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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-9050

Rapid Screening Analysis of On-Line Purified Residual Pesticides in Crop Extract Using Accurate Mass Information

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User Benefits

- Enables analysis using positive/negative ion polarity switching by the LCMS-9050.
- Enables comprehensive analysis of compounds using the accurate mass and retention time information.
- This method using an online purification column enables quick and easy sample preparation.

■ Introduction

The pesticide residue limits in food are strictly regulated by each region or country in order to protect the people's health. In recent years, the number of pesticides regulated around the world is on the rise.

The QuEChERS method published by the USDA (United States Department of Agriculture) in 2003, which has been adopted as an official method by organizations such as the Association of Analytical Communities (AOAC) and Committee of European Normalization (CEN), enables efficient extraction of pesticides without the need for special equipment. However, there are problems with reproducibility being dependent on operators and the time required for the work.

In this article, an example of comprehensive analysis of residual pesticides in spinach using a Revive In-Line Sample Preparation (ILSP) column[1)] (RESTEK Co.) and the quadrupole time-of-flight mass spectrometer LCMS-9050 (Fig. 1) is introduced. The Revive ILSP column, which enables on-line cleanup of compounds derived from the matrix, makes the sample preparation quicker and easier. It is also expected that this method can contribute to the reduction of waste and costs associated with pretreatment.

Fig. 1 Exterior of LCMS-9050

■ Sample Preparation

Commercially available spinach and pesticides standard solution 74, 75 (Kanto Chemical Co., Inc.) were used for this analysis. The detailed preparation processes are shown in Fig. 2. 1.0 g of spinach that was frozen with dry ice and crushed was put in a 15 mL tube, and 3 mL of 1 % acetic acid in acetonitrile was added, then the tube was shaken. Subsequently, the tube was centrifuged after waiting for 2 minutes, and the supernatant was collected for sampling. Compared with the QuEChERS method, this method enables quicker and easier sample preparation. For example, it is expected that the time needed for sample preparation can be reduced to approximately one third by using this method when fourteen samples of spinach are pretreated[1)].

The recovery rate for losses in the preparation process and matrix effects were evaluated by adding a fixed concentration of pesticides standard solution to the spinach before extraction.

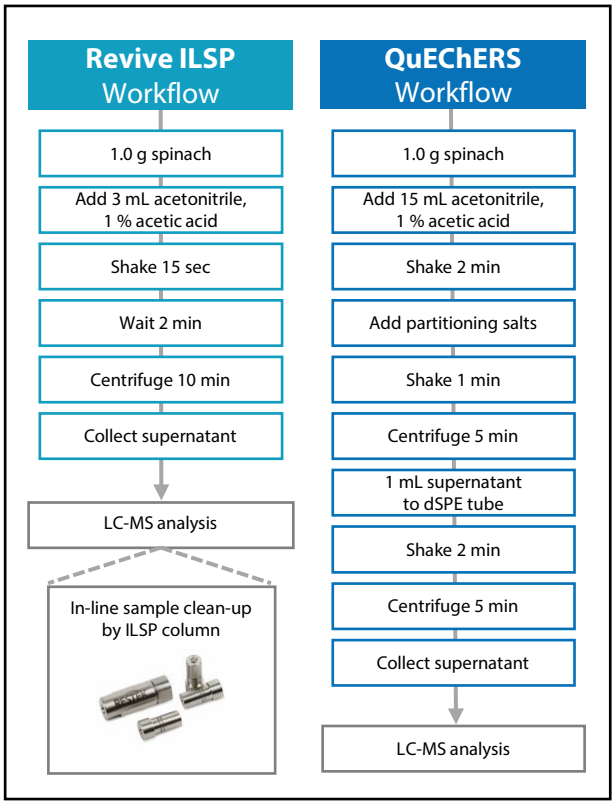

Fig. 2 Workflow for Sample Preparation (Left: Revive ILSP, Right: QuEChERS)

■ Analytical Conditions

For the analysis of pesticides, the method included in the LC/MS/MS Method Package Residual Pesticides Ver. 3 was applied to the LCMS-9050. The HPLC and MS conditions are shown in Table 1, and the outline of this system and the LC time program are shown in Figs. 3 and 4 respectively.

