농학석사 학위논문

Establishment of Analytical Method for Pesticide Multiresidue in Soil and Water using HPLC-UVD/FLD

HPLC-UVD/FLD 를 이용한 토양 및 용수 중 잔류농약 다성분 동시 분석법 확립

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Establishment of Analytical Method for Pesticide Multiresidue in Soil and Water using HPLC-UVD/FLD

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ABSTRACT

Establishment of Analytical Method for Pesticide Multiresidue in Soil and Water using HPLC-UVD/FLD

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To ensure safety of the soil and water directly related to food safety at large, 85 pesticides multiresidue analysis using HPLC-UVD/FLD for soil and water was established. Based on 73 pesticides (3 UVD-groups, 2 FLD-groups) on the list of the NAQS (National Agricultural Products Quality Management Service) for crops and vegetable, 12 pesticides were newly added considering the type of groups and retention time. UVD-group 3 and FLD-group 1, 2

sensitivity was enhanced by modifying detection wavelengths. Soil wetting (NH_4Cl) and various extraction solvents (acetonitrile, dichloromethane, ethyl acetate) were attempted for the optimization of sample treatment of soil and water. Acetonitrile+saturated solution 30 mL (for soil) and acetonitrile+NaCl 20 g (for water) in general had the best recovery result. Established method was validated with linearity, selectivity, sensitivity, statistical LOD/LOQ, working range, method LOD/LOQ, trueness and precision, measurement uncertainty and ruggedness. Also system suitability test was performed by retention factor, separation factor, number of theoretical plate, resolution and symmetry.

Key words : soil, water, pesticide, multiresidue analysis, method validation, system suitability

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I. Introduction

1.1. Pesticides and environmental safety

Food safety is significantly important these days. As it is directly related to human health, the need for producing highly qualified and safe crops is ever growing. Pesticide analysis is one of the ways to fulfill this need.

Pesticide is defined as any substance or mixture of substances intended for preventing, destroying, repelling or migrating any pests. Main purpose of using pesticides is to prevent the pest or diseases. By doing so, total production of crops and vegetables are expected to increase. In MFDS (Ministry of food and drug safety), there are 416 pesticides MRLs (Maximum Residue Levels) for crops and vegetables with no regulation or guideline for soil and water as of date. However, there's some research for the pesticide analysis for the soil(1). For better production of crops and vegetables, pesticides are usually sprayed repeatedly on the field. Pesticide degrades but

slowly, therefore it is easily accumulated on soil and water. These accumulated pesticides can penetrate to the crops and contaminate them(2). This penetration can mean possible health hazards on our daily tables. The argument made is that by analyzing soil and water for the pesticides residue, it can be prevented. Healthy environment will provide healthy agricultural products. Therefore, analysis for soil and water for pesticide residue is very important.

There's another objective for the soil and water monitoring. Organic agricultural product and pesticide-free product attract many people. To get a credit for the organic agricultural product and pesticide-free product, it has to follow the notification provided by NAQS (National Agricultural Products Quality Management Service). In this notification, it is mentioned that pesticide's LOD (Limit of Detection) on the soil has to be under 0.01 ppm to get a certification of organic agricultural product and certification of pesticide-free product(3). This is significantly important because it means that not

only crops but also soil and other factors are concerned in giving out the credit. Even if a farmer did not use any pesticide directly on the crops, pesticide residue on the soil can prevent farmers from selling their products as organic product or pesticide-free product. In some research showed that pesticides were detected on the organic agricultural product(4). The research didn't showed pesticide penetration to the crops but it can be assumed by other researches on the pesticide penetration from soil to the crops(5).

1.2. Pesticide multiresidue analysis

For the pesticide analysis, single residue analysis and multiresidue analysis are most commonly performed. Both methods are slightly different but the overall scheme follows the same procedure. Both follow extraction, separation, clean up and instrumental analysis.

The first step in pesticide analysis is extraction. Soil residue analysis method for herbicide(6), soil residue analysis method for

insecticide(7) and soil residue analysis method for fungicide(8) describe the single pesticide compound analysis for soil. The most used solvents for extraction in these 3 books are acetone, acetonitrile and methanol. Among these 3 extraction solvents, acetone was the most used one and also in other research(9).

In pesticide analysis, accuracy is important but also time-efficiency is significant. Agricultural products have to be delivered quickly to the customers, so products can be provided at its freshest. For this reason, sample treatment and instrumental analysis have to be less time consuming. And one of the ways to fulfill this need is the pesticide multiresidue analysis. Instead of single residue analysis, multiresidue analysis can save many hours when all other conditions are controlled. Couple researches showed that acetonitrile was used for the extraction solvent(10, 11) while others used ethyl acetate(12) and methanol(13) for the extraction solvent. For the environmental concerns, organo-chlorine solvent like dichloromethane is not

preferably used(14). Recent pesticide multiresidue analysis tends to use acetonitrile as the extraction solvent since QuEChERS is used for the sample treatment method along with MS instrumental analysis(15, 16) (17-19).

In pre-harvest, NAQS is taking care of pesticide analysis and in post-harvest, MFDS is dealing with post-harvest pesticide analysis. Both organizations have their own pesticide multiresidue analysis method. In NAQS, 273 pesticides are on the pesticide multiresidue analysis list.(3). There are 286 pesticides on the pesticide multiresidue analysis list in Korean Food Standard Codex(20). But other notification in MFDS, there's another pesticides multiresidue analysis method which is revised version of NAQS pesticide multiresidue analysis method(21). In Korean food standard codex, acetone and acetonitrile are introduced for the extraction solvent in pesticide multiresidue analysis method. But in NAQS, only

acetonitrile is used for the extraction solvent in pesticide multiresidue analysis method.

1.3. System suitability test

Purpose of the system suitability test is to prove the proper performance of the HPLC system(22). It not only indicates the adequate performance but also provides a diagnostic information on the problem(23). For example, problem can be fixed by checking tailing factor or symmetry(24). And system suitability can be used as the quality control of chromatogram assay(23).

System suitability factors are resolution, number of theoretical plate, symmetry, retention factor and so on. Some paper involve LOD and linearity as well(23). It can be assumed that system suitability factors and method validation factors overlap. They overlap but highlighted factors are different. Resolution, tailing factor, peak

asymmetry were the mostly mentioned factors in system suitability test(23).

Method validation guideline of MFDS introduce a system suitability(25). In the guideline, capacity factor, resolution, relative retention, symmetry factor, and number of theoretical plate are explained.

1.4. Method validation

Method development has to be followed by method validation. Main purpose for the method validation is to ensure the quality and comparability of analytical results(26). In South Korea, KOLAS (Korea Laboratory Accreditation Scheme) is used for the method validation guidelines and it referred NATA guideline. In ICH Q2A guideline, there are 7 validation factors. They are specificity, linearity, quantitation limit, detection limit, range, accuracy and precision(27). ICH Q2B explained these 7 factors in detail.(28).

Figure 1. Domain for a HPLC analytical system showing relationship between system suitability, validation and calibration

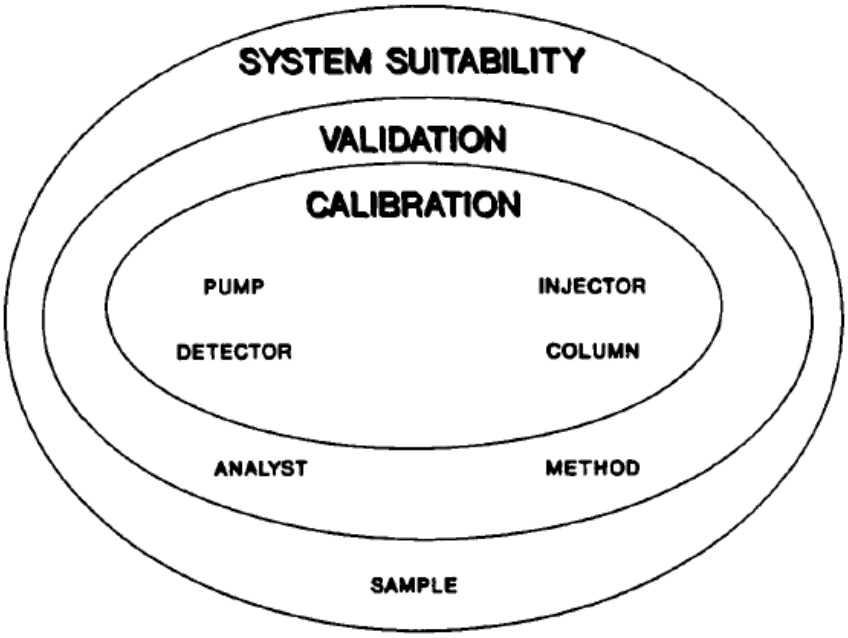


Table 1. Validation parameters in organizations

KOLAS	Sanco	NATA
Linearity	Linearity	Selectivity
Sensitivity	Matrix effect	Linearity
Selectivity	LOQ	Sensitivity
Trueness, Bias	Specificity	Accuracy
LOD, LOQ	Accuracy	LOD, LOQ
Working range	Precision1	Range
Ruggedness	Precision2	Ruggedness
Measurement Uncertainty	Robustness	Measurement Uncertainty

Table 1 shows validation parameters in 3 different organizations. Their parameters are almost similar. In the table, there's no measurement uncertainty in Sanco guideline, but they also dealt with the measurement uncertainty.

In ICH Q2(R1), linearity is defined as ability to obtain test results which are directly proportional to the concentration of analyte in the sample(29). In analytical method, linearity refers to the relationship between the instrumental response and the concentration(30). To

achieve linearity, there should be 5 concentrations or more. Range has to be around 0~150% of the target compound concentration(30). If necessary, correlation coefficient, y-intercept, slope of the regression line and residual sum of square has to be provided(29). In other document, linearity is categorized with the working range(31).

Sensitivity is the ratio of the concentration change responding to the instrumental response(32). It is easy to recognize the change of the concentration if sensitivity is big. It should be checked as part of the laboratory's ongoing quality assurance and quality control procedures(30)

Other guidelines or documents define selectivity as specificity. Main purpose of selectivity is distinguishing and quantifying the response of the target substance from the response of all other substances(33). Mass spectrometry and chromatography can be very selective but method like colorimetric measurements can be

affected by colored samples(30). Before the recovery test, selectivity has to be performed with control and fortified sample.

In KOLAS guideline, accuracy possesses trueness and precision. But in Sanco guideline, accuracy and precision are two different parameters of method validation. These parameters can be differentiated conceptually, but what they each mean are far from different.

Precision is the measurement of the random error. Repeatability and reproducibility are the parameters for representing precision. Normally standard deviation is used for precision.

Trueness describes how close the test sample is to the accepted reference of quantify measurements. Bias is the quantitative expression of trueness. And lack of trueness express systematic error(30). Certified reference material (CRM) is basically used for the trueness. When CRM is not available, Reference material (RM) is used to estimate trueness. If both are not available, trueness will be

achieved by spiked sample with known concentration on the analyte(32).

The limit of detection (LOD) is the amount of the concentration that can be distinguished from 0. Also it has to be bigger than measurement uncertainty(30).

The limit of quantitation (LOQ) is the lowest concentration of analyte that can be determined with an acceptable level of uncertainty(30).

The most generally recommended method is determining LOQ as 3 fold of LOD. It has to be distinguished with the lowest instrument response and LOD of methods. Signal to ratio is good criterion for the instrument performance but not suitable for the LOD of methods. To calculate the LOD of method, 7 replicate samples at each of 3 concentrations are needed. Then standard deviation vs concentration is extrapolated and estimated the standard deviation at the zero concentration (S_0). LOD of methods is the $b+3S_0$ and gives 95%

confidence. Among 3 different concentrations, lowest concentration has to be close to 0. And b represents a sample blank value(30).

Another way to calculate LOD of methods is that standard deviation of 7 replicate of about twice the LOQ in the single concentration is achieved. And this becomes S_0. Then it will calculate the LOD of methods same as described above(30).

Range is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity(29). It depends on the type of analyte, but normally 70 ~120% of the target analyte concentration is considered to be the working range(25, 29). The LOQ becomes the lowest concentration of the range. The upper limit can be the saturated concentration but it has to be within the validated range.

