STUDY ON ANALYTICAL METHOD FOR SIMULTANEOUS DETERMINATION OF 40 PESTICIDES RESIDUE IN GOLF COURSE

Kim HS[1], Yoon JK[1], Noh H-J[1], Kim TS[1]*, Boo M-H[2], Kang Y-S[2]

[1]National Institute of Environmental Research, Environmental Research Complex, Seo-Gu, Kyoungseo-Dong, Incheon 404-708, Republic of Korea; [2]Lab Frontier Co., Ltd, Younglin Bldg, #899-6, Hogye-dong, Dongan-gu, Anyan, Gyeonggi-do 431-836, Republic of Korea

Introduction

In recent years golf courses have greatly increased the number in South Korea. More than 420 golf courses has been operating and annually about 118 ton of pesticides are being used in South Korea. 40 kinds of pesticides residue monitoring program is carried out by the regulatory agencies. Monitoring is performed on lawn, soil and effluent water collected in pond every twice a year.

Various analytical method and screening test for food types are reported, but is not environmental media such as soil and water. Analytical method for environmental media used by regulatory agencies in Korea is very cumbersome and inefficient. The goal of this study is to develop efficient methods for pesticides residue on lawn, soil and water.

We try to develop simple and simultaneous analytical method that can be replaced by complex process such as distillation or manual derivatization procedure with commonly used gas chromatography low resolution quadrupole mass spectrometry(GC/RLMS) and high pressure liquid chromatography(HPLC) UV detector and fluorescence detector using auto derivatization device.

Materials and methods

Reagents and apparatus

The standard compounds of 40 pesticides were obtained from Chem Service and AccuStandard. Analytical and residue grades of acetonitrile, dichloromethane, and hexane were purchased from J.T. Baker. Analytical reagent grad anhydrous sodium chloride(NaCl) and hydrochloric acid were purchased from Sigma Aldrich . SPE was obtained from Agilent Technologies.

Sample preparation

The grass samples were chopped and homogenized, and the soil samples were sieved through 200 meshes. Appropriate amounts of pesticide standards were spiked onto the samples to obtain spiking levels of 0.01 mg kg^{-1} or mg L^{-1}. The samples were mixed homogenously and left for 1 h to allow the solvent to evaporate.

33 pesticides simultaneous method with GC/MS

In case of solid samples such as soil and grass, a set of seven 50g of soil samples and 10g of grass samples were placed in 250 ml flasks. The samples were mixed with 50 ml of D.I. water and 100 ml of acetonitrile were added and then vertically extracted for 30 min. 50g of NaCl were added into the flasks and centrifuged for 10 min at relative centrifugal force 2000g. 20 ml of supernatant layer was transferred into a new concentration tube and concentrated with 1 ml for clean-up procedure.

In case of effluent water, simply liquid-liquid extraction method was used. A set of seven 500 ml of water were placed in 1000 ml separatory funnel. 50g of NaCl were added to ensure the extraction of pH dependent compounds and to minimize the degradation of basic and acid pesticides. 50 ml of dichloromethane was added and then vertically extracted for 30 min. The dichloromethane was separated and the extraction was repeated. Collected dichloromethane was concentrated with 1ml for clean-up procedure.

For clean-up, 1 ml of extracts was loaded on 3g of Florisil SPE and 20 ml of 50/50 acetone/hexane mobile phase was eluted. Eluted solution was evaporated to 1 ml for GC/MS determinations.

methamidophos, oemthoate and monochrotophs
Methamidophos, oemthoate and monochrotophs are high-polarity compounds and recoveries of those pesticides were low when used general solvent extraction. In case of effluent water, 200 ml of effluent water was directly evaporated to dryness and reconstituted in 2 ml of acetone and then applied to the upper same clean-up procedure.

In case of soil and grass, 50g of soil samples and 10g of grass samples were placed in 250 ml flasks. The samples were mixed with 20 ml of D.I. water and 100 ml of acetone were added and then vertically extracted for 30 min. Centrifuged for 10 min at relative centrifugal force 2000g. All of supernatant layer was transferred into a new flask and evaporated to dryness and reconstituted in 2 ml of acetone and then applied to the upper same clean-up procedure.