Hydrophobic compounds from the matrix in injected samples are separated from pesticides by the Revive ILSP column, and the pesticides are eluted to the analytical column (upper of Fig. 3). On the other hand, the compounds from the matrix are back-flushed by a pump for washing and eluted to a drain, after switching a 6-port valve when the targeted pesticides have been eluted from the Revive ILSP column (bottom of Fig. 3 and Fig. 4). In this way, the Revive ILSP column can be reused.

Table 1 Analytical Conditions

UHPLC (Nexera™ X3 system)	
Analytical Column:	Shim-pack™ Velox Biphenyl (100 mm L × 2.1 mm I.D., 2.7 µm) P/N: 227-32015-03
In-Line Sample: Preparation Column:	Revive ILSP Pesticides Single 5 × 2.1 mm Cartridge (RESTEK)
Mobile Phase A:	2 mM Ammonium formate-0.002 % Formic acid-Water
Mobile Phase B, C (Wash):	2 mM Ammonium formate-0.002 % Formic acid-Methanol
Gradient Program:	B conc. 3 % (0 min)-10 % (1 min)-55 % (3 min)-100 % (10.5-12 min)-3 % (12.01-15 min)
Flowrate (A & B):	0.4 mL/min
Flowrate (C):	0 mL/min (0-5.49 min) – 1 mL/min (5.5-7.5 min) – 0.4 mL/min (7.51-11 min) - 0 mL/min (11.01-15 min)
Switching Valve Position:	load (0-6.49 min) – wash (6.5-10.5 min) – load (10.51-15 min)
Injection Volume:	2 µL (Co-injection 40 µL Water)

MS (LCMS-9050)	
Ionization:	ESI (Positive, Negative)
TOF-MS:	*m/z* 50-800
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
DL Temp.:	150 °C
Block Heater Temp.:	300 °C
Interface Temp.:	200 °C
Probe Position:	+2 mm

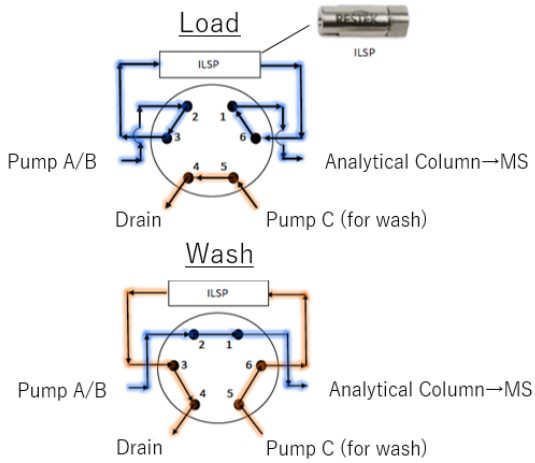

Fig. 3 Outline of ILSP System

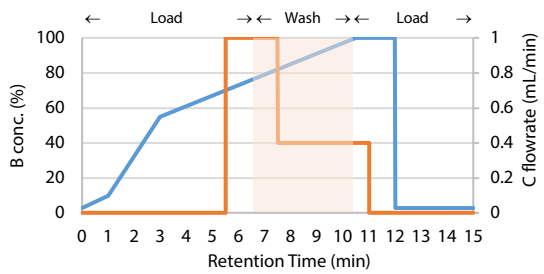

Fig. 4 LC Time Program

■ Analysis of Pesticides Standard Solution by the LCMS-9050

Table 2 shows the mass error of 79 compounds in pesticides standard solution diluted to 50 ppb. All the compounds were detected within ±1 mDa mass error. Theoretical *m/z* values of pesticides were calculated using LabSolutions Insight Explore™.

Fig. 5 shows the total ion current chromatogram (TICC) of the pesticides standard solution. Also, Fig. 6 shows the extracted ion chromatogram (XIC) of 79 compounds in pesticides standard solution and blank solvent.

