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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-9030

Analysis of Residual Pesticides in Strawberries Using the Quadrupole Time-of-Flight Mass Spectrometer

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User Benefits

- It is possible to identify residual pesticides from the accurate mass and retention time information obtained by the LCMS-9030.
- Comprehensive analysis of residual pesticides can be performed with this analytical method.
- This method combining QuEChERS (EN 15662) and a SPEEDIA residual pesticides purification kit enables quick and easy sample preparation.

■ Introduction

Many pesticides are currently used around the world to meet the growing demand for food along with rapid population increase. While pesticides can enable stable food supply, there are risks to health due to residual pesticides. For that reason, each region and country has established maximum residue levels (MRLs) for pesticides in food and strictly regulates them.

Currently, triple quadrupole mass spectrometers, that can perform quantitative analysis highly selectively and highly sensitively, are widely used for the analysis of residual pesticides in food. However, this method can only detect the envisaged target compounds, and there is a limit to the number of compounds that can be measured at one time. Therefore, comprehensiveness is limited for use in screening applications. Against this background, comprehensive analysis for residual pesticides in full scan mode using a high-resolution mass spectrometer is attracting attention.

In this article, an example of comprehensive analysis of residual pesticides in strawberries using the quadrupole time-of-flight mass spectrometer LCMS-9030 (Fig. 1) is introduced.

Fig. 1 Exterior of Nexera™ X3 and LCMS-9030

■ Sample Preparation

Commercially available strawberries and a pesticides mixture standard solution (Hayashi Pure Chemical Ind., Ltd.) were used for this analysis. The strawberries were pretreated according to the QuEChERS (EN 15662) method. 10.0 g of strawberry was put in a 50 mL tube, and 10 mL of acetonitrile was added, then the tube was shaken. Subsequently, the QuEChERS extraction salt kit was added and mixed, and the tube was centrifuged. A purification process was performed by the membrane filtration method using the SPEEDIA residual pesticides purification kit (Miura Co., Ltd.). Finally, 0.45 mL of filtrate and 0.55 mL of acetonitrile were transferred to a vial as an LC/MS sample. The detailed preparation processes are shown in Fig. 2. In addition, by adding a fixed concentration of pesticide standard solution to the strawberries, the recovery rate for losses in the preparation process and matrix effects were also evaluated.

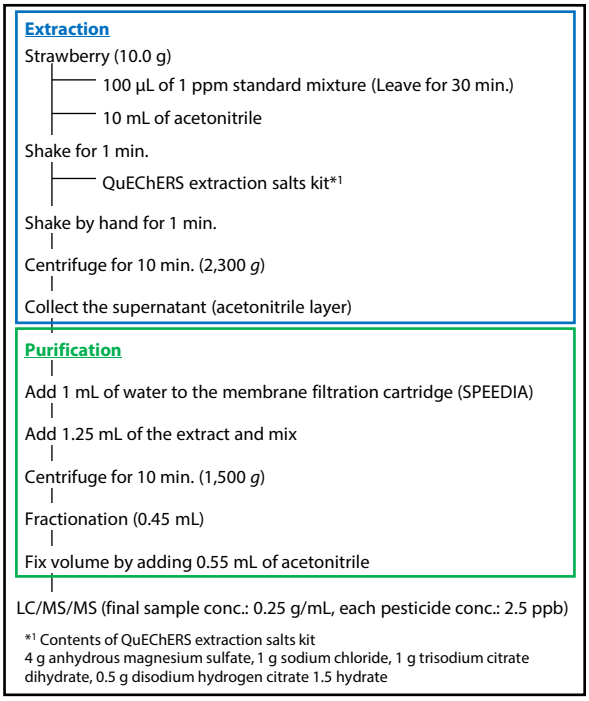

Fig. 2 Workflow for Sample Preparation

■ 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-9030. The HPLC and MS conditions are shown in Table 1.

Table 1 Analytical Conditions

UHPLC (Nexera™ X3 system)	
Column:	Shim-pack™ Velox Biphenyl (100 mmL × 2.1 mmI.D., 2.7 µm) P/N: 227-32015-03
Mobile Phase A:	2 mM Ammonium formate-0.002 % formic acid-water
Mobile Phase B:	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:	0.4 mL/min
Injection Volume:	2 µL (Co-injection 40 µL water)

MS (LCMS-9030)	
Ionization:	ESI (positive)
TOF-MS:	*m/z* 50-950
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
DL Temp.:	250 °C
Block Heater Temp.:	400 °C
Interface Temp.:	300 °C

■ Creation of Compound List for Pesticides

Table 2 shows the compound list of pesticides used in this experiment. Theoretical *m/z* values of pesticides were calculated using LabSolutions Insight Explore™.