Oxin-copper, thiram and bensulide
Analytical methods of oxin-copper, thiram and bensulide are very cumbersome and reported only individual test method. For those pesticides, extraction procedure was applied with solid phase extractor(SPE) packed with material of styrene divinylbenzene copolymer. 250 ml of effluent water was adjusted with hydrochloric acid to pH 3.5. SPE was conditioned with 5 ml acetonitrile and 5 ml D.I. water. Samples were passed slowly through SPE using vacuum pressure. The flow rate was not exceed 10 ml min^{-1}. SPE tube was dried with passing air for 10 min. 5 ml of elution solvent was eluted and added 0.5 ml of 2% diethylglycal/acetone as a concentration keeper. Eluted solution was evaporated to 1 ml for HPLC analysis.

GC/MS analysis
An Agilent 6890N gas chromatograph was equipped with Agilent 5975 mass selective detector and DB-5MS column(30m x 0.25mm I.D. x 0.25㎛ film thickness, J&W). MSD system was operated in selective ion monitoring(SIM) mode using one target and two qualifier ions. The column was initially maintained for 2 min at 80℃, and then the temperature was increased to 200℃ at a rate of 10℃ min^{-1} and held for 2 min, then increased to 260℃ at a rate of 5℃ min^{-1} and held for 2 min, then increased to 300℃ at a rate of 10℃ min^{-1} and held for 2 min. The injector temperature was kept at 260℃. Helium was used as a carrier gas with a flow rate of 1 ml min^{-1}.

HPLC analysis
An Agilent 1200 series liquid chromatography system equipped with G1322A degasser, a G1311A pump, a G1316A autosampler, a G1365D UV detector, a G1321A fluorescence detector, and a Pickering Vector PCX post column derivatization was employed in this study. A 250 mm x 4.6 mm x 5㎛ Shisheido Capcell pack C18 was operated at 40℃. The elution was carried out with a binary gradient which was composed of 50 mM phosphate buffer(A) and acetonirile(B). The initial composition was 40%B hold for 0.5 min, followed by linear gradient to 60% B from 0.5 to 20 min, and re-equilibrated at initial condition from 20 to 40 min. The flow rated was 0.7 ml min^{-1}. The wavelength of UV detector was 270 nm for thiram, and 235 nm for oxine-copper and bensulide, and 254 nm for thiophanate-methyl. The reaction temperature of post column derivatization was 100℃ and regent flow was 0.3 ml min^{-1}. The wavelengths of fluorescence detector were 330 nm for exciting, 446 nm for emission.

Results and discussion
In this study, the simultaneous analytical method using GC/RLMS was considered as a priority. HPLC analytical method development only had been taken into account when GC analytical method development was not available. Relatively High-polarity pesticides such as oxine-copper, bensulide, thiram, methomyl, carbofuran, thiophanate-methyl are analyzed with HPLC. Especially, oxine-copper was skipped complex distillation procedure as well as carbofuran was converted manual derivatization to automatic derivatization with post column derivatization device(Vector PCX).

The established methods were validated in terms of linearity, limit of detection(LOD) and limit of quantification(LOQ), reproducibility and recovery. Soil, grass and effluent water samples were analyzed seven times for calculation of linearity, LOD, LOQ and recover. Recoveries for pesticides were in the range of 75~116% . The limit of quantitation(LOQ) varies between 0.0001 and 0.0015 mg/L. Regression equations, the regression coefficients, the LODs and LOQs for 40 pesticides are shown in table 1. Recovery of Oxine-copper was lowest, but was usually acceptable at ~ 70%.

Table 1 Regression equations, the regression coefficients, the LODs and LOQs for 40 pesticides in the effluent water.