Table 2 List of Pesticide Compounds

Compound	Molecular Formula	Selected Ion	Theoretical *m/z*	Mass Error (mDa)	Retention Time (min)
Acetamiprid	$C_{10}H_{11}ClN_4$	$[M+H]^+$	223.0745	-0.2	4.81
Dimethoate	$C_5H_{12}NO_3PS_2$	$[M+H]^+$	230.0069	0.0	4.18
Bromacil	$C_9H_{13}BrN_2O_2$	$[M-H]^-$	259.0087	0.4	4.80
Propoxur	$C_{11}H_{15}NO_3$	$[M+H]^+$	210.1125	-0.2	5.01
Isouron	$C_{10}H_{17}N_3O_2$	$[M+H]^+$	212.1394	-0.1	4.97
Fluometuron	$C_{10}H_{11}F_3N_2O$	$[M+H]^+$	233.0896	-0.1	4.81
Pyraclonil	$C_{15}H_{15}ClN_6$	$[M+H]^+$	315.1120	-0.1	7.07
Metalaxyl/Metalaxyl-M*	$C_{15}H_{21}NO_4$	$[M+H]^+$	280.1544	-0.1	6.51
Methidathion	$C_6H_{11}N_2O_4PS_3$	$[M+H]^+$	302.9692	-0.2	7.17
Flumioxazin	$C_{19}H_{15}FN_2O_4$	$[M+H]^+$	355.1089	0.0	8.50
Chlorbufam	$C_{11}H_{10}ClNO_2$	$[M+H]^+$	224.0473	-0.5	5.91
Ethiprole	$C_{13}H_9Cl_2F_3N_4OS$	$[M+H]^+$	396.9899	0.3	5.95
Paclobutrazol	$C_{15}H_{20}ClN_3O$	$[M+H]^+$	294.1368	-0.1	6.18
Barban	$C_{11}H_9Cl_2NO_2$	$[M+H]^+$	258.0083	-0.3	6.68
Benthiavalicarb-isopropyl	$C_{18}H_{24}FN_3O_3S$	$[M+H]^+$	382.1595	-0.1	6.74
Tiadinil	$C_{11}H_{10}ClN_3OS$	$[M-H]^-$	266.0160	0.0	6.55
Triadimenol	$C_{14}H_{18}ClN_3O_2$	$[M+H]^+$	296.1161	-0.4	6.30
Triflumizole Metabolite	$C_{12}H_{14}ClF_3N_2O$	$[M+H]^+$	295.0820	-0.2	4.81
Prometryn	$C_{10}H_{19}N_5S$	$[M+H]^+$	242.1434	-0.2	6.66
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$	$[M+H]^+$	372.0288	-0.2	6.90
Flusilazole	$C_{16}H_{15}F_2N_3Si$	$[M+H]^+$	316.1076	0.0	7.65
Bensulide	$C_{14}H_{24}NO_4PS_3$	$[M+H]^+$	398.0678	-0.1	7.99
Flubendiamide	$C_{23}H_{22}F_7IN_2O_4S$	$[M-H]^-$	681.0160	0.1	7.27
Kresoxim-methyl	$C_{18}H_{19}NO_4$	$[M+H]^+$	314.1387	-0.1	8.30
Pyrazoxyfen	$C_{20}H_{16}Cl_2N_2O_3$	$[M+H]^+$	403.0611	0.1	8.90
Famoxadone	$C_{22}H_{18}N_2O_4$	$[M+NH_4]^+$	392.1605	0.1	8.60
Phoxim	$C_{12}H_{15}N_2O_3PS$	$[M+H]^+$	299.0614	-0.2	8.47
Trichlamide	$C_{13}H_{16}Cl_3NO_3$	$[M-H]^-$	338.0123	0.6	7.40
Metconazole	$C_{17}H_{22}ClN_3O$	$[M+H]^+$	320.1524	-0.1	7.56
Pyraclofos	$C_{14}H_{18}ClN_2O_3PS$	$[M+H]^+$	361.0537	0.1	8.44