Table 2 List of Pesticide Compounds

Compound	Molecular Formula	Selected Ion	*m/z*	Retention Time (min)
(E)-Fenpyroximate	$C_{24}H_{27}N_3O_4$	$[M+H]^+$	422.2074	9.794
(Z)-Fenpyroximate	$C_{24}H_{27}N_3O_4$	$[M+H]^+$	422.2074	9.391
Acibenzolar-S-methyl	$C_8H_6N_2OS_2$	$[M+H]^+$	210.9994	7.334
Aldicarb-sulfone (Aldoxycarb)	$C_7H_{14}N_2O_4S$	$[M+NH_4]^+$	240.1013	3.282
Anilofos	$C_{13}H_{19}ClNO_3PS_2$	$[M+H]^+$	368.0305	8.179
Azamethiphos	$C_9H_{10}ClN_2O_5PS$	$[M+H]^+$	324.9809	5.939
Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$	$[M+H]^+$	318.0131	7.499
Azoxystrobin	$C_{22}H_{17}N_3O_5$	$[M+H]^+$	404.1241	7.978
Benzofenap	$C_{22}H_{20}Cl_2N_2O_3$	$[M+H]^+$	431.0924	9.444
Boscalid	$C_{18}H_{12}Cl_2N_2O$	$[M+H]^+$	343.0399	6.724
Carbaryl (NAC)	$C_{12}H_{11}NO_2$	$[M+H]^+$	202.0863	5.105
Carpropamid	$C_{15}H_{18}Cl_3NO$	$[M+H]^+$	336.0499	6.872
Chloridazon	$C_{10}H_8ClN_3O$	$[M+H]^+$	222.0429	4.091
Chloroxuron	$C_{15}H_{15}ClN_2O_2$	$[M+H]^+$	291.0895	6.585
Clofentezine	$C_{14}H_8Cl_2N_4$	$[M+H]^+$	303.0199	8.424
Cloquintocet-mexyl	$C_{18}H_{22}ClNO_3$	$[M+H]^+$	336.1361	9.096
Clothianidin	$C_6H_8ClN_5O_2S$	$[M+H]^+$	250.0160	3.767
Cumyluron	$C_{17}H_{19}ClN_2O$	$[M+H]^+$	303.1259	6.624
Cyazofamid	$C_{13}H_{13}ClN_4O_2S$	$[M+H]^+$	325.0521	7.672
Cyprodinil	$C_{14}H_{15}N_3$	$[M+H]^+$	226.1339	7.375
Dimethomorph (E, Z)	$C_{21}H_{22}ClNO_4$	$[M+H]^+$	388.1310	7.688
Diuron (DCMU)	$C_9H_{10}Cl_2N_2O$	$[M+H]^+$	233.0243	4.561
Epoxiconazole	$C_{17}H_{13}ClFN_3O$	$[M+H]^+$	330.0804	7.414
Fenamidone	$C_{17}H_{17}N_3OS$	$[M+H]^+$	312.1165	6.626
Fenobucarb	$C_{12}H_{17}NO_2$	$[M+H]^+$	208.1332	5.582
Fenoxaprop-ethyl	$C_{18}H_{16}ClNO_5$	$[M+H]^+$	362.0790	8.722
Flufenacet	$C_{14}H_{13}F_4N_3O_2S$	$[M+H]^+$	364.0737	7.059
Flufenoxuron	$C_{21}H_{11}ClF_6N_2O_3$	$[M+H]^+$	489.0435	8.670
Fluridone	$C_{19}H_{14}F_3NO$	$[M+H]^+$	330.1100	7.105
Hexythiazox	$C_{17}H_{21}ClN_2O_2S$	$[M+H]^+$	353.1085	9.266
Imazalil	$C_{14}H_{14}Cl_2N_2O$	$[M+H]^+$	297.0556	6.959
Imidacloprid	$C_9H_{10}ClN_5O_2$	$[M+H]^+$	256.0596	4.354
Indanofan	$C_{20}H_{17}ClO_3$	$[M+H]^+$	341.0939	7.972
Iprovalicarb	$C_{18}H_{28}N_2O_3$	$[M+H]^+$	321.2173	6.312
Lactofen	$C_{19}H_{15}ClF_3NO_7$	$[M+NH_4]^+$	479.0827	8.978
Mepanipyrim	$C_{14}H_{13}N_3$	$[M+H]^+$	224.1182	7.040
Methabenzthiazuron	$C_{10}H_{11}N_3OS$	$[M+H]^+$	222.0696	5.813
Methomyl	$C_5H_{10}N_2O_2S$	$[M+H]^+$	163.0536	3.673
Monolinuron	$C_9H_{11}ClN_2O_2$	$[M+H]^+$	215.0582	4.879
Novaluron	$C_{17}H_9ClF_8N_2O_4$	$[M+H]^+$	493.0196	7.539
Oxaziclomefone	$C_{20}H_{19}Cl_2NO_2$	$[M+H]^+$	376.0866	8.930
Oxycarboxin	$C_{12}H_{13}NO_4S$	$[M+H]^+$	268.0638	4.367
Pirimicarb	$C_{11}H_{18}N_4O_2$	$[M+H]^+$	239.1503	5.814
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	$[M+H]^+$	388.1059	8.737
Pyrazolynate	$C_{19}H_{16}Cl_2N_2O_4S$	$[M+H]^+$	439.0281	8.986
Pyriftalid	$C_{15}H_{14}N_2O_4S$	$[M+H]^+$	319.0747	7.539
Simeconazole	$C_{14}H_{20}FN_3OSi$	$[M+H]^+$	294.1432	6.039
Spinosyn A	$C_{41}H_{65}NO_{10}$	$[M+H]^+$	732.4681	8.970
Spinosyn D	$C_{42}H_{67}NO_{10}$	$[M+H]^+$	746.4838	9.353
Tebuthiuron	$C_9H_{16}N_4OS$	$[M+H]^+$	229.1118	4.802
Thiacloprid	$C_{10}H_9ClN_4S$	$[M+H]^+$	253.0309	5.219
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	$[M+H]^+$	292.0266	3.937
Thiodicarb	$C_{10}H_{18}N_4O_4S_3$	$[M+H]^+$	355.0563	7.162
Triflumuron	$C_{15}H_{10}ClF_3N_2O_3$	$[M+H]^+$	359.0405	7.213