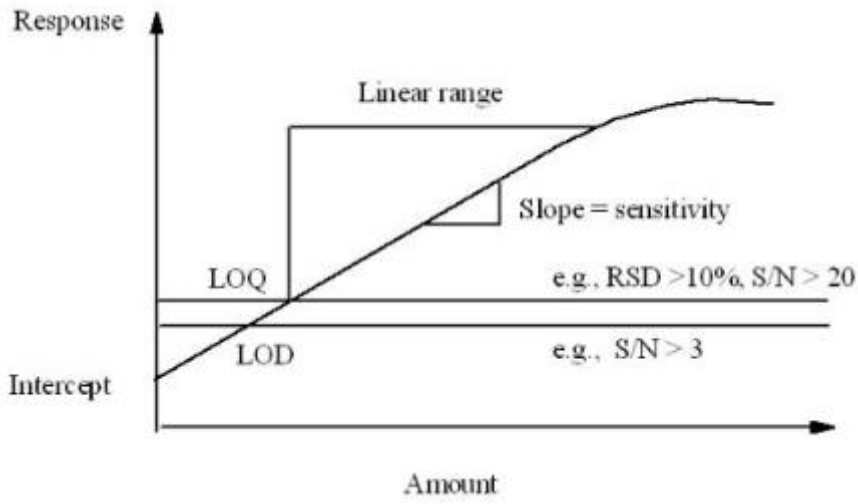


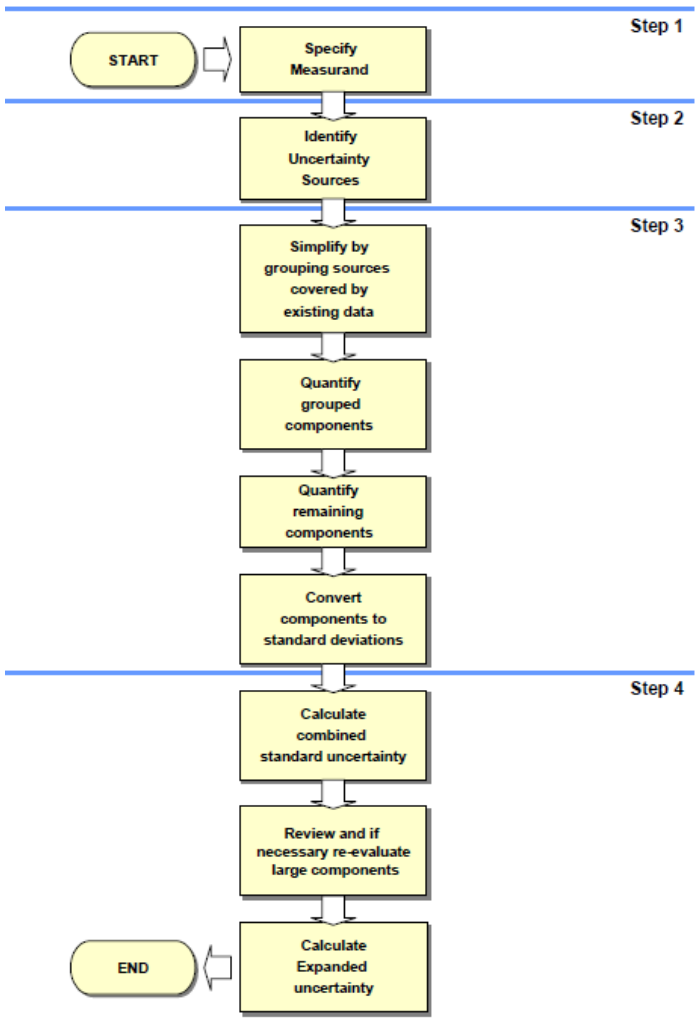
Figure 2. Definition for linearity and range

Some guidelines define ruggedness as robustness. They are two different names for the same measure. Ruggedness of the method is the degree to which results are unaffected by minor changes from the experimental conditions such as temperature, pH, reagent concentration, extraction time and so on(30). If the results are easily varied sue to the shifts in the condition analysis is under, it has to be mentioned in the analytical methods(25). Research should assume

the factor which can affect the result and should control those factors(32).

Measurement uncertainty is the parameter consists of all the factor that might affect the results. Also it is defined as the expression of given measure and given result of measurement(34). And measure uncertainty is the property of the measurement, not a method(30). There are many factors which can be attributed to the measurement uncertainty such as volumetric equipment, reference values and masses (35). EURACHEM CITAC guideline emphasize the traceability. In the guideline, traceability is defined as the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty(35). Figure 3 shows the process of measurement uncertainty estimation.

Figure 3. The Uncertainty Estimation Process



II. Materials and Methods

1. Materials and reagents

1.1. Pesticides standard

All the pesticides were purchased from Wako Pure Chemical Industries, Ltd., Dr. Ehrenstorfer GmbH, Chem Service, Inc., Sigma-Aldrich Co. LLC. All the standard stock solutions except carbendazim and ametoctradin (100ppm) were made in 1000ppm. All the pesticides and stock solutions were stored under −20℃.

1.2. Reagents and apparatus

Acetonitrile, acetone, n-hexane, dichloromethane and methanol were of HPLC grades purchased from Burdick & Jackson® and sodium chloride were of extra-pure grades purchased from Samcheon chemical. For fluorescent derivatization, o-phthalaldehyde, N, N-dimethyl-2-mercaptoethylamine

hydrochloride(Thiofluor^{TM}), carbamate hydrolysis reagent (0.05M NaOH/C47^{TM}) and o-phthaladehyde diluent were purchased from Pickering Laboratories. Aminopropyl (NH_2, 1 g, 6 mL, Strata^{TM}) SPE cartridge was purchased from Phenomenex.

2. Instruments and analytical condition

2.1. Instruments

SR-2w shaker was purchased from Taitec company and Hanil combi 408 centrifuge was used. SPE manifold from Pierce company was used and Hurricane-Lite concentrator was used.

Agilent 1100 series HPLC-UVD/FLD were used and Pinnacle PCX was connected for fluorescent derivatization. In HPLC-UVD/FLD analysis, Phenomenex Gemini NX C18(150 x 4.6 mm, 3 μm) column was used.

2.2. Analytical condition

As for solvent A, Acetonitrile was used while diluted water was the solvent B in the HPLC-UVD/FLD analysis. Gradient start from 30% of solvent A and 0.7 mL/min flow. Than it changes to 0 min ~5min = A : 30%, B : 70% flow : 0.7 mL/min, 5min~20min = A :70%, B : 30% flow : 0.7 mL/min, 20min~30min = A : 90% B : 10% flow : 0.7 mL/min, 30min~31min = A ; 100% B : 0% flow : 1 mL/min, 31min~40min = A : 100% B : 0% 1 mL/min(10min postrun).

Detection wavelength for UVD-group1 and group UVD-group2 was 254nm. Detection wavelength for UVD-group3 was 225nm. Excitation wavelength for FLD-group was 240nm and Emission wavelength for FLD-group was 450nm.

3. Standard solution mixture

In HPLC-UVD/FLD analysis, 73 pesticides in the NAQS's list were made for the sample treatment and addition of new pesticide

experiment. The number of pesticides in each group was 19 (UVD-group1), 20 (UVD-group2, UVD-group3) and 7 (FLD-group1, 2). The total number of pesticides was 73. After adding 12 pesticides, total of 85 pesticides were made into 5 different mixtures (UVD-1, UVD-2, UVD-3, FLD-1, FLD-2) and concentration of mixture was 100LOQ. UVD-1 contains 22 pesticides. UVD-2 also contains 22 pesticides. UVD-3 contains 26 pesticides. FLD-1 contains 8 pesticides. And FLD-2 contains 7 pesticides. To make this mixture, LOQ of each pesticide was obtained. Based on this LOQ, 100LOQ mixture was prepared.

4. Sampling for soil and water

Sampling was performed following the guidelines provided by 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 brought from organic farms in Ansung. All the organic farms in the area has certification from NAQS (National Agriculture products Quality management Service). Paddy soils, orchard soils and upland soils were collected.

Water control samples were brought from river, lake and groundwater in Ansung. Also distilled water was used as control sample as well.

5. Establishment of analytical condition of pesticide multiresidue analysis in soil and water

5.1. Analysis of 73 pesticides in the NAQS's pesticide multiresidue method

Based on pesticide multiresidue analysis for crops and vegetables according to NAQS's method, 73 pesticides were analyzed. Analytical method is described in table 2. And 73 pesticides used are listed in table 3.

Table 2. Analytical condition in NAQS for multiresidue pesticides analysis method

Detector	DAD (Agilent 1100) FLD (Agilent 1100)			
Reactor	Post-column OPA reactor(Pinnacle PCX) is connected to FLD			
Column	Phenomenex Gemeni-NX C18 (150 mm × 4.6 mm, 3 μm)			
Injector	Injection volume: 10 μL			
Detector wavelength	UVD-1, 2 : 254 nm, UVD-3 : 235 nm	Excitation : 330 nm, Emission : 446 nm		
Gradient	Time	Mobile phase		Flow (mL/min)
		D.W.	Acetonitrile	
	0	70	30	0.7
	5	70	30	0.7
	20	30	70	0.7
	30	10	90	0.7
	31	0	100	1
	40	0	100	1

Table 3. List of the 73 pesticide from NAQS multiresidue pesticides analysis

Group	Name	No
LC-UVD1	Chromafenozide, Clothianidin, Cyhalofop-butyl, Dimethomorph, Dimethylvinphos(Z), Ferimzone, Flumioxazin, Hexaflumuron, Mepanipyrim, Metamifop, Novaluron, Pirimicarb, Pyributicarb, Pyriproxyfen, Quinoclamine, Tebufenozide, Thiabendazole, Thiacloprid, Trifloxystrobin	19
LC-UVD2	Acetamiprid, Boscalid, Cyazofamid, Cymoxanil, Diethofencarb, Diflubenzuron, Diuron, Fenpyroximate, Fluacrypyrim, Forchlorfenuron, Imibenconazole, Imidacloprid, Pentoxazone, Pyraclostrobin, Pyribenzoxim, Pyrimethanil, Pyroquilon, Spirodiclofen, Teflubenzuron, Uniconazole	20
LC-UVD3	Amisulbrom, Bendiocarb, Benthiavalicarb-isopropyl, Benzoximate, Carbendazim (Benomyl included), Chloraniliprole, Ethaboxam, Etofenprox, Flubendiamide, Flupicolide, Hexythiazox, Mandipropamid, Methoxyfenozide, Oxaziclomefon, Pencycuron, Silafluofen, Spiromesifen, Thiophanate-methyl, Tiadinil, Tricyclazole	20
LC-FLD1	Carbaryl, Carbofuran, Fluquinconazole, Isoprocarb, Methiocarb, Methomyl, Thiodicarb	7
LC-FLD2	Aldicarb, Ethiofencarb, Fenobucarb, Metolcarb, Oxamyl, Propoxur, Thiamethoxam	7
	Total	73

5.2. Optimization of UV absorption wavelength

Following the analytical condition set forth by NAQS, UVD-groups were analyzed with 254 nm (UVD-group1, 2) and 225 nm (UVD-group3). Comparing UV wavelength for each group was performed by using agilent 1100 series UVD.

5.3. Optimization of FLD absorption wavelength

According to analytical method by NAQS, excitation wavelength was 330 nm and emission wavelength was 446 nm. Scan for the excitation wavelength and emission wavelength was performed. And the wavelength most suitable was selected.

5.4 Addition of new pesticides

Based on 73 pesticide in NAQS's analysis list (LC part), 12 pesticides were newly added. 9 pesticides were from the list of GC analysis from NAQS and 3 pesticides were new pesticides chosen by

NAQS in 2012. Single standard solution was made and its selectivity was compared with that of each groups.

6. System suitability test

Based on MFDS method validation guideline, system suitability test factors were decided. Resolution, retention factor, separation factor, peak width and symmetry were obtained.

$$5.545 \times (\frac{Tr}{Whalf})^2$$

Above is the formula of the number of theoretical plate. Tr refers to the retention time of target peak. Whalf referes to the peak width at the 1/2height of the peak.

$$(\mathrm{Tr} - \mathrm{To})/\mathrm{To}$$

Above is the formula of retention factor. To refers to the retention time of first peak in the chromatogram. Tr refers to the retention time of target peak.

$$k2/k1$$

It is the formula for the separation factor. K refers to the retention factor. K2 is the farther peak and k1 is the closer peak.

$$\frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{K}{K+1}\right)$$

This is the formula of the resolution. N means number of plate, α means separation factor, K means retention factor.

$$b/a$$

This is the formula of the symmetry. A means the width of front half of peak and b is the width of back half of peak.