Compound	Molecular Formula	Selected Ion	Theoretical *m/z*	Mass Error (mDa)	Retention Time (min)
Bitertanol	$C_{20}H_{23}N_3O_2$	$[M+H]^+$	338.1863	-0.2	7.94
Pyrazophos	$C_{14}H_{20}N_3O_5PS$	$[M+H]^+$	374.0934	0.0	9.38
Diflufenican	$C_{19}H_{11}F_5N_2O_2$	$[M+H]^+$	395.0814	0.0	8.26
Pentoxazone	$C_{17}H_{17}ClFNO_4$	$[M+NH_4]^+$	371.1168	0.1	9.24
Tolfenpyrad	$C_{21}H_{22}ClN_3O_2$	$[M+H]^+$	384.1473	0.0	9.08
Pyributicarb	$C_{18}H_{22}N_2O_2S$	$[M+H]^+$	331.1475	0.1	9.47
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	$[M+H]^+$	349.9336	-0.2	9.25
Etoxazole	$C_{21}H_{23}F_2NO_2$	$[M+H]^+$	360.1770	0.1	9.39
Cyenopyrafen	$C_{24}H_{31}N_3O_2$	$[M+H]^+$	394.2489	0.1	9.45
Spirodiclofen	$C_{21}H_{24}Cl_2O_4$	$[M+H]^+$	411.1125	-0.1	9.62
3-Hydroxycarbofuran	$C_{12}H_{15}NO_4$	$[M+H]^+$	238.1074	-0.1	4.06
Cymoxanil	$C_7H_{10}N_4O_3$	$[M+H]^+$	199.0826	-0.4	4.35
Phosphamidon	$C_{10}H_{19}ClNO_5P$	$[M+H]^+$	300.0762	-0.1	5.04
Terbacil	$C_9H_{13}ClN_2O_2$	$[M-H]^-$	215.0593	-0.2	4.86
XMC (3,5-xylyl methylcarbamate)	$C_{10}H_{13}NO_2$	$[M+H]^+$	180.1019	-0.2	5.25
Flutriafol	$C_{16}H_{13}F_2N_3O$	$[M+H]^+$	302.1100	0.1	5.78
Fensulfothion	$C_{11}H_{17}O_4PS_2$	$[M+H]^+$	309.0379	0.0	6.62
Triforine (isomer-1)	$C_{10}H_{14}Cl_6N_4O_2$	$[M+H]^+$	432.9321	0.2	5.68
Triforine (isomer-2)	$C_{10}H_{14}Cl_6N_4O_2$	$[M+H]^+$	432.9321	0.4	5.79
Diethofencarb	$C_{14}H_{21}NO_4$	$[M+H]^+$	268.1544	-0.3	6.29
Fludioxonil	$C_{12}H_6F_2N_2O_2$	$[M-H]^-$	247.0324	0.2	6.00
Mandipropamid	$C_{23}H_{22}ClNO_4$	$[M+H]^+$	412.1310	0.0	7.53
Pyriminobac-methyl (E)	$C_{17}H_{19}N_3O_6$	$[M+H]^+$	362.1347	-0.1	7.88
Malathion	$C_{10}H_{19}O_6PS_2$	$[M+H]^+$	331.0434	-0.1	7.34