Pesticides	Regression equations	Regression coefficients	Recovery (%)	RSD (%)	LOD (mg/L)	LOQ (mg/L)
Dichlorvos[1)]	y=1010000x -103000	0.998	112.0	1.8	0.00008	0.00025
Diazinon[1)]	y=416000x -75100	0.996	107.3	2.8	0.00003	0.00010
Daconil[1)]	y=774000x -153000	0.998	112.7	2.7	0.00025	0.00080
Phoshamidone[1)]	y=232000x -44500	0.997	96.0	2.1	0.00006	0.00019
Chlorpyrifos-methyl[1)]	y=706000x -128000	0.996	110.7	2.1	0.00005	0.00015
Tolclofos-methyl[1)]	y=1410000x -179000	0.998	113.3	2.7	0.00008	0.00025
Fenitrothion[1)]	y=205000x -38300	0.998	108.7	3.8	0.00007	0.00023
Chlorpyrifos[1)]	y=37000x -6240	0.994	108.0	3.2	0.00007	0.00022
Parathion[1)]	y=208000x -41000	0.998	109.3	2.8	0.00006	0.00020
Dicofol[1)]	y=1200000x -148000	0.998	109.3	4.2	0.00013	0.00043
Pendimethalin[1)]	y=364000x -82700	0.997	114.0	1.8	0.00006	0.00020
Captan[1)]	y=255000x -55400	0.991	110.7	4.5	0.00008	0.00025
Phenthoate[1)]	y=535000x -127000	0.997	105.3	2.9	0.00005	0.00015
Methidathion[1)]	y=666000x -157000	0.997	104.7	2.2	0.00005	0.00016
Endosulfan1[1)]	y=91600x -5540	0.999	116.0	1.7	0.00014	0.00044
Endosulfan2[1)]	y=109000x -9250	0.999	116.7	2.0	0.00010	0.00031
Triazophos[1)]	y=439000x -104000	0.997	105.3	2.9	0.00004	0.00014
Endosulfan-sulfate[1)]	y=235000x -28000	0.999	116.0	1.7	0.00009	0.00028
Propargite[1)]	y=536000x -96000	0.996	108.0	1.9	0.00008	0.00025
Iprodione[1)]	y=259000x -50300	0.998	108.0	1.9	0.00006	0.00019
Bromopropylate[1)]	y=616000x -134000	0.997	104.0	1.9	0.00004	0.00012
EPN[1)]	y=346000x -82100	0.995	110.7	3.8	0.00011	0.00035
Fenpropathrin[1)]	y=517000x -97100	0.997	110.7	3.8	0.00007	0.00022
Tetradifon[1)]	y=457000x -31400	0.999	110.7	3.8	0.00006	0.00018
Furathiocarb[1)]	y=653000x -130000	0.998	102.0	2.0	0.00005	0.00016
Phosalon[1)]	y=437000x -98400	0.998	104.7	2.2	0.00006	0.00019
Lamda-cyhalothrin[1)]	y=363000x -71700	0.998	101.3	3.0	0.00007	0.00021
Amitraz[1)]	y=276000x -51500	0.998	96.7	4.3	0.00006	0.00020
gamma-cyhalothrin[1)]	y-827000x -166000	0.998	108.0	1.9	0.00006	0.00020
Benfuracarb[1)]	y=154000x -26300	0.999	98.0	3.5	0.00009	0.00028
Pyraclofos[1)]	y=281000x -62700	0.998	101.3	3.0	0.00005	0.00016
Deltamethrin [1)]	y=95400x -19600	0.997	98.7	6.2	0.00005	0.00015

Demeton-S-methyl[1)]	y=240000x -24700	0.997	94.0	2.1	0.00019	0.00062
Methamidophos[2)]	y=441000x -7430	0.992	92.0	2.2	0.00043	0.00138
Omethoate[2)]	y=755000x -73200	0.999	96.7	5.2	0.00044	0.00141
Monocrotophos[2)]	y=698000x -55700	0.999	86.7	5.3	0.00046	0.0146
Oxine-copper[3)]	y=117.259x + 4.518	0.998	75.7	1.4	0.00052	0.00165
Bensulide[3)]	y=12.905x - 1.872	0.999	100.8	1.2	0.00044	0.00142
Thiram[3)]	y=93.594x + 21.198	0.999	106.3	2.7	0.00021	0.00068
Methomyl[4)]	y=57340x + 0	0.999	101.7	0.4	0.00008	0.00026
Carbofuran[4)]	y=0.00118x + 0	0.999	110.6	1.8	0.00037	0.00116
Thiophanate-methyl[4)]	y=4.659x -0.533	0.999	103.1	15.2	0.00008	0.00027