Most factors were calculated by agilent HP1100 offline software.

7. Establishment of sample treatment of pesticide multiresidue in soil and water

Based on NAQS's sample treatment method, optimization for the soil and water was performed.

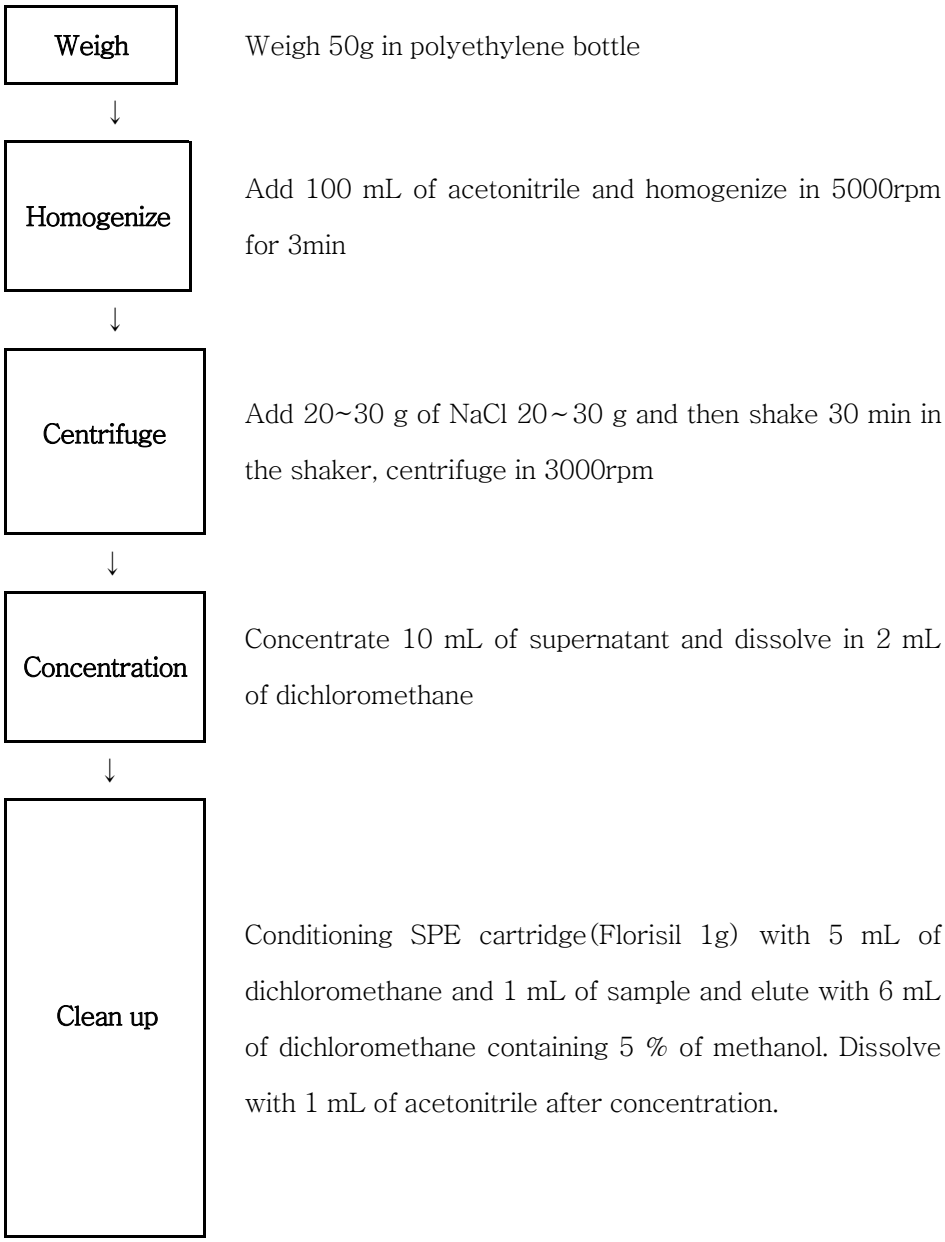


Figure 4. NAQS multiresidue pesticide analysis for crop and vegetable

7.1. Confirmation of SPE cartridge’s efficiency

NH_2 SPE purification was confirmed. First, SPE cartridge was conditioned with 5 mL of dichloromethane, than 1 mL of standard solution was loaded on the SPE cartridge. After that, it was eluted with dichloromethane containing 5% of MeOH (3times with 3 mL). UVD-2 standard solution was used for SPE cartridge’s efficiency test.

7.2. Comparing wetting condition in soil extraction

In wetting comparing, 4 different conditions were used. UVD-2 standard solution was used for this experiment

① Sample (50 g of soil) spiked with standard solution was wetted with 30 mL of 2N NH_4Cl for 2hours before extraction of acetonitrile.

② NH_4Cl (2N, 30 mL) was added to 50 g of soil spiked with standard solution and immediately extracted with acetonitrile.

③ Diluted water (30 mL) was added to 50 g of soil spiked with standard solution and immediately extracted with acetonitrile.

④ Sample (50 g of soil) spiked with standard solution was directly extracted with acetonitrile.

7.3. Comparing soil extraction condition

In soil extraction comparing, 3 different conditions were used. UVD-1 standard solution was used for this experiment.

① Sample (50 g of soil) spiked with standard solution was extracted with 100 mL of acetone.

② Sample (50 g of soil) spiked with standard solution was extracted with 100 mL of acetonitrile.

③ Sample (50 g of soil) spiked with standard solution was extracted with 100 mL of acetonitrile, 30 mL of saturated solution.

7.4. Comparing water extraction condition

In water extraction comparing, 3 different conditions were used. UVD-1 standard solution was used for this experiment.

① Sample (50 mL of water) spiked with standard solution was extracted with 100 mL of dichloromethane and 20 g of NaCl.

② Sample (50 mL of water) spiked with standard solution was extracted with 100 mL of ethyl acetate and 20 g of NaCl.

③ Sample (50 mL of water) spiked with standard solution was extracted with 100 mL of acetonitrile and 20 g of NaCl.

8. Method validation on pesticide multiresidue analysis in soil and water followed by KOLAS-G-015 guideline

By using KOLAS-G-015 guideline, method validation was carried out. Linearity, selectivity, sensitivity, statistic LOD/LOQ, working range, trueness and precision, method LOD/LOQ, ruggedness, measure uncertainty, these 9 factors were analyzed for the method validation. Also some other guidelines such as Sanco, NATA were referred.

Linearity, sensitivity, statistic LOD/LOQ and working range were achieved from instrumental pesticide analysis. Trueness and precision, method LOD/LOQ and selectivity were obtained by recovery test. Uncertainty measure was calculated from field sample with selected pesticide.

8.1. Linearity

Stock solutions were dissolved in acetonitrile. The most of pesticides were at the concentration of 1000ppm. The mixture of UVD-group standard solution's concentration was 10ppm and FLD-group standard solution's concentration was 5ppm. 0.01, 0.02, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10 ppm(UVD) were used for the linearity and they were all diluted serially. These concentrations were analyzed two times and their average area was used for Y-axis. And X-axis was concentration. By doing this process, correlation coefficient of all pesticide were calculated. All the data were obtained by Agilent 1100 series UVD/FLD.

8.2. Sensitivity

Sensitivity was achieved from regression equation. Slope in the regression equation is the sensitivity.

8.3. Statistic LOD/LOQ

Statistic LOQ means a signal to noise ratio is higher than 10 in the chromatogram. And one third of LOQ is LOD. Each pesticide's statistic LOD/LOQ was calculated from standard solution of pesticide analyzed by Agilent 1100 series UVD/FLD

8.4. Working range

The lowest value of working range is statistical LOQ / 5(concerning sample method). The maximum value of working range was obtained by the highest concentration of mixture (5 ppm in FLD, 10 ppm in UVD). In each mixture, the highest peak was selected. The highest peak' s height was extrapolated into the calibration curve of each pesticide in mixture. And then it was divided by 5(concerning sample method).

8.5. Selectivity

Standard solution chromatogram and control chromatogram were compared. Pesticide peak should not be overlaid with other pesticide peak and unknown peak from the control.

8.6. Trueness and Precision

Recovery test was performed 7times. 3 soil samples (paddy soil, upland soil, orchard soil) and 4 water samples (diluted water, river, groundwater, lake) were used for the recovery test. Average recovery and CV(coefficient of variance) of each sample were obtained from the result of 7 repeated recovery test. Trueness is defined as average recoveries and precision is defined as CV(coefficient of variance).

8.7. Method LOD/LOQ

Method LOD was achieved by 7 repeated recovery tests. Standard deviation was obtained from 7 repeated recovery tests, defined as S_0. Average sample blank was defined as b. $b+S_0$ is the method LOD. Method LOQ is multiple 3 on method LOD.

8.8. Ruggedness

5 different columns were used for ruggedness. Phenomenex Gemini-NX(150 x 4.6 mm, 3 μm), phnomenex Gemini-NX(250 x 4.6 mm, 3 μm), shiseido capcellpak C18MG(150 x 4.6 mm, 3 μm), Agilent Eclipse XDB-C18(150 x 4.6 mm, 5 μm), and Agilent Eclipse XDB-C8(150 x 4.6 mm, 5 μm) were used to identify different peak shapes, heights and resolutions. UVD-1 was used for this experiment.

8.9. Measurement uncertainty

Measure uncertainty was calculated based on KOLAS and EURACHEM guideline(35). As measure uncertainty data is achieved through 'result±uncertainty,' it has been calculated by monitoring samples. Monitoring samples, soil and water, were collected from all over the country and were analyzed by developed method. In each detector, one pesticide was chosen. In UVD, teflubenzuron was chosen. In FLD, fluquinconazole was chosen. All the steps for measure uncertainty were determined by considering NAQS measurement uncertainty estimation method.

Ⅲ. Results and Discussion

1. Establishment of analytical condition in HPLC-UVD/FLD

1.1 Addition of 12 pesticides in UVD and FLD groups

Table 4 shows the name of pesticides newly added in each group. To make the most suitable chromatogram, wavelengths and resolutions were considered. 3 pesticides were added in UVD-group1. 3 pesticides were added in UVD-group2. 6 pesticides were added in UVD-group3. 1 pesticide was added in FLD-group1. As Pyroquilon, newly added to UVD-group3 is originally from UVD-2 group, it was not counted as newly added pesticide. By adding 12 new pesticides, total number of pesticides changed from 73 to 85.

Table 4. List of the new pesticide added on each group

Group	Compound name	No
LC-UVD1	Ametoctradin, Clofentezin, Lepimectin	3
LC-UVD2	Chlorofenvinphos, Diniconazole, Picoxystrobin	3
LC-UVD3	Flufenoxuron, Metalaxyl, Metolachlor, Paclobutrazole, Simazine,	5
LC-FLD1	Furathiocarb	1
	Total	12

1.2 New analytical condition in HPLC-UVD/FLD

Detector UVD and FLD are commonly used with liquid chromatography. UVD refers to 'ultraviolet detector'. Sometimes terms like DAD, PDA or VWD are used but they are different names for fundamentally the same. PDA (photodiode array detector) and DAD (diode array detector) are multichannel instruments. They use diode array to detect all the wavelengths possible for the instrument simultaneously. Also identification of spectrum of wavelength and multi-wavelength detection is possible.

Spectrophotometer measures transmittance of light. When substance absorbs the light, radiant power of light will decreases. Normally absorbance is proportional to the length of the substance's pass channel. And this can be expressed as the Beer's law. Beer's law indicates that absorbance is proportional to the concentration of analyte. In case of change in temperature in substance and too many

stray light reaching to the detector, Beer's law can't be followed. Also too high concentration of analyte is leading same result.

Organic compounds are usually absorbed in UV-Vis wavelength. To be able to absorb in those wavelength, functional group has to be exist. Chromophores are the compound which can absorb UV-Vis wavelength and has functional group in it.