Bromobutide-dibromo	$C_{15}H_{22}BrNO$	$[M+H]^+$	234.1852	0.0	6.10
Fluopicolide	$C_{14}H_8Cl_3F_3N_2O$	$[M+H]^+$	382.9727	0.1	6.98
Triadimefon	$C_{14}H_{16}ClN_3O_2$	$[M+H]^+$	294.1004	-0.1	6.79
Flamprop-methyl	$C_{17}H_{15}ClFNO_3$	$[M+H]^+$	336.0797	-0.1	7.35
Bromobutide	$C_{15}H_{22}BrNO$	$[M+H]^+$	312.0958	0.0	6.67
Carfentrazone-ethyl	$C_{15}H_{14}Cl_2F_3N_3O_3$	$[M+NH_4]^+$	429.0702	0.1	8.00
Dimethametryn	$C_{11}H_{21}N_5S$	$[M+H]^+$	256.1591	-0.2	7.16
Penthiopyrad	$C_{16}H_{20}F_3N_3OS$	$[M+H]^+$	360.1352	0.0	6.88
Tebuconazole	$C_{16}H_{22}ClN_3O$	$[M+H]^+$	308.1524	0.0	7.24
Benalaxyl	$C_{20}H_{23}NO_3$	$[M+H]^+$	326.1751	0.0	8.40
Oxadiargyl	$C_{15}H_{14}Cl_2N_2O_3$	$[M+H]^+$	341.0454	-0.2	8.19
Isoxathion	$C_{13}H_{16}NO_4PS$	$[M+H]^+$	314.0611	0.0	8.56
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	$[M+H]^+$	376.0381	0.0	8.70
Pirimiphos-methyl	$C_{11}H_{20}N_3O_3PS$	$[M+H]^+$	306.1036	-0.1	8.25
Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$	$[M+H]^+$	406.0720	-0.5	9.00
Trifloxystrobin	$C_{20}H_{19}F_3N_2O_4$	$[M+H]^+$	409.1370	0.1	8.75
Triflumizole	$C_{15}H_{15}ClF_3N_3O$	$[M+H]^+$	346.0929	0.0	8.18
Amisulbrom	$C_{13}H_{13}BrFN_5O_4S_2$	$[M+H]^+$	467.9628	0.3	9.05
Profenofos	$C_{11}H_{15}BrClO_3PS$	$[M+H]^+$	374.9402	-0.2	8.63
Buprofezin	$C_{16}H_{23}N_3OS$	$[M+H]^+$	306.1635	0.0	8.80
Piperonyl butoxide	$C_{19}H_{30}O_5$	$[M+NH_4]^+$	356.2432	-0.2	9.05
Butachlor	$C_{17}H_{26}ClNO_2$	$[M+H]^+$	312.1725	-0.1	8.90
Quinoxyfen	$C_{15}H_8Cl_2FNO$	$[M+H]^+$	308.0040	0.0	9.26
Pyridaben	$C_{19}H_{25}ClN_2OS$	$[M+H]^+$	365.1449	0.2	10.13
Fenpropimorph	$C_{20}H_{33}NO$	$[M+H]^+$	304.2635	-0.2	7.10

