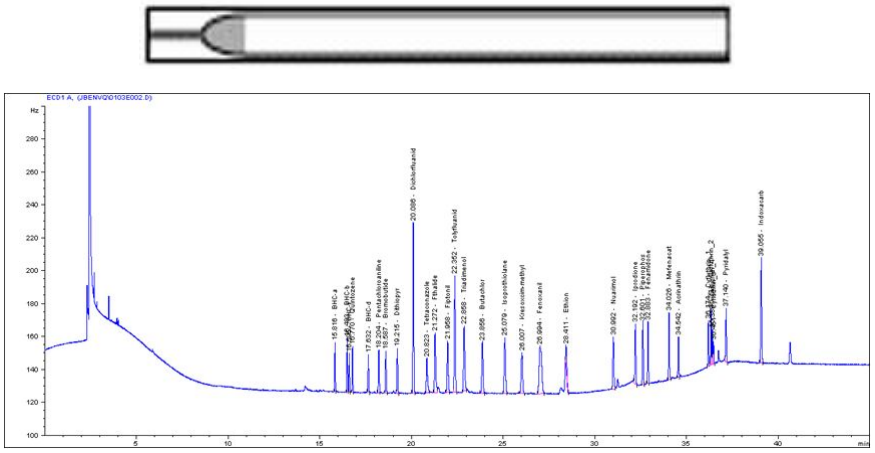
For evaluation of the GC inlet liner, 3 liners used for the analysis of the GC-ECD3. 2 Splitless liners (Single taper splitless liner와 Single taper splitless liner, with glass wool) which generally use single taper spilt liner with glass wool and focus liner which is similar feature of the current liner are used to achieve ruggedness. Selectivity didn't showed difference but sensitivity showed difference among inlet liners. Single taper spilt liner with glass wool liner showed highest sensitivity and focus liner also showed similar sensitivity.



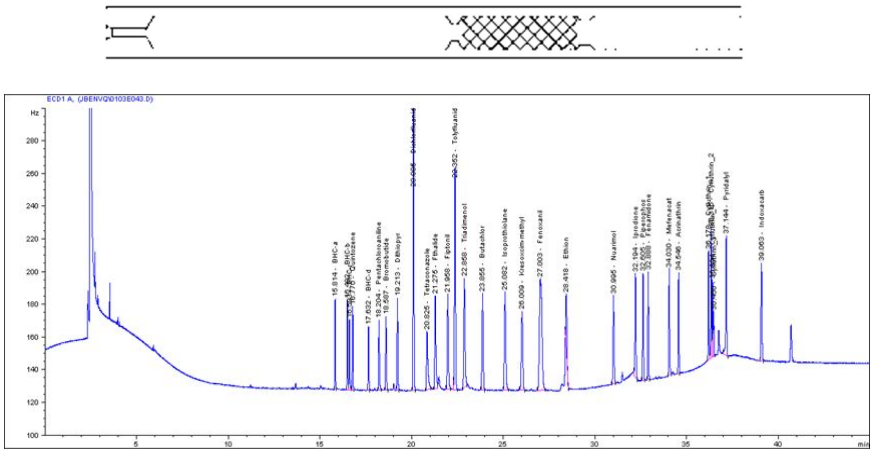
(a) ECD-3 chromatogram with single taper split liner with glass wool



(b) ECD-3 chromatogram with single taper splitless liner



(c) ECD-3 chromatogram with single taper splitless liner with glass wool



(d) ECD-3 chromatogram with focus liner

Figure 8. Ruggedness by inlet liner

3.9 Sample analysis using GC-ECD/NPD

Paddy soil, upland soil, orchard soil, groundwater, stream water, lake water from 9 province (Gyeonggi-do, Gangwon-do, Chungcheongbuk-do, Chungcheongnam-do, Gyeongsangbuk-do, Gyeongsangnam-do, Jeollabuk-do, Jeollanam-do, Jeju Special Self-Governing Province) were collected and analyzed by developed method. In some sample, pesticides were detected but retention time was same. So it was unable to identify pesticide. For this additional experiment is needed to identify these pesticide. In water sample, some pesticides were detected on river of Gangwon-do, river and lake water of Chungcheongnam-do, underground and lake water of Jeollanam-do, river of Gyeongsangbuk-do, river, lake, underground water of Gyeongsangnam-do when concentration factor is 5 same as the developed method. So concentration factor is changed to 10 and it was reanalyzed. In soil sample, orchard of Gangwon-do, orchard of Chungcheongbuk-do, field soil of Jeollabuk-do were analyzed by GC-MS/MS, and river of Gangwon-do, river of Chungcheongnam-do which was detected by GC-ECD/NPD was analyzed by GC-MS/MS. In the result of MS analysis, among pesticide which was unable to distinguish because of the same retention time, some were detected and by improvement of the sensitivity some pesticide which was not detected from GC was also detected.