Table 5. Absorption characteristics of some common chromophores

Chromophore	Solvent	γ_{max},nm	ε max	Transition Type
Alkene	n-Heptane	177	13000	$\pi \rightarrow \pi^*$
Alkyne	n-Heptane	178	10000	$\pi \rightarrow \pi^*$
		196	2000	-
		225	160	-
Carbonyl	n-Hexane	186	1000	n $\rightarrow \sigma^*$
		280	16	n $\rightarrow \pi^*$
		180	large	n $\rightarrow \sigma^*$
		293	12	n $\rightarrow \pi^*$
Carboxyl	Ethannol	204	41	n $\rightarrow \pi^*$
Amido	Water	214	60	n $\rightarrow \pi^*$
Azo	Ethanol	339	5	n $\rightarrow \pi^*$
Nitro	Isooctane	280	22	n $\rightarrow \pi^*$
Nitroso	Ehtyl ether	300	100	-
		665	20	n $\rightarrow \pi^*$
Nitrate	Dioxane	270	12	n $\rightarrow \pi^*$

These analyte are usually dissolved in solvent for the LC analysis. So solvent has to be considered. Solvent has so called cutoff, it indicate that below cutoff wavelength, it can't be used. Therefore suitable solvent has to be used not to interrupt the analysis of analyte.

Table 6. Solvents for the ultraviolet and visible regions

Solvent	Lower wavelength Limit, nm	Solvent	Lower wavelength Limit, nm
Water	180	Diethyl ether	210
Ethanol	220	Acetone	330
Hexane	200	Dioxane	320
Cyclohexane	200	Cellosolve	320
Carbon tetrachloride	260		

Luminescence can be divided into three type; fluorescence, phosphorescence and chemiluminescence. Fluorescence and phosphorescence both absorb the light source, but the difference is that fluorescence life-time is much shorter than phosphorescence and when electronic energy transition occurs, electron spin does not involves in that phenomenon.

When analyte absorb the certain wavelength, it changes to the excited state. And after real short term (10^{-8}s), it returns to the ground state emitting certain wavelength. Resonance fluorescence means when excitation wavelength and emission wavelength are same. But more often, emission wavelength is longer than excitation wavelength. This is called Stokes shift. In excited state, normally molecule loses its energy through heat or vibration so emission wavelength is longer than excitation wavelength.

In some cases, post column is used for the fluorescence detector analysis. Compound hydrolyzes by special reagent and run through

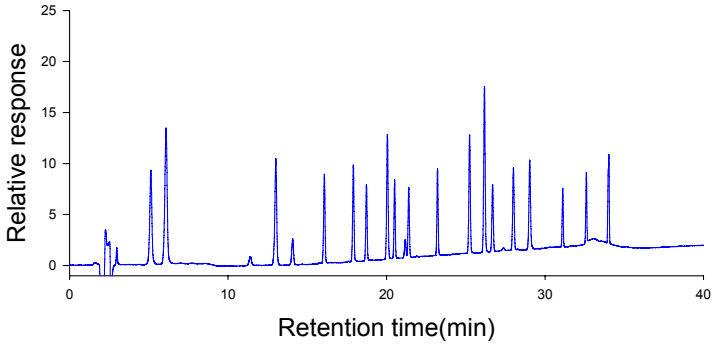
derivatization step. In derivatization, fluoresceence reagent adhere to the decomposed compound. This reagent makes it possible to fluorescence detector to analyze the compound.

Wavelength for each group was already set up by NAQS. But for the better sensitivity, λmax for the all pesticide was considered. By this procedure, best suitable wavelength was able to achieve for the UVD-group 3 and FLD-groups.

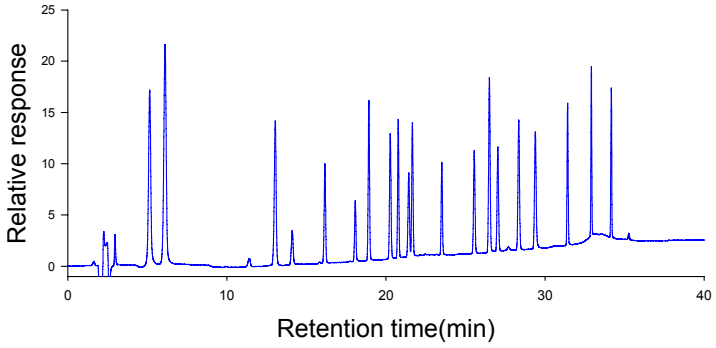
Table 7 shows newly established analytical condition. As described above in material and method, UVD-group3 and FLD-group wavelength has been changed. Main purpose for changing wavelength is for better sensitivity. Figure 5 and 6 show big difference in different wavelengths. And figure 7 shows the scan of excitation wavelength and emission wavelength.

Table 7. New analytical condition for UVD and FLD

Detector	DAD (Agilent 1100)		FLD (Agilent 1100)	
Reactor	Post-column OPA reactor(Pinnacle PCX) is connected to FLD			
Column	Phenomenex Gemeni-NX C18 (150 mm × 4.6 mm, 3 μm)			
Injector	Injection volume: 10 μL			
Detector wavelength	UVD-1, 2 : 254 nm, UVD-3 : 225 nm		Excitation : 240 nm, Emission : 450 nm	
Gradient	Time	Mobile phase		Flow
		D.W.	Acetonitrile	(mL/min)
	0	70	30	0.7
	5	70	30	0.7
	20	30	70	0.7
	30	10	90	0.7
	31	0	100	1
	40	0	100	1

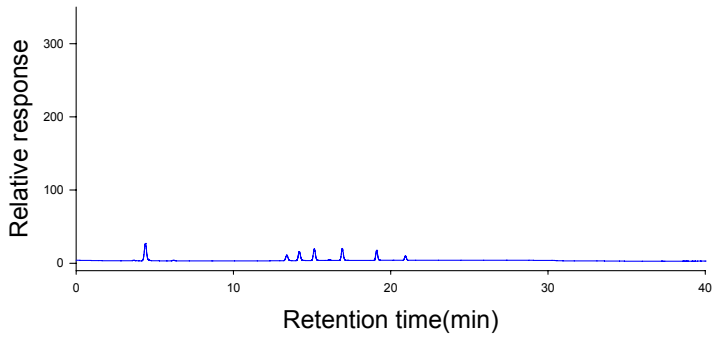


(a)

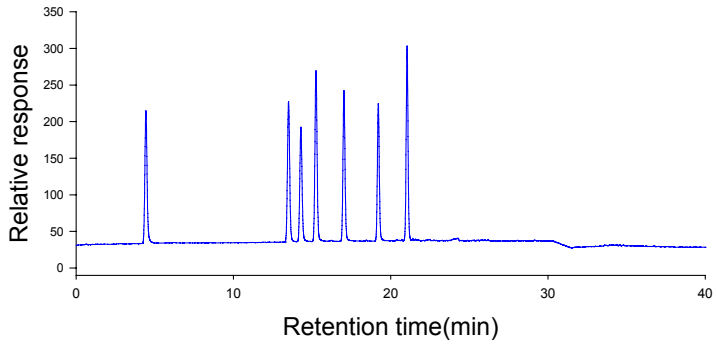


(b)

Figure 5. Chromatogram of UVD-3; (a) 235 nm (b) 225 nm

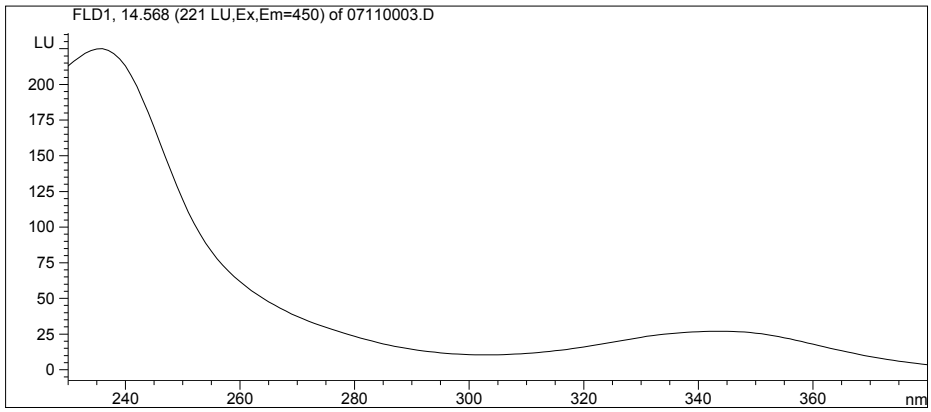


(a)

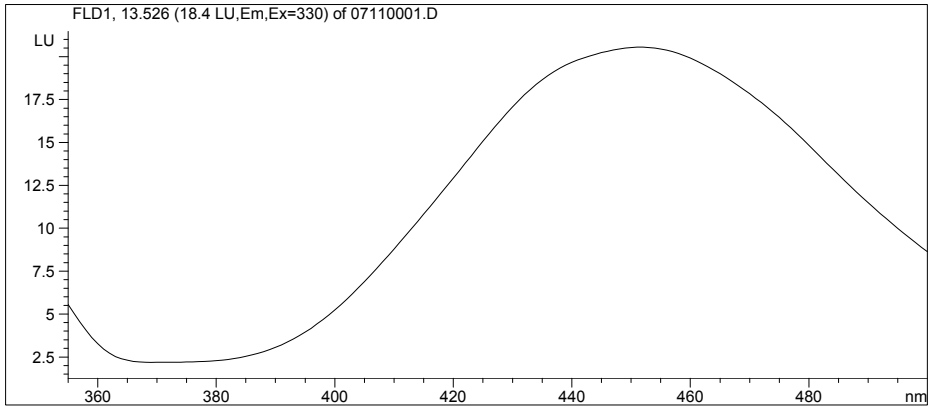


(b)

Figure 6. Chromatogram of FLD; (a) excitation : 330 nm, emission : 446 nm (b) excitation : 240 nm, emission : 450nm



(a)



(b)

Figure 7. Scan of FLD; (a) scan of excitation wavelength (b) scan of emission wavelength

2. Establishment of sample treatment of pesticide multiresidue in soil and water analyzing by HPLC-UVD/FLD

NAQS's pesticide multiresidue analysis method for the crop and vegetable was referred. Most of the steps were not changed. But in the homogenizing steps and concentrating supernatant of extraction were revised. And for the FLD analysis, clean up step was changed to the filtration.

Established sample treatment is as follows; weigh 50 g of sample in the polyethylene bottle, add 100 mL of acetonitrile, shake for 30min, add 20~30 g of NaCl, shake for 30min, concentrate 20 mL of supernatant, dissolve in 2 mL of dichloromethane, conditioning NH_2 SPE cartridge with 6 mL of dichloromethane, load 1mL of dissolved dichloromethane, elute with 6 mL of dichloromethane (5% of methanol), concentrate 6 mL of elution and then dissolve with 1 mL of acetonitrile. Generally development of analysis is performed in the opposite order of real analysis