*Pesticides standard solution contains 50 ppb each of metalaxyl and metalaxyl-M.

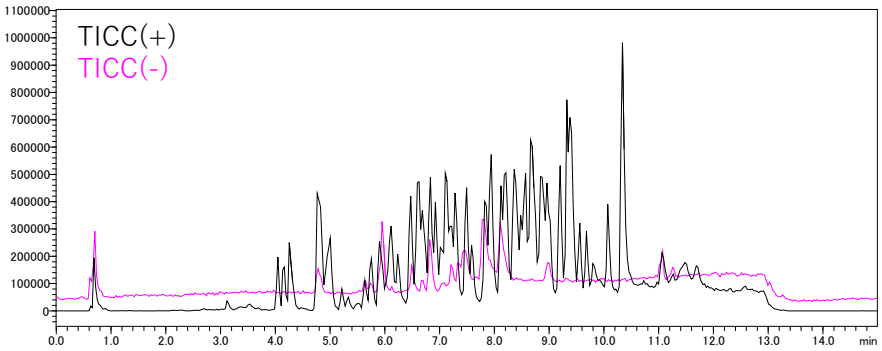

Fig. 5 Total Ion Current Chromatogram (TICC) of Pesticides Standard Solution

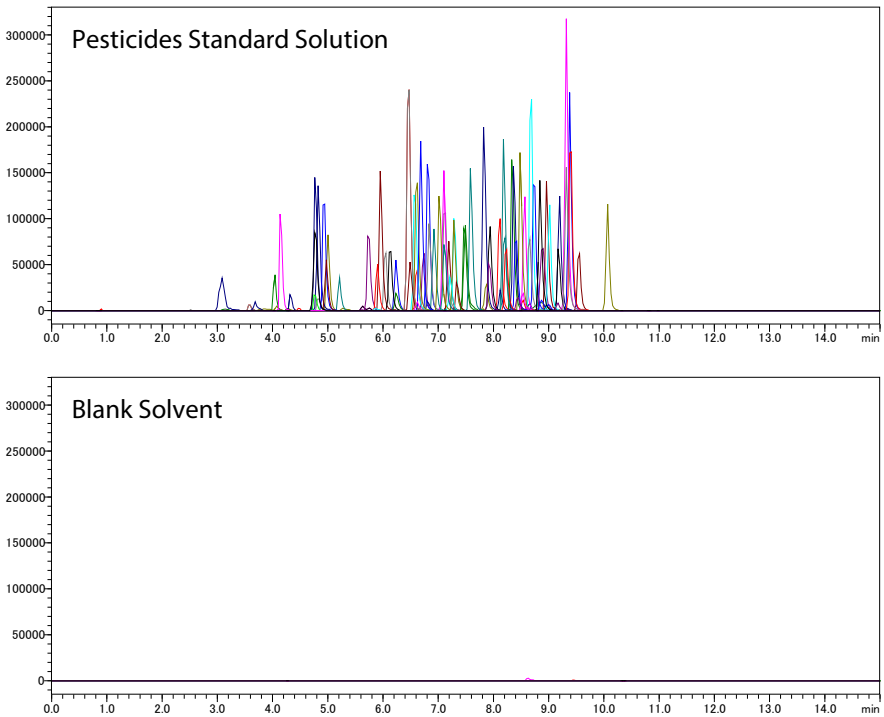

Fig. 6 Extracted Ion Chromatograms (XICs) of 79 Pesticide Compounds

■ Linearity of Calibration Curve

Linearity of the calibration curve for each pesticide was evaluated by generating a 7-point calibration curve with the range 0.5-50 ppb in solvent (acetonitrile) and in spinach extract. Both in solvent and in extract, linearity showed very good results (coefficient of determination R^2: 0.99 or more) for all the compounds. For 68 out of 79 compounds detection was within 2.5 ppb or less. Calibration curves for fludioxonil, which was detected in negative mode, in solvent and in extract are shown in Fig. 7 as an example, and calibration ranges for all the compounds are shown in Table 3.

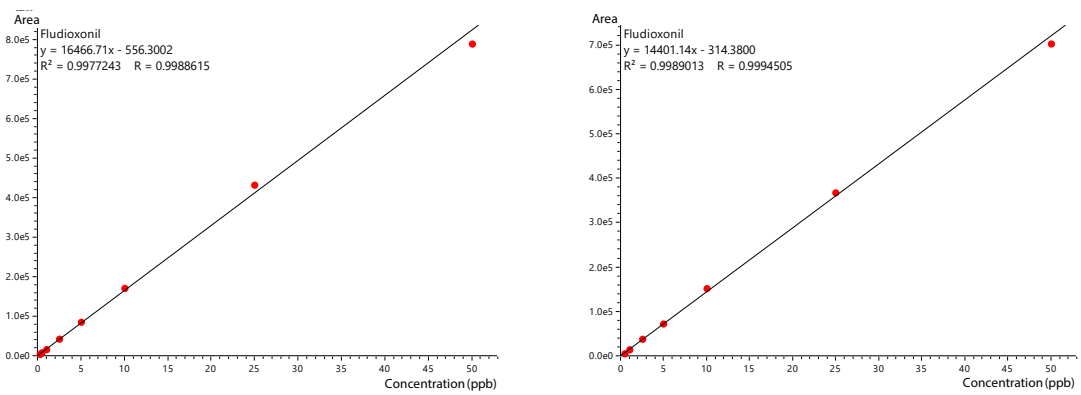

Fig. 7 Calibration Curve of Fludioxonil (Left: in Solvent, Right: in Spinach Extract)