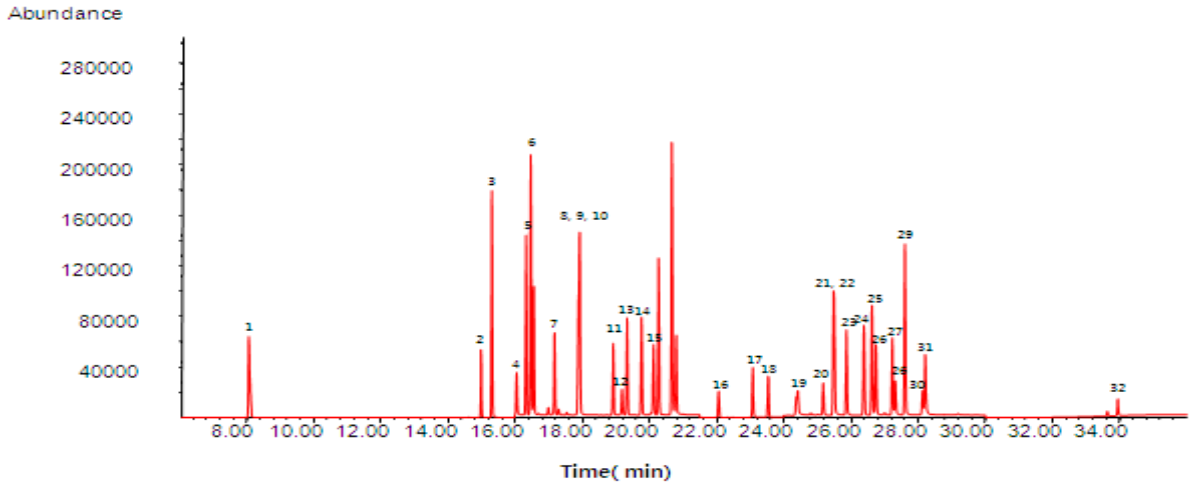

Fig. 1 SIM chromatogram of the effluent water spiked with 0.1mg/kg of target analyte. Peaks: 1. Dichlorvos; 2. Diazinon; 3. Daconil; 4. Phosphamidone; 5. Chlorpyrifos-methyl; 6. Tolclofos-methyl; 7. Fenitrothion; 8. Chlorpyrifos; 9. Parathion; 10. Dicofol; 11. Pendimethalin; 12. Captane; 13. Phenthoate; 14. Methidathion; 15. Endosulfan Ⅰ; 16. Endosulfan Ⅱ; 17. Endosulfan sulfate; 18. Triazophos; 19. Propargite; 20. Iprodione; 21. Bromopropylate; 22. EPN; 23. Fenpropathrin; 24. Tetradifon; 25. Furathiocarb; 26. Phosalone; 27. Lamda-Cyhalothrin; 28. Amitraz; 29. Gamma-Cyhalothrin; 30. Benfuracarb; 31. Pyraclofos; 32. Deltamethrin

Acknowledgements
This study was financially supported by project of Soil and Groundwater Division, Ministry of Environment Korea.

References
1. K. Mastovska, K.J. Dorweiler, S.J. Lehotay, J.S. Wegscheid, K.A. Szpylka, J. Aric. (2010); *Food Chem*. 58: 5959
2. E.G. Amvrazi, T.A. Albanis, J. Agric. Food Chem. (2008*); Food Chem*. 56: 5700
3. C. Blasco, G. Ffont, Y. Pico(2004) ; *J. Chromatogr. A* 1028 ; 267