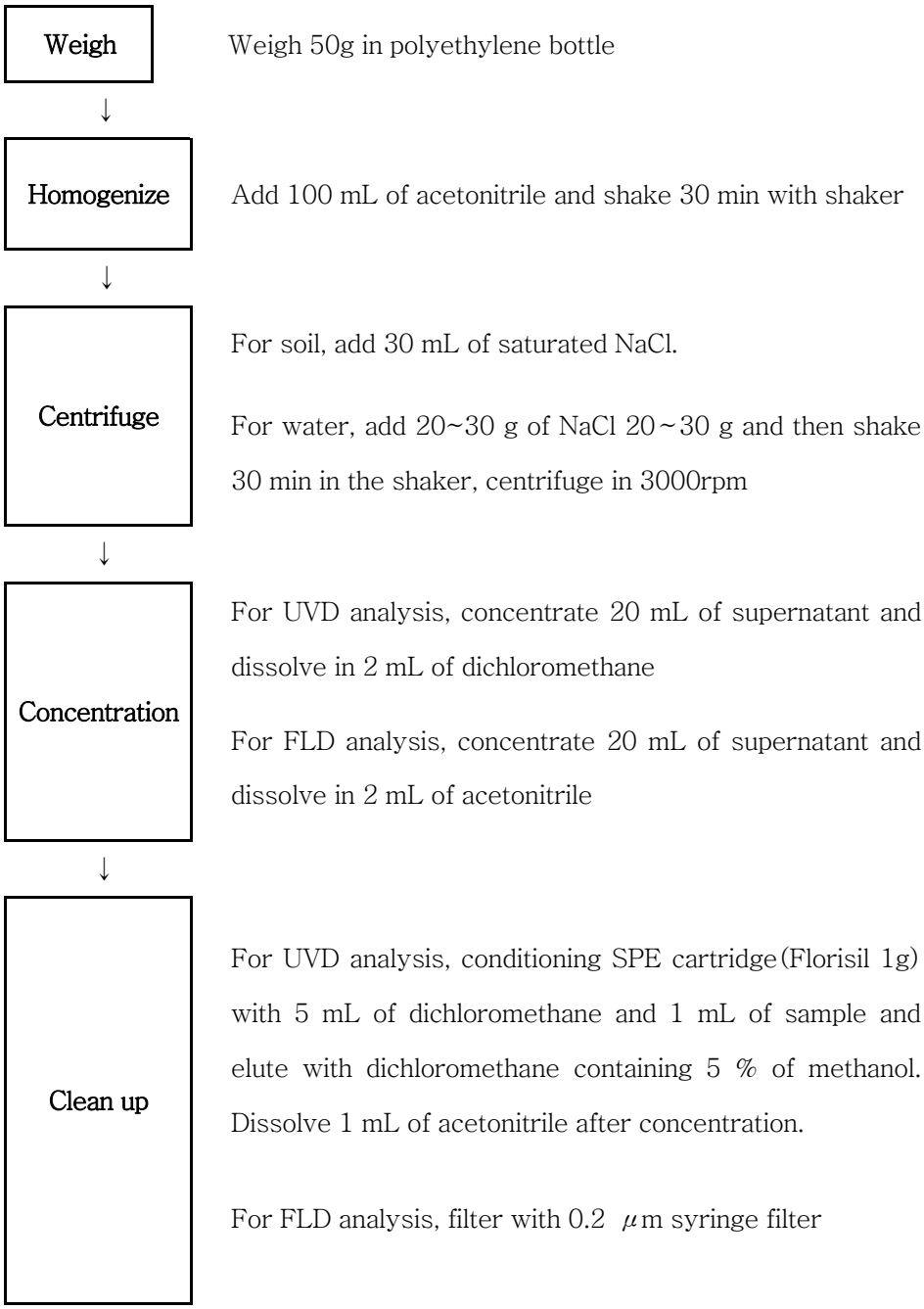


Figure 8. Established preparation condition for soil and water

2.1 Establishing SPE cartridge's efficiency

Usually SPE cartridge is used in the step of clean-up. Normally clean-up is performed to eliminate matrix and get a more pure compound. Also clean-up can prevent target peak to overlay with other unknown peak.

SPE cartridge (NH2, 1g) was used to confirm the efficiency. It followed steps of NAQS's pesticide multiresidue analysis for crops and vegetables. Cartridge was conditioned with 5 mL of dichloromethane. After conditioning, 1 mL of stock solution(UVD-2 group mixture, 1 ppm) was loaded to the cartridge. Dichloromethane with 5 % of methanol was used for elution. Elution was 3times repeated with 3 mL of dichloromethane with 5 % of methanol. Eluted solution was concentrated with NH_2 gas than dissolved with 1 mL of acetonitrile. UVD-2 was used for this efficiency test.

The result showed that most of the pesticides were come out in the 6 mL of elution solvent. And 6 mL is also used for crops and

vegetables analysis. It is decided to use 6 mL of elution solvent in the newly established method for soil and water. Recovery results for SPE cartridge’ s efficiency are on the Table 8.

Table 8. Recovery of the SPE cartridge (UVD-2)

Recovery (%)	0-3 mL	3-6 mL	6-9 mL	Total (%)
Imidacloprid	64.9	28.6		93.5
Acetamiprid	66.9	24.9		91.9
Cymoxanil	71.3	19.8		91.1
Forchlorfenuron		23	64.9	87.9
Diuron	69.8	23.4	0.2	93.4
Pyrimethanil	88.9	2.3		91.3
Diethofencarb	93.9	1.2		95.1
Uniconazole	62.2	28.5		90.8
Boscalid	92.8	3.1		95.8
Diflubenzuron	79.2	27.6	1.6	108.4
Diniconazole	65.4	29.6		95
Chlorfenvinphos	92.9	1.7	0.2	94.7
Cyazofamid	96.2	4.2		100.5
Picoxystrobin	94	0.8		94.7
Pyraclostrobin	93.1	1.3		94.5
Teflubenzuron	66	25.1	0.1	91.3
Imibenconazole	83.7	10.2	0.2	94.2
Fluacrypyrim	93.1	0.8	0.1	94
Pentoxazone	95	1.7	0.2	96.9
Pyribenzoxim	92.6	2.1	0.1	94.8
Fenpyroximate	93.5	1.8		95.2
Spirodiclofen	167.7			167.7

2.2 Establishing wetting condition in soil extraction

In soil analysis, usually experiment called 'soil wetting' is performed. This step can increase extraction efficiency of polar pesticide since it can easily stick to the soil particle. Usually NH_4Cl is used for soil wetting. This is highly ionized solution, so it will minimize the electrostatic attraction between soil particle and polar pesticide.

4 different conditions were used in this experiment. Among 4 different conditions, extracting without adding nothing was chosen for the new method. Result wasn't the best but there wasn't a big different and it was less time consuming. The results are on Table 9.

Table 9. Recovery on the wetting condition on soil extraction(UVD-2)

① Extract with 2N NH_4Cl with 2hours wetting, ② Extract with 2N NH_4Cl without wetting,

③ Extract with 30 mL of water and 20 g of NaCl without wetting, ④ Extract with only solvent

Recovery (%)	①	②	③	④
Imidacloprid	104.3	96.3	86	85.7
Acetamiprid	94.1	93	74.1	74.1
Cymoxanil	104.4	100.1	63.5	76.4
Forchlorfenuron	85	91.4	3.1	4.3
Diuron	95.9	96.3	76.8	87.9
Pyrimethanil	92.3	94.3	59.3	75.2
Diethofencarb	96.5	92.2	80.8	87.6
Uniconazole	95.2	94.2	77.6	87
Boscalid	122.7	119	93.4	110.6
Diflubenzuron	104.9	112.9	81.1	118.8
Diniconazole	90.9	89.6	92.7	88.9
Chlorofenvinphos	106	104.7	72.6	91.3
Cyazofamid	107.7	105.3	65.1	71.5
Picoxystrobin	92.3	91.1	82.3	89.8
Pyraclostrobin	94.2	10.9	10.5	15.3
Teflubenzuron	106.5	105.7	87.7	101
Imibenconazole	88.8	90.1	95.6	109.2
Fluacrypyrim	96.6	96.3	73.8	85.8
Pentoxazone	98.4	94.7	78.3	90.3
Pyribenzoxim	95.6	93.5	65	67.7
Fenpyroximate	110.9	113.5	106.5	134.6
Spirodiclofen	111.6	107.1	89.1	98.6

2.3 Establishing soil extraction condition

In this experiment, 3 different solvents were used. And these selected soil solvent were referred from soil residue analysis method for herbicide(6), soil residue analysis method for insecticide(7), soil residue analysis method for fungicide(8) published by NAQS. First condition was extracting with 100 mL of acetone. Second condition was extracting with 100 mL of acetonitrile. Last condition was extracting with 100 mL of acetonitrile with adding 30 mL of saturated NaCl. 100 mL of acetonitrile with adding 30 mL of saturated NaCl showed the best result. The results are shown in Table 10. In Table 10, results are shown as before clean up and after clean up.

Table 10. Recovery of the before and after soil clean up (UVD-1)

① Extract with 100 mL of acetone, ② Extract with 100mL of acetonitrile, ③ Extract with 100mL of acetonitrile and 30 mL of saturated NaCl

Recovery(%)	Before clean up			After clean up		
	①	②	③	①	②	③
Clothianidin	77.8	95.9	100.8	30.3	62.1	92.2
Thiabendazole	0	0	49.1	0	0	45.3
Thiacloprid	81.5	89.2	103.5	34.9	72.2	100.4
Quinoclamine	90.9	89.5	91.8	38.2	87.9	93.7
Pirimicarb	65.3	17.2	94.7	22.7	0	86.4
Ferimzone	0	0	70.5	0	0	86.7
Dimethomorph1	101.3	89	94.7	35.6	93.1	105.4
Dimethomorph2	96.8	95.6	113.8	31.7	86.1	96
Flumioxazin	101	103.3	102	37.8	95.3	99
Dimethylvinphos	100.1	98.5	106.9	38.7	96.8	93.9
Ametoctradin	86.1	91.2	115.1	0	101.2	88.3
mepanipyrim	99.8	98.6	98.7	40.4	100.8	100.6
Chromafenozide	105.9	105	111.9	39.5	93	102.4
Tebufenozide	100.9	107.5	104.2	40.6	96.3	98.1
Clofentezine	91.9	111.4	104.6	32.9	95.1	97.2
Hexaflumuron	101.2	106.9	104.2	0	99.8	113.9
Metamifop	114.9	124.5	112.3	36.1	108.3	97.5
Novaluron	108.3	98.2	96.6	0	98.2	102.7
Trifloxystrobin	102.1	102.2	99.1	37.3	97.6	99
Cyhalofop-butyl	99.7	99.5	99.8	43.8	100.8	102.7

Pyriproxyfen	100.1	98.5	94.6	57.3	99.7	101.2
Pyributicarb	95.1	117.6	106.2	39.9	101.6	101.5
Lepimectin1	111.6	98	133.8	28.9	101.6	114.2
Lepimectin2	108.2	77.5	110.8	28.8	72.4	99.1

2.4 Establishing water extraction condition

3 different extraction conditions were used in this experiment. First condition was extracting with 100 mL of dichloromethane with 20 g of NaCl. Second condition was extracting with 100 mL of ethyl acetate with 20 g of NaCl. Last condition was extracting with 100 mL of acetonitrile with 20 g of NaCl. It was analyzed without clean up since there can be an interference in clean up steps. 5 fold concentration was followed because of developed method's concentration. The results are shown in Table 11.

Table 11. Recovery of water extraction

① Extract with 100 mL of dichloromethane with 20 g of NaCl,② Extract with 100 mL of dichloromethane with 20 g of NaCl, ③ Extract with 100 mL of acetonitrile with 20 g of NaCl

Recovery(%)	Before clean up			After clean up		
	①	②	③	①	②	③
Clothianidin	77.8	95.9	100.8	30.3	62.1	92.2
Thiabendazole	0	0	49.1	0	0	45.3
Thiacloprid	81.5	89.2	103.5	34.9	72.2	100.4
Quinoclamine	90.9	89.5	91.8	38.2	87.9	93.7
Pirimicarb	65.3	17.2	94.7	22.7	0	86.4
Ferimzone	0	0	70.5	0	0	86.7
Dimethomorph1	101.3	89	94.7	35.6	93.1	105.4
Dimethomorph2	96.8	95.6	113.8	31.7	86.1	96
Flumioxazin	101	103.3	102	37.8	95.3	99
Dimethylvinphos	100.1	98.5	106.9	38.7	96.8	93.9
Ametoctradin	86.1	91.2	115.1	0	101.2	88.3
mepanipyrim	99.8	98.6	98.7	40.4	100.8	100.6
Chromafenozide	105.9	105	111.9	39.5	93	102.4
Tebufenozide	100.9	107.5	104.2	40.6	96.3	98.1
Clofentezine	91.9	111.4	104.6	32.9	95.1	97.2
Hexaflumuron	101.2	106.9	104.2	0	99.8	113.9
Metamifop	114.9	124.5	112.3	36.1	108.3	97.5
Novaluron	108.3	98.2	96.6	0	98.2	102.7
Trifloxystrobin	102.1	102.2	99.1	37.3	97.6	99
Cyhalofop-butyl	99.7	99.5	99.8	43.8	100.8	102.7

Pyriproxyfen	100.1	98.5	94.6	57.3	99.7	101.2
Pyributicarb	95.1	117.6	106.2	39.9	101.6	101.5
Lepimectin1	111.6	98	133.8	28.9	101.6	114.2
Lepimectin2	108.2	77.5	110.8	28.8	72.4	99.1

3. System suitability test

It can be said that the most important factor in the system suitability test is the resolution, especially in multiresidue analysis. Because in the chromatogram, there are many peaks therefore not overlaying with other peak is very important.