Table 3 Linear Ranges of 79 Pesticides

Compound	Calibration Range (ppb)	
	in solvent	in spinach extract
Acetamiprid	1-50	2.5-50
Dimethoate	1-50	0.5-50
Bromacil	5-50	5-50
Propoxur	1-50	1-50
Isouron	0.5-50	0.5-50
Fluometuron	0.5-50	0.5-50
Pyraclonil	0.5-50	0.5-50
Metalaxyl/Metalaxyl-M*	0.5-50	0.5-50
Methidathion	1-50	1-50
Flumioxazin	5-50	5-50
Chlorbufam	25-50	25-50
Ethiprole	1-50	1-50
Paclobutrazol	1-50	1-50
Barban	5-50	5-50
Benthiavalicarb-isopropyl	0.5-50	0.5-50
Tiadinil	1-50	1-50
Triadimenol	2.5-50	2.5-50
Triflumizole Metabolite	0.5-50	0.5-50
Prometryn	0.5-50	0.5-50
Tetraconazole	0.5-50	0.5-50
Flusilazole	0.5-50	0.5-50
Bensulide	1-50	1-50
Flubendiamide	1-50	2.5-50
Kresoxim-methyl	0.5-50	1-50
Pyrazoxyfen	0.5-50	0.5-50
Famoxadone	2.5-50	2.5-50
Phoxim	1-50	0.5-50
Trichlamide	2.5-50	2.5-50
Metconazole	1-50	0.5-50
Pyraclofos	0.5-50	0.5-50
Bitertanol	2.5-50	2.5-50
Pyrazophos	0.5-50	0.5-50
Diflufenican	1-50	1-50
Pentoxazone	10-50	10-50
Tolfenpyrad	0.5-50	1-50
Pyributicarb	0.5-50	0.5-50
Chlorpyrifos	1-50	1-50
Etoxazole	0.5-50	0.5-50
Cyenopyrafen	0.5-50	0.5-50
Spirodiclofen	1-50	1-50

Compound	Calibration Range (ppb)	
	in solvent	in spinach extract
3-Hydroxycarbofuran	1-50	1-50
Cymoxanil	5-50	5-50
Phosphamidon	0.5-50	0.5-50
Terbacil	5-50	5-50
XMC (3,5-xylyl methylcarbamate)	2.5-50	2.5-50
Flutriafol	1-50	1-50
Fensulfothion	0.5-50	0.5-50
Triforine (isomer-1)	10-50	10-50
Triforine (isomer-2)	10-50	10-50
Diethofencarb	1-50	1-50
Fludioxonil	0.5-50	0.5-50
Mandipropamid	0.5-50	0.5-50
Pyriminobac-methyl (E)	0.5-50	0.5-50
Malathion	0.5-50	0.5-50
Bromobutide-debromo	1-50	1-50
Fluopicolide	0.5-50	1-50
Triadimefon	1-50	1-50
Flamprop-methyl	0.5-50	0.5-50
Bromobutide	1-50	2.5-50
Carfentrazone-ethyl	1-50	1-50
Dimethametryn	0.5-50	0.5-50
Penthiopyrad	0.5-50	0.5-50
Tebuconazole	1-50	0.5-50
Benalaxyl	0.5-50	0.5-50
Oxadiargyl	2.5-50	2.5-50
Isoxathion	0.5-50	0.5-50
Prochloraz	1-50	1-50
Pirimiphos-methyl	0.5-50	0.5-50
Difenoconazole	1-50	0.5-50
Trifloxystrobin	0.5-50	0.5-50
Triflumizole	0.5-50	0.5-50
Amisulbrom	25-50	25-50
Profenofos	0.5-50	0.5-50
Buprofezin	0.5-50	0.5-50
Piperonyl butoxide	0.5-50	0.5-50
Butachlor	5-50	5-50
Quinoxyfen	0.5-50	1-50
Pyridaben	0.5-50	0.5-50
Fenpropimorph	0.5-50	0.5-50

*Metalaxyl and metalaxyl-M were not distinguished.

■ Spike and Recovery Test

A spike and recovery test was performed using spinach extract to which 79 pesticides standard solution was spiked at 0.01 mg/kg per sample (concentration in pretreated sample solution was 2.5 ppb). The recovery rate was calculated at the concentration of 2.5 ppb, if not possible, at the concentration of 50 ppb. The results of recovery rate calculated by external standard method and reproducibility (n=5) are shown in Table 4, and the breakdown of the recovery rate is shown in Fig. 8.

Recovery rates were 70-120 % for 77 of the 79 compounds and %RSDs were less than 20 % for all the compounds. Good recovery rate and reproducibility were obtained without significant matrix inhibition by using the Revive ILSP column in sample preparation.