IV. Conclusion

From this experiment, simultaneous multiple pesticide residue analysis method is developed and method validation is performed based on KOLAS guide line. And by applying developed method for analyzing whole country soil and water sample, this experiment confirmed possibility for application for the developed method. Developed and validated simultaneous multiple pesticide residue analysis method in soil and water can attribute to conduct comprehensive pesticides management system for the nation beside our nation don't have standard or monitoring system for soil and water.

KOLAS guide line deals with comprehensive method for chemical experiment method, so it has difficulty to adjust to the pesticide. Sensitivity, linearity, ruggedness, and uncertainty of the measurement don't have precise standard decision in related government department, therefore there's different on calculating MDL, MQL. In case of assessment characteristic which don't have specific experimental method or judgement standard, setting a suitable standard is necessary after discussed with NAQS or MFDS. This will fit to the International standard like ISO/IEC 17025 and also will be helpful to develop method and mange it suitable for nation's circumstance.

However, there's limitation for quantifying pesticides which showed low recovery or similar retention time. GC-MS/MS showed fast and accurate analysis therefore problems mentioned might be able to solve if analyze by GC-MS/MS.

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국문 요약

토양 및 농업용수에서 농약 다성분 동시분석법의 확립 및 유효성 검증 (GC-ECD/NPD)

이 진 범

농산물의 안전성을 궁극적으로 보장하기 위해서는 농산물뿐 아니라 농산물에 직.간접적으로 영향을 미치는 토양, 용수 등의 재배환경에 대한 관리가 이루어져야 한다. 이를 위하여 본 연구에서는 현재 국립농산물품질관리원에서 농산물에 대해 사용하는 잔류농약 다성분 분석법을 토양과 용수에 확대 적용하여 166성분(GC-ECD : 그룹1 26종, 그룹2 23종, 그룹3 24종, 그룹4 24종, GC-NPD : 그룹1 18종, 그룹2 18종, 그룹3 17종, 그룹4 16종)에 대하여 분석법을 개발하고 KOLAS 기준에 따라 유효성을 검증하였다. 이전 연구에서 확립한 기기조건을 바탕으로 추출, 팽윤, 정제 과정을 최적화하였다. 추출 용매로 acetonitrile, dichloromethan, ethyl acetate 등을 비교한 결과 acetonitirle의 효율이 가장 높았다. 토양 시료 추출 과정에서 NaCl 20 g을 넣어주는 대신에 포화소금물 30 mL로 대체하였다. 용수 시료는 SPE를 이용한 정제 과정을 생략하고 단순

여과하는 것으로 대체하였다. 개발된 다성분 잔류분석법을 용수 4종(증류수, 하천수, 호소수, 지하수)과 토양 3종 (논토양, 밭토양, 과수원토양)에 적용하여 7반복한 회수율을 구하고, 이 자료를 바탕으로 KOLAS 기준에 따른 유효성 평가 특성(직선성, 재현성, 진도(편의) 시험방법의 LOD, LOQ, 감도, 선택성, 적용범위, 둔감도)값을 산출하였다. 측정불확도는 검출기마다 농약을 하나씩 선정하여 전국 9개도에서 채취한 모니터링 시료에서의 잔류량을 계산하였다. GC-ECD의 경우 endosulfan sulfate를 사용하였으며 측정불확도는 1.662 ± 0.121 μg/kg (k=2.042)로 계산되었다. GC-NPD의 경우 chlorpyrifos의 측정 불확도는 0.0076 ± 0.0011 μg/kg (k=2.086) 이었다.

주요어 : 토양, 농업용수, 다성분 분석, 유효성 검증, GC-ECD, GC-NPD, 측정불확도

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