Retention factor represent how well analyte is remained in the chromatogram. For the single peak in the chromatogram, 1−10 is good for the retention factor. Separation factor is calculated by retention factor. It indicates that how compounds are well separated. Appropriate range for the separation factor is about 1.5. The number of theoretical plate indicates the column efficiency. Resolution shows how compounds are well separated. It seems same factor compare to the separation factor but in here, many other sources are considered (peak width, separation factor, the number of theoretical plate). Generally, bigger than 1.5 is good result for the resolution. By symmetry, fronting and tailing can be checked. Smaller than 1 is

fronting and bigger than 1 is tailing. Normally 0.8 – 1.2 is appropriate for the peak.

Table 12. System suitability of UVD-1

Name	T_o(min)	T_R(min)	K	Width	N(Plate)	R	α	As
Clothianidin	2.52	4.96	0.97	0.19	3821	8.60	x	1.28
Thiabendazole	2.52	6.40	1.54	0.22	4886	4.19	1.59	1.11
Thiacloprid	2.52	8.90	2.53	0.27	5872	6.00	1.64	1.39
Quinoclamine	2.52	11.93	3.74	0.16	30178	8.19	1.48	1.08
Pirimicarb	2.52	14.25	4.66	0.12	73112	9.51	1.25	0.93
Ferimzone	2.52	16.26	5.46	0.11	116769	10.03	1.17	0.81
Dimethomorph1	2.52	17.52	5.96	0.09	190639	7.17	1.09	0.94
Dimethomorph2	2.52	18.01	6.15	0.09	201394	3.03	1.03	0.95
Flumioxazin	2.52	19.13	6.60	0.09	232715	7.02	1.07	0.94
Dimethylvinphos	2.52	19.85	6.88	0.10	233658	4.47	1.04	0.90
Amectotradin	2.52	20.40	7.10	0.09	264739	3.40	1.03	0.90
Mepanipyri	2.52	21.13	7.39	0.10	240884	4.39	1.04	0.91
Chromafenozide	2.52	21.80	7.66	0.09	324887	4.08	1.04	0.91
Tebufenozide	2.52	22.78	8.05	0.09	363952	6.49	1.05	0.92
Clofentezin	2.52	24.83	8.86	0.10	335909	12.67	1.10	0.99
Hexaflumuron	2.52	25.01	8.93	0.09	451494	1.15	1.01	0.94
Metamifop	2.52	25.56	9.15	0.09	408280	3.56	1.02	0.93
Novaluron	2.52	25.82	9.25	0.09	491684	1.66	1.01	0.96
Trifloxystrobin	2.52	26.13	9.38	0.09	434323	2.05	1.01	0.94
Cyhalofop-butyl	2.52	26.56	9.55	0.09	438062	2.66	1.02	0.95
Pyriproxyfen	2.52	28.05	10.14	0.10	402856	8.81	1.06	0.96
Pyributicarb	2.52	28.66	10.38	0.10	443233	3.53	1.02	0.95
Lepimectin1	2.52	30.95	11.29	0.08	809985	14.77	1.09	0.96
Lepimectin2	2.52	31.91	11.67	0.08	902707	7.01	1.03	0.95

4. Method validation based on KOLAS-G-015 guideline

After the development of method, method validation has to be done. Main purpose of validation is to prove that it is suitable for the designed purpose. By doing this method validation, other researcher can use this method without any doubt. By giving certain result of these 9 factors, this newly developed method can be validated and result obtained by this method can be trustful.

4.1. Linearity

In the Quantification, there has to be a standard to compare with the sample, so it can calculate the amount in the sample. Calibration curve is used to calculate the amount of sample and the linearity is the factor which can be achieved by calibration curve. When Linearity' s R^2 is getting close to the 1, it means their quantification result is much more accurate. Generally in screening method, R^2>0.99 is suitable for the method. And in quantitation method,

R^2>0.999 is suitable for the method. In this experiment most of the pesticide showed R^2 higher than 0.99. Table 13 shows linearity of UVD-group1 and UVD-group3.

Table 13. Linearity of UVD-1 and UVD-3

HPLC-UVD1	R^2	HPLC-UVD3	R^2
Clothianidin	0.9998	Carbendiazim	0.9933
Thiabendazole	0.9999	Tricyclazole	0.9999
Thiacloprid	0.9998	Pyroquilon	1
Quinoclamine	0.9998	Simazine	0.9999
Pirimicarb	0.9998	Thiophanate-methyl	0.9929
Ferimzone	0.9998	Bendiocarb	0.9999
Dimethomorph1	0.9998	Metalaxyl	0.9999
Dimethomorph2	0.9998	Ethaboxam	0.9998
Flumioxazin	0.9997	Chlorantraniliprole	0.9999
Dimethylvinphos	0.9999	Paclobutrazole	0.9997
Ametoctradin	0.9997	Benthiavalicarb-isopropyl	0.9998
mepanipyrim	0.9998	Tiadinil	0.9999
Chromafenozide	0.9998	Mandipropamid	0.9999
Tebufenozide	0.9998	Fluopicolide	0.9994
Clofentezine	0.9998	Methoxyfenozide	1
Hexaflumuron	0.9997	Metolachlor	0.9999
Metamifop	0.9998	Flubendiamide	0.9999
Novaluron	0.9998	Pencycuron	1
Trifloxystrobin	0.9998	Benzoximate	0.9999
Cyhalofop-butyl	0.9998	Amisulbrom	0.9999

Pyriproxyfen	0.9997	Hexythiazox	0.9907
Pyributicarb	0.9997	Flufenoxuron	0.9999
Lepimectin1	0.9998	Oxaziclomefon	0.9999
Lepimectin2	0.9998	Spiromesifen	1
		Etofenprox	0.9999
		Silafluofen	0.9999

4.2 Sensitivity

By calibration curve, formula in the form of 'y=ax+b' is achieved. In this formula, 'a' is usually called as slope. And this is the sensitivity. The better slope means a better sensitivity. It means that with much lower concentration, much higher peak can be obtained when it has higher sensitivity than the other. The results are shown in the Table 14. The result shows that pesticides in the FLD-groups have much higher sensitivity than pesticides in the UVD-groups.

Table 14. Sensitivity range of pesticides

Slope	Number of pesticides (UVD)	Number of pesticides (FLD)
1 - 25	21	-
25-50	39	-
50-100	11	-
100-500	1	-
500-1000	-	3
1000-2000	-	6
2000-3000	-	6
3000-	-	-
Total	72	15

4.3 Statistic LOD/LOQ

When the S/N ratio of peak is about 3, it becomes LOD and when it is about 10, it becomes LOQ. Definition of LOD is the limit of detection. If chromatogram shows lower than LOD, it is assumed as not detected. Definition of LOQ is the limit of quantitation. To quantify, target peak has to be the height of the LOQ. If target peak shows lower than LOQ but higher than LOD, it can be said that it is detected but not able to quantify. These LOD and LOQ are listed in Table 15.

Table 15. Number of pesticides of statistical LOQ in certain concentration range

ppm	UVD1	UVD2	UVD3	FLD1	FLD2
<0.01	-	-	-	-	-
0.01-0.05	-	-	-	6	4
0.05-0.1	13	18	12	1	3
0.1-1	11	4	14	1	-
>1	-	-	-	-	-

4.4 Working range

As described above in the linearity, R^2 closed to 1 is better for quantifying target compound. But calibration curve can't maintain linearity endlessly. In some part, it will lose its linearity. So we use only straight part of the calibration curve and in other term, it is called as working range. In some high concentration, calibration curve start to bend. In other term, curve is saturated. And in low concentration, certain concentration will be decided to be the lowest concentration for the calibration curve which is available to quantify.

In this experiment, the lowest concentration is set to 1/5 of the statistical LOQ. Statistical value has to be divided by 5 because developed method's concentration factor is the 5. In some high concentration, calibration curve start to bend. In other term, curve is saturated. This saturated concentration becomes the highest concentration of the working range. Working range for pesticide are described in Table 16.

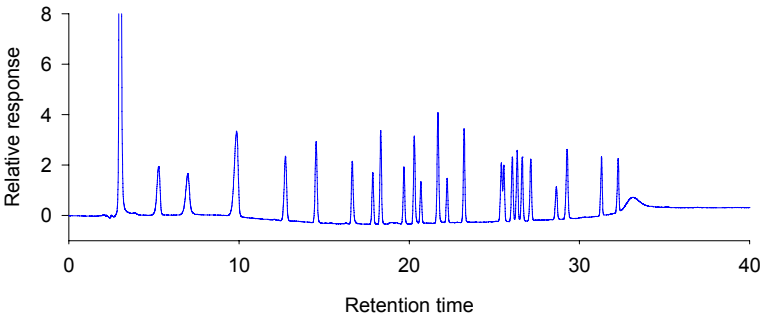
Table 16. Working range of UVD-1

HPLC-UVD1	Working range (ppm)	
	min	max
Clothianidin	0.01	4.402
Thiabendazole	0.1	30.55
Thiacloprid	0.02	6.44
Quinoclamine	0.01	3.54
Pirimicarb	0.01	3.42
Ferimzone	0.01	3.63
Dimethomorph1	0.02	9.1
Dimethomorph2	0.01	5.44
Flumioxazin	0.02	8.93
Dimethylvinphos	0.02	5.77
Ametoctradin	0.02	9.54
mepanipyrim	0.01	2.08
Chromafenozide	0.01	5.71
Tebufenozide	0.02	5.54
Clofentezine	0.01	3.76
Hexaflumuron	0.01	3.8
Metamifop	0.02	8.23
Novaluron	0.02	6.73
Trifloxystrobin	0.01	3.76
Cyhalofop-butyl	0.01	4.11
Pyriproxyfen	0.02	14.52
Pyributicarb	0.02	6.39
Lepimectin1	0.01	3.86
Lepimectin2	0.01	3.81

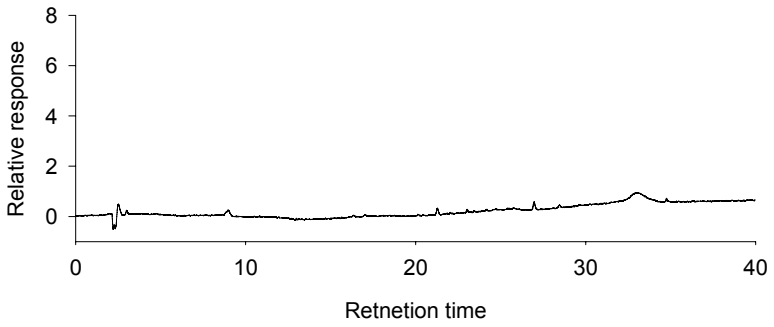
4.5 Selectivity

Selectivity is the accuracy of the measurement when there's interference. The target pesticide should not be overlaid with interference or other target pesticides. If it is overlaid, it would be impossible to quantify the target pesticide.

In this experiment some pesticide are added from GC list and new pesticide list of NAQS. All the pesticides are well separated and not overlaid. Chromatogram of UVD-1 is shown in Figure 9.



(a)



(b)

Figure 9. Chromatogram of UVD-1

(a) Standard, (b) control of DW

4.6 Trueness and Precision

In this part, 7 repeated recovery test was used to achieve Trueness and Precision. In KOLAS guideline, trueness defined as how closed to the accepted reference value(32). Bias is represented as the systematic error. It is quantitative expression of trueness. When bias is increased, trueness starts to decrease.. Precision is the random error which achieved by repeated experiment under certain condition. Generally Precision is expressed as relative standard deviation. In this experiment, Precision is defined as CV(coefficient of variation)

In this experiment, acceptable average recovery is 50%~150% and acceptable CV is under 30%. This criteria is used in NAQS and MFDS(Ministry of food and drug safety)(36). The results are shown in the Table 17. As shown in the table, waters show better results compare to the soil especially in recovery 70%~120%.

Table 17. Recovery and CV of soil and water

(a)Recovery of soil and water, (b)CV of soil and water

Recovery(%)	Paddy soil	Upland soil	Orchard soil	Diluted water	River	Ground water	Lake
50>	3	4	6	4	9	8	8
50-70	5	3	11	5	2	1	1
70-120	64	67	62	71	71	75	69
120-150	11	11	6	6	2	1	6
150<	4	2	2	1	3	2	3
Total	87	87	87	87	87	87	87

(a)

CV(%)	Paddy soil	Upland soil	Orchard soil	Diluted water	River	Ground water	Lake
nd							
10>	51	26	24	12	51	24	49
10~20	18	49	47	56	30	49	30
20-35	16	10	11	18	3	11	4
35<	2	2	5	1	3	3	4
Total	87	87	87	87	87	87	87

(b)

4.7 Method LOD/LOQ

Method LOD is the minimum amount or concentration of the target compound that is definitely distinguished with 0(32). Method LOQ is generally the minimum concentration of target compound which can be reliably quantified with a certain degree(30). Normally 3 times of LOD is defined as LOQ.