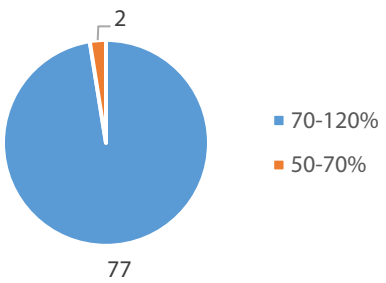

Fig. 8 Breakdown of Recovery Rate

Table 4 Recovery Rate and Reproducibility (%RSD) (n=5)

Compound	Recovery Rate (%)	%RSD
Acetamiprid	82.6	11.4
Dimethoate	87.1	7.6
Bromacil	73.1	2.5
Propoxur	103.9	9.9
Isouron	107.1	1.5
Fluometuron	105.4	4.7
Pyraclonil	107.8	5.6
Metalaxyl/Metalaxyl-M*	108.6	3.6
Methidathion	110.5	10.3
Flumioxazin	65.5	1.7
Chlorbufam	85.0	12.7
Ethiprole	104.3	7.9
Paclobutrazol	103.2	8.4
Barban	92.1	4.0
Benthiavalicarb-isopropyl	108.2	1.3
Tiadinil	99.3	7.1
Triadimenol	100.4	18.6
Triflumizole Metabolite	103.9	4.0
Prometryn	107.2	5.7
Tetraconazole	98.1	5.1
Flusilazole	97.2	5.9
Bensulide	102.3	3.6
Flubendiamide	95.3	14.4
Kresoxim-methyl	95.9	9.9
Pyrazoxyfen	116.9	4.9
Famoxadone	84.6	18.6
Phoxim	85.2	12.5
Trichlamide	80.7	17.2
Metconazole	95.2	5.3
Pyraclofos	115.7	5.9
Bitertanol	77.4	6.4
Pyrazophos	112.3	2.1
Diflufenican	83.6	5.6
Pentoxazone	97.9	3.1
Tolfenpyrad	101.3	4.6
Pyributicarb	102.9	2.6
Chlorpyrifos	104.2	13.3
Etoxazole	108.0	2.1
Cyenopyrafen	98.8	4.7
Spirodiclofen	110.5	6.7
3-Hydroxycarbofuran	93.4	14.6
Cymoxanil	81.9	2.6
Phosphamidon	96.8	6.5
Terbacil	74.9	3.7
XMC (3,5-xylyl methylcarbamate)	88.2	7.7
Flutriafol	99.6	8.4
Fensulfothion	104.6	4.0
Triforine (isomer-1)	97.9	7.5
Triforine (isomer-2)	101.8	4.3
Diethofencarb	100.2	6.8
Fludioxonil	88.4	4.5
Mandipropamid	104.5	3.2
Pyriminobac-methyl (E)	100.3	3.5
Malathion	105.2	5.1
Bromobutide-debromo	107.0	12.2
Fluopicolide	96.2	7.2
Triadimefon	96.5	6.0
Flamprop-methyl	105.6	8.4
Bromobutide	112.5	9.9
Carfentrazone-ethyl	77.3	6.0
Dimethametryn	106.5	5.2
Penthiopyrad	97.9	5.7
Tebuconazole	109.9	6.8
Benalaxyl	93.3	3.0
Oxadiargyl	66.0	12.0
Isoxathion	94.3	3.9
Prochloraz	98.3	6.2
Pirimiphos-methyl	100.4	5.1
Difenoconazole	95.7	7.7
Trifloxystrobin	96.7	3.1
Triflumizole	83.4	3.1
Amisulbrom	98.1	4.4
Profenofos	92.1	3.2
Buprofezin	94.1	4.1
Piperonyl butoxide	87.6	7.6
Butachlor	100.9	3.0
Quinoxyfen	100.4	3.2
Pyridaben	95.0	4.9
Fenpropimorph	103.7	3.9

*Metalaxyl and metalaxyl-M were not distinguished.

■ Conclusion

Screening analysis of pesticides was performed using positive/negative ion polarity switching by the LCMS-9050. The on-line sample preparation method with a Revive ILSP column made it possible to speed up and simplify the preparation process. Screening analysis of pretreated spinach samples with pesticides standard spiked provided good results for recovery rate, reproducibility and linearity. For sensitivity, 68 out of 79 compounds were detected at 2.5 ppb or less. This method is applicable for the analysis of residual pesticides in other crops.

<References>
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