Researcher should not confuse with instrumental (statistical) LOD/LOQ and method LOD/LOQ. Instrumental LOD/LOQ is usually achieved by S/N ratio in the chromatogram. It is valuable factor but not suitable for the method LOD/LOQ.

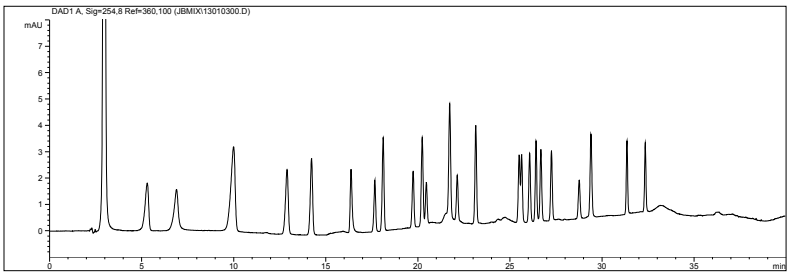
To calculate method LOD/LOQ, all the procedure has to be concern. The result of Method LOQ is shown in the table 18.

Table 18. The number of pesticides in in certain range Method LOQ

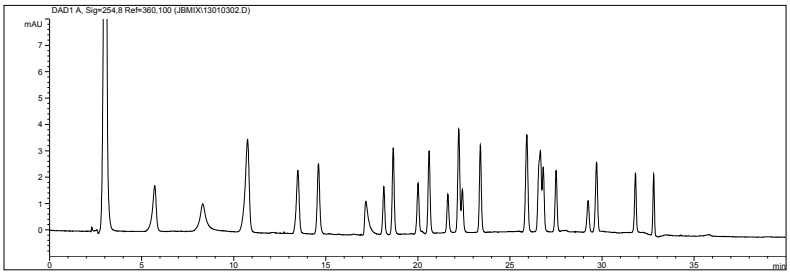
Mg/kg	Paddy soil	Upland soil	Orchard soil	Diluted water	River	Ground water	Lake
ND							
0.01>	2		1	1	1		4
0.01−0.05	23	10	11	43	47	40	35
0.05−0.1	19	19	16	26	26	32	28
0.1−1	41	58	59	17	13	14	20
1<	2					1	
Total	87	87	87	87	87	87	87

4.8 Ruggedness

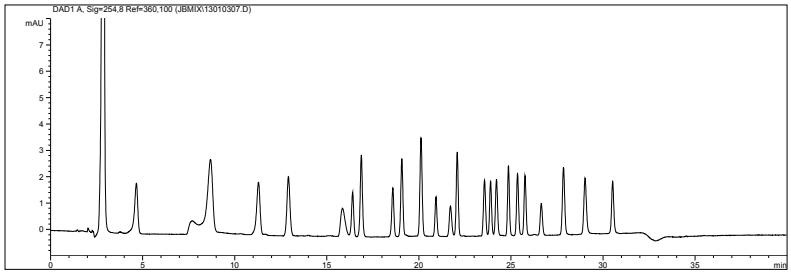
In this experiment 5 different columns were used for the Ruggedness. The result's chromatograms are shown in the figure 10. All the chromatograms show some different in the result.



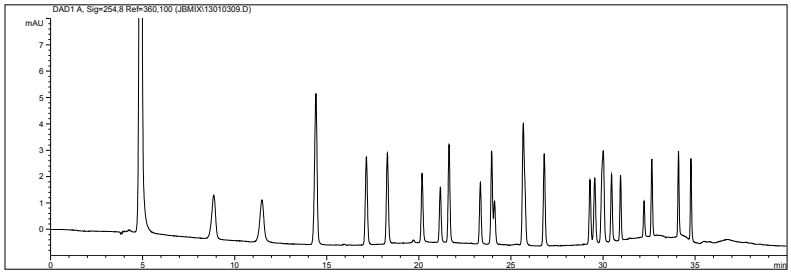
(a) Phenomenex Gemini-NX (150 × 4.6 mm, 3 μm)



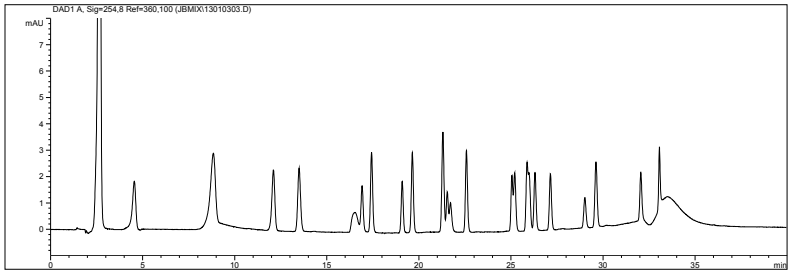
(b) Shiseido Capcellpak C18 MG (150 × 4.6 mm, 3 μm)



(c) Agilent Eclipse XDB-C8 (150 × 4.6 mm, 5 μm)



(d) Phenomenex Gemini-NX (250 × 4.6 mm, 3 μm)



(e) Agilent Eclipse XDB-C18 (150 × 4.6 mm, 5 μm)

Figure 10. Chromatogram of UVD-1 in 5 different columns

4.9 Measurement Uncertainty

In this experiment, to calculate the measure uncertainty, monitoring samples were gathered from 9 province of the nation. Detailed calculation was followed by NAQS method and EURACHME. The results are shown in the figure 11.

■ Uncertainty of Sample

1. Uncertainty of Sample weight (u_{weight})

(1) Accuracy(calibration)

$u_{calibration}$ = 0.0002 g

0.0003 g at 95 % confidence level

k = 2

NOTE:

Detailed calculations for uncertainties in mass can very intricate, and it is important to refer to calibration certificate.

(2) Readability

0.0001 g

$u_{readability}$ = 0.000029 g

(3) Stability

Sp of 9 repeat weighings of 50 g

$u_{stability}$ = 0.000091 g

number of trial	weigh(g)		
	12/10	12/11	12/12
1	50.0015	50.0012	50.0014
2	50.0017	50.0015	50.0013
3	50.0017	50.0014	50.0014
average	50.0016	50.0014	50.0014
Si	0.0001	0.0002	0.0001
Vi	2	2	2
Sp	0.0003	n	9
Uc	0.000091	vi	8

Combined Uncertainty of Stock weight

$u_{weight} = \sqrt{(u_{calibration}^2 + u_{readability}^2 + u_{stability}^2)} =$ 0.00018

2. Uncertainty of 100 mL Graduated Cylinder(u_{volum})

(1) Tolerance

manufacturer's specification : 100 mL ± 0.2 mL at 20 degree ℃ k = 2

assuming a **rectangular distribution**

$u_{tolerance}$ = **0.10** mL

(2) Temperature

calibrated temp by manufacturer : 20 degree ℃

laboratory temp variation : 5 degree ℃

vol	100	
temp var.	5	
exp. Coef.	0.00021	
vol * temp * expansion coefficient =		0.1050

assuming a **rectangular distribution**

$u_{temperature}$ = **0.061** mL

(3) Repeatability

SD of 5 repeat weighings of 100 mL filled to mark with water

$u_{repeatability}$ = **0.039** mL

number of trial	measurement	
1	98.5639	
2	98.4084	
3	98.3694	
4	98.4422	
5	98.5511	
c. value	0.2000	
n	5	
average	98.4670	
Si	0.0867	
Vi	4	0.0075
Sp	0.0867	
Uc	0.0388	

Combined Uncertainty of 100 mL Graduated Cylinder

$u_{graduated\ cylinder} = \sqrt{(u_{tolerance}^2 + u_{temperature}^2 + u_{repeatability}^2)}$ = **0.123**

3. Uncertainty of 20 mL pipette(uvolum)

(1) Tolerance

manufacturer's specification : 20 mL ± 0.06 mL at 20 degree ℃ k = 2

assuming a **rectangular distribution**

$u_{tolerance}$ = **0.030** mL

(2) Temperature

calibrated temp by manufacturer : 20 degree ℃

laboratory temp variation : 5 degree ℃

vol	20
temp var.	5
exp. Coef.	0.00021
vol * temp * expansion coefficient =	0.0210

assuming a **rectangular distribution**

$u_{temperature}$ = **0.0121** mL

(3) Repeatability

SD of 5 repeat weighings of 20 mL filled to mark with water

$u_{repeatability}$ = **0.0112** mL

number of trial	measurement
1	19.8180
2	19.7990
3	19.8648
4	19.8412
5	19.8228
c. value	0.0140
n	5
average	19.8292
Si	0.0249
Vi	4
Sp	0.0249
Uc	0.0112

0.0006

Combined Uncertainty of 20 mL pipette

$u_{pipette} = \sqrt{(u_{tolerance}^2 + u_{temperature}^2 + u_{repeatability}^2)}$ = **0.0342**

4. Uncertainty of 2 mL pipette(uvolum)

(1) Tolerance

manufacturer's specification : 2 mL ± 0.02 mL at 20 degree ℃ k = 2

assuming a **rectangular distribution**

$u_{tolerance}$ = 0.0100 mL

(2) Temperature

calibrated temp by manufacturer : 20 degree ℃

laboratory temp variation : 5 degree ℃

vol	2
temp var.	5
exp. Coef.	0.00021
vol * temp * expansion coefficient =	0.0021

assuming a **rectangular distribution**

$u_{temperature}$ = 0.00121 mL

(3) Repeatability

SD of 5 repeat weighings of 1 mL filled to mark with water

$u_{repeatability}$ = 0.0020 mL

number of trial	measurement
1	2.0017
2	2.0016
3	1.9984
4	2.0067
5	1.9944
c. value	-0.0230
n	5
average	2.0006
Si	0.0045
Vi	4
Sp	0.0045
Uc	0.0020

0.0000207

Combined Uncertainty of 2 mL pipette

$u_{pipette} = \sqrt{(u_{tolerance}^2 + u_{temperature}^2 + u_{repeatability}^2)}$ = 0.01028

5. Uncertainty of 1 mL pipette(uvolum)

(1) Tolerance

manufacturer's specification : 1 mL ± 0.01 mL at 20 degree ℃ k = 2

assuming a **rectangular distribution**

$u_{tolerance}$ = 0.005 mL

(2) Temperature

calibrated temp by manufacturer : 20 degree ℃

laboratory temp variation : 5 degree ℃

vol	1
temp var.	5
exp. Coef.	0.00021
vol * temp * expansion coefficient =	0.0011

assuming a **rectangular distribution**

$u_{temperature}$ = 0.0006 mL

(3) Repeatability

SD of 5 repeat weighings of 1 mL filled to mark with water

$u_{repeatability}$ = 0.0012 mL

number of trial	measurement
1	1.0199
2	1.0171
3	1.0144
4	1.0135
5	1.0177
c. value	0.0140
n	5
average	1.0165
Si	0.0026
Vi	4
Sp	0.0026
Uc	0.0012

0.0000

Combined Uncertainty of 1 mL pipette

$u_{pipette} = \sqrt{(u_{tolerance}^2 + u_{temperature}^2 + u_{repeatability}^2)}$ = 0.0052

■ Uncertainty of Standards Solution

1. Uncertainty of Sample weight (u_{weight})

(1) Accuracy(calibration)

$u_{calibration}$ = **0.0002** g

0.0003 g at 95 % confidence level

NOTE: k = 2

Detailed calculations for uncertainties in mass can very intricate, and it is important to refer to calibration certificate.

(2) Readability

0.0001 g

$u_{readability}$ = **0.000029** g

(3) Stability

Sp of 9 repeat weighings of 50 g

$u_{stability}$ = **0.000091** g

number of trial	weigh (g)		
	12/10	12/11	12/12
1	50.0015	50.0012	50.0014
2	50.0017	50.0015	50.0013
3	50.0017	50.0014	50.0014
average	50.0016	50.0014	50.0014
Si	0.0001	0.0002	0.0001
Vi	2	2	2
Sp	0.0003	n	9
Uc	0.000091	vi	8

Combined Uncertainty of Stock weight

$u_{weight} = \sqrt{(u_{calibration}^2 + u_{readability}^2 + u_{stability}^2)}$ = **0.00018**

2. Uncertainty of 25 mL Volumetric Flask(uvol

(1) Tolerance

manufacturer's specification : 25 mL ± 0.06 mL at 20 degree ℃ k = 2

assuming a **rectangular distribution**

$u_{tolerance}$ = **0.030** mL

(2) Temperature

calibrated temp by manufacturer : 20 degree ℃

laboratory temp variation : 5 degree ℃

vol	25
temp var.	5
exp. Coef.	0.00021
vol * temp * expansion coefficient =	0.0263

assuming a **rectangular distribution**

$u_{temperature}$ = **0.015** mL

(3) Repeatability

SD of 5 repeat weighings of 25 mL filled to mark with water

$u_{repeatability}$ = **0.0396** mL

number of trial	measurement
1	24.7016
2	24.7964
3	24.8852
4	24.7987
5	24.9304
c. value	-0.0400
n	5
average	24.8225
Si	0.0887
Vi	4
Sp	0.0887
Uc	0.0396

0.0079

Combined Uncertainty of 25 mL Volumetric Flask

$u_{volumetric\ flask} = \sqrt{(u_{tolerance}^2 + u_{temperature}^2 + u_{repeatability}^2)}$ = **0.0520**

3. Uncertainty of Purity (u_{purity}) Fluquinconazole. 99.4%

Purity of Standard = 99.4 % (100 % ± 1.2 %) = 1.0 ± 0.012
assuming a rectangular distribution

u_{purity} = 0.0069

Combined Uncertainty of Stock Standard Solution-A

	value		u	ur
Weight(mg)	0.0253		0.0002	0.00703
Vol(mL)	25		0.0520	0.0021
Purity(%)	0.994		0.0069	0.007
Conc =	1	g/L		

$u_{standard} = conc\sqrt{((u_{weight}/Weight)^2 + (u_{vol}/Vol)^2 + (u_{purity}/Purity)^2)}$ **0.0102** g/L

u_{std}/Std = **0.0101**

■ Uncertainty of Repeatability

1. Uncertainty of Recovery measurement from QC samples (n=21)

	QC samples		
No.	paddy	upland	orchard
1	102.100	101.500	103.400
2	101.000	131.700	110.300
3	102.000	129.600	101.900
4	99.500	107.800	96.400
5	94.600	117.500	104.100
6	95.300	127.500	101.100
7	92.900	123.400	95.700
Mean(%)	98.200	119.857	101.843
Standard deviation(%)	3.844	11.504	4.949
Vi	6	6	6
Sp	0.076	Mean.Total	1.066
R.Sp	0.070928		

Mean. Recovery	**1.066**
Standard uncertainty	**0.076**
Relative standard uncertatinty	**0.070928**

■ Uncertainty of Calibration curve

standard	concentration(L	1	2	3	mean	slope	22.51
STD-0	0.00	0			0.00	Y-axis	-0.31
STD-1	0.50	10.72467			10.72	X-axis	-0.01
STD-2	1.00	22.00443			22.00	Correl	1.000
STD-3	5.00	112.77273			112.77		
STD-3	10.00	224.15941			224.16		
STD-4	20.00	450.05377			450.05	Degree of freedom	4

b1 = Slope	22.5086
b0 =Intercept	-0.308
p = number of analysis for sam	1
m = total number of measurem	6
y = Area	8.78
x = measurement of sample (r	505.16
$\bar{x}$ = average concentration of standard solution(ng)	6.08
S	0.4718
Sxx	304.2083
$u_c(x)$ =	0.6002
$u_{STDcurve}$ /Conc =	0.0012
content(mg/kg) =	50.516046

formula

$$u_c(x) = \frac{s}{b_1}\sqrt{\frac{1}{p} + \frac{1}{m} + \frac{(x-\bar{x})^2}{S_{xx}}}$$

s

Aj = STD - j Level's result

b0 =Intercept

b1 = Slope

Cj = STD - j Level's concentration

n-2 = number of total measurement(m)-2

$$S = \sqrt{\frac{\sum_{j=1}^{n} [A_j - (B_0 + B_1 \times C_j)]^2}{n-2}}$$

numerator in formula	((Ai-(b0+b1xci))^2	No.1
STD-0's concentration and result		0.09491
STD-1's concentration and result		0.04908
STD-2's concentration and result		0.03845
STD-3's concentration and result		0.28930
STD-4's concentration and result		0.38242
STD-5's concentration and result		0.03613
sum of total concentration ∑	0.8903	
n-2 = number of total measure	4	
S =	0.4718	

Sxx

Cj = STD - j Level's concentration

$\overline{C}$ = average concentration of std solution(Mean)

Sxx 304.21

$$S_{xx} = \sum_{j=1}^{n} (C_j - \overline{C})^2$$

example

formula	Sxx= (Cj - C)^2	calculate
((STD-0value) - (average std solution))^2		37.0069
((STD-1value) - (average std solution))^2		31.1736
((STD-2value) - (average std solution))^2		25.8403
((STD-3value) - (average std s		1.1736
((STD-4value) - (average std s		15.3403
((STD-5value) - (average std solution))^2		193.6736
sum(Sxx) =		304.21

B_1 : Slope ,

B_0 : Intercept

P : Number of measurements to determine conc.

n : Number of measurements for the calibration

C0 : Determined concentration

$\overline{C}$: Mean value of the different calibration standards

I : Index for the number of calibration standards to obtain the calibration Curve

J : Index for the number of measurements

1. combined uncertainty of sample treatment

1) weigh sample 50 g

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Accuracy	x	0.000150	x	∞
Readability	x	0.000029	x	∞
Stability	x	0.000091	x	8.00
combined	50	0.000178	0.000004	116.79

2) 100mL measuring cylinder uncertainty

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Tolerance	x	0.100000	x	∞
Temperature	x	0.060622	x	∞
Repeatablity	100	0.038754	0.000388	4.00
combined	100	0.123194	0.001232	408.47

3) 20mL pipette uncertainty

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Tolerance	x	0.030000	x	∞
Temperature	x	0.012124	x	∞
Repeatablity	20	0.011157	0.000558	4.00
combined	20	0.034227	0.001711	354.22

4) 2mL pipette uncertainty

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Tolerance	x	0.010000	x	∞
Temperature	x	0.001212	x	∞
Repeatablity	2	0.002033	0.001017	4.00
combined	2	0.010276	0.005138	2609.51

5) 1mL pipette uncertainty

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Tolerance	x	0.005000	x	∞
Temperature	x	0.000606	x	∞
Repeatablity	1	0.001157	0.001157	4.00
combined	1	0.005168	0.005168	1592.58

6) combined uncertainty in sample treatment

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
50g	50	0.000178	0.000004	116.79
100mL	100	0.123194	0.001232	408.47
20mL	20	0.034227	0.001711	354.22
2mL	2	0.010276	0.005138	2609.51
1mL	1	0.005168	0.005168	1592.58
1mL	1	0.005168	0.005168	1592.58
combined	0.2	0.001836	0.009179	5952.98

2. Combined uncertainty in making stock solution

1) weigh standard

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Accuracy	x	0.000150	x	∞
Readability	x	0.000029	x	∞
Stability	x	0.000091	x	8.00
combined	0.0253	0.000178	0.007027	116.79

2) 25mL measuring flask uncertainty

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Tolerance	x	0.030000	x	∞
Temperature	x	0.015155	x	∞
Repeatablity	25	0.039647	0.001586	4.00
combined	25	0.051977	0.002079	11.82

3) Standard purity uncertainty

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
uncertainty	0.994	0.006928	0.006970	∞

4) Combined uncertainty in making stock solution

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
Weight(g)	0.0253	0.000178	0.007027	116.79
Volume(mL)	25	0.051977	0.002079	11.82
Purity	0.994	0.006928	0.006970	∞
uncertainty	1.006	0.010173	0.010113	465.86

3. Measurement uncertainty in repeatability

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
uncertainty	1.000	0.075633	0.070928	18.00

4. Measurement uncertainty in calibration cure

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
uncertainty	505.160	0.600175	0.001188	4.00

5. Calculating expanded uncertainty

	measurd	standard uncertainty	relative uncertainty	Degree of freedom
sample treatmen	0.2	0.001836	0.009179	5952.98
stock solution	1.006	0.010173	0.010113	465.86
repeatability	1.000	0.075633	0.070928	18.00
Calbration curve	505.160	0.600175	0.001188	4.00
LOQ	0.05	0	0	∞
combined	5.082	0.367094	0.072241	19.37

K value of 95% confidence 2.093

Expanded standard uncertainty 0.768328

(5.082 ± 0.768) μg/kg (k=2.093)

Figure 11. Measurement uncertainty of Fluquinconazole

Ⅳ. Conclusion

Interest in food safety is getting bigger. Pesticide is necessary for the agricultural products and for this reason, soil and water have to be managed.

For the environment safety, pesticide multiresidue analysis for the soil and water was developed. Based on NAQS's crop and vegetable pesticide HPLC analysis, method was developed. 12 pesticides were added to the list of 73 pesticide HPLC-UVD/FLD analysis on NAQS. Total 85 pesticides were analyzed for the soil and water. In the extraction solvent compare experiment, acetonitrile, dichloromethane and ethyl acetate were used. Among these solvent, acetonitrile showed the most suitable result. In soil wetting condition experiment, 30 mL of saturated solution was decided to use. In case of analyzing with FLD, filtration was performed instead of SPE clean-up. Developed method was validated by using 7 repeated recovery test

based on KOLAS guideline. Linearity, selectivity, sensitivity, statistical LOD/LOQ, method LOD/LOQ, working range, trueness and precision, ruggedness, measurement uncertainty were the factor for the method validation. All the result values were well obtained. Also system suitability test was performed. Retention factor, separation factor, theoretical number of plate, resolution and symmetry was used for the system suitability test. Achieved results were prospered for the test. MFDS's validation guideline was used for the system suitability test.

These days, pesticides analysis using QuEChERS along with MS is popular. QuEChERS means quick, easy, cheap, effective, rugged and safe (37). Compare to the conventional pesticide analysis method, it is less time consuming, less expensive and much convenient (38). It is better coupled with MS since MS has the high selectivity. But when using MS, matrix effect has to be concerned since it cause different on the results (39).

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국문요약

HPLC-UVD/FLD 를 이용한 토양 및 용수 중 잔류농약 다성분 동시 분석법 확립

김 병 준

본 연구에서는 식품 안전과 직접 관련이 있는 농작물 재배 토양 및 농업용수의 안전성을 확보하기 위해 토양 및 용수에 대해 HPLC-UVD/FLD 를 사용하여 85 종 농약 다성분 분석법을 확립하였다. 농산물 품질관리원에서 작물 중 잔류농약 다성분 분석대상인 73 종 농약 (UVD 3 그룹, FLD 2 그룹)을 바탕으로 그룹의 종류와 retention time 을 조사하여 12 종의 농약을 새로 추가하였다(UVD 그룹 1 22 종, UVD 그룹 2 22 종, UVD 그룹 3 26 종, FLD 그룹 1 8 종, FLD 그룹 2 7 종). UVD 그룹 3 과 FLD 그룹 1, 2 의 파장을 변경하여 감도를 높였다

(UVD 그룹 3 : 235 nm → 225 nm, FLD 그룹 : excitation 330 nm → 240 nm, emission 446 nm → 450nm). 토양 및 용수의 전처리법확립을 위해 습윤법 (NH4Cl), 다양한 추출용매 (acetonitrile, dichloromethane, ethyl acetate)의 조합을 시도하였고, 토양은 acetonitrile+포화 NaCl 30 mL, 용수는 acetonitrile+NaCl 20 g 을 사용할 때 전반적으로 가장 회수율이 좋았다. 확립된 분석법에 7 반복한 회수율을 구하고 이 자료를 바탕으로 직선성, 선택성, 감도, ILOD/ILOQ, 적용범위, MLOD/MLOQ, 진도와 정밀성, 측정불확도, 둔감도등 총 9 인자에 대한 분석법의 유효성을 검증하였다. 또한 retention factor, separation factor, number of theoretical plate, resolution, symmetry 의 값을 구하여 시스템 적합성 시험을 평가하였다.

주요어 : 토양, 용수, 농약, 다성분 분석, 유효성 검증, 시스템적합성

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