■ Full Scan Analysis by LCMS-9030

Full scan analysis of the 54 pesticide standard mixture diluted to 2.5 ppb and acetonitrile as blank solution was performed. Fig. 3 shows the total ion current chromatogram (TICC) of the pesticide standard solution, and Fig. 4 shows the extracted ion chromatogram (XIC) of each of the 54 compounds in the standard solution and blank solution. All 54 pesticides were detected at a concentration of 2.5 ppb from the standard solution.

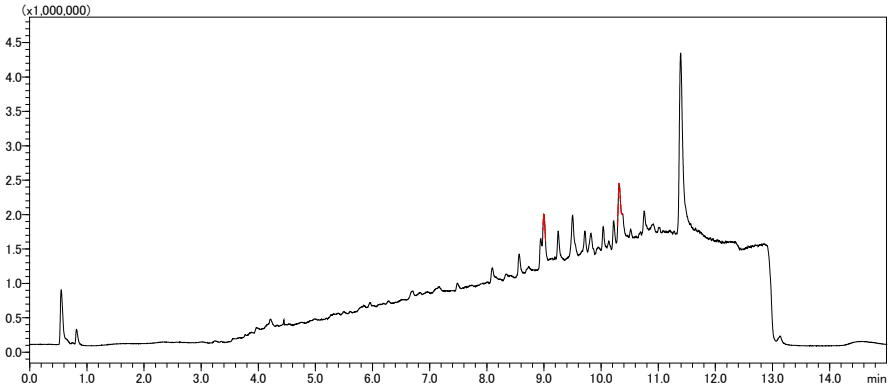

Fig. 3 Total Ion Current Chromatogram of Pesticides Mixture Standard Solution

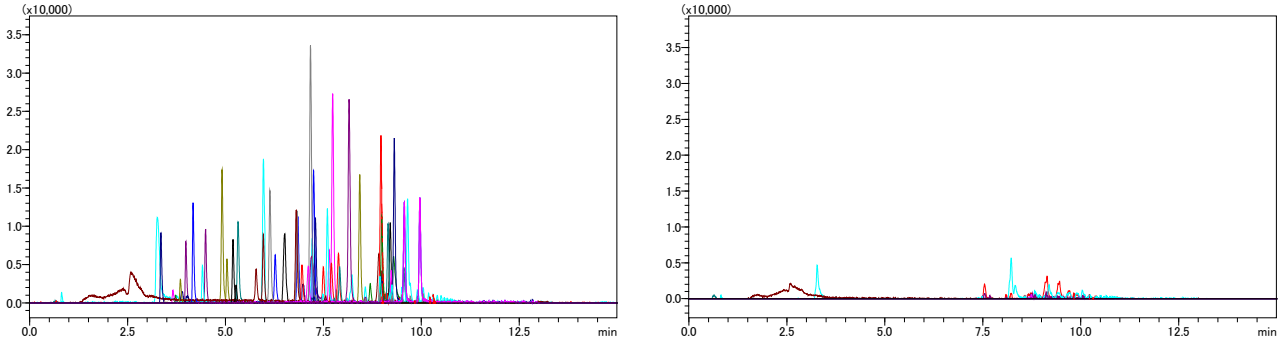

Fig. 4 Extracted Ion Chromatograms of 54 Pesticide Compounds in Standard Solution (Left) and Blank (Right)

■ Linearity of Calibration Curve

Linearity of the calibration curve for each pesticide was evaluated by generating a 6-point calibration curve with the range 0.25-50 ppb (in solvent) or a 5-point calibration curve with the range 0.25-25 ppb (in strawberry extract). Both in solvent and in strawberry extract, linearity showed very good results (coefficient of determination R^2: 0.99 or more) for all compounds. Calibration curves for Boscalid in solvent and in extract are shown in Fig. 5 as an example, and calibration ranges for all 54 compounds are shown in Table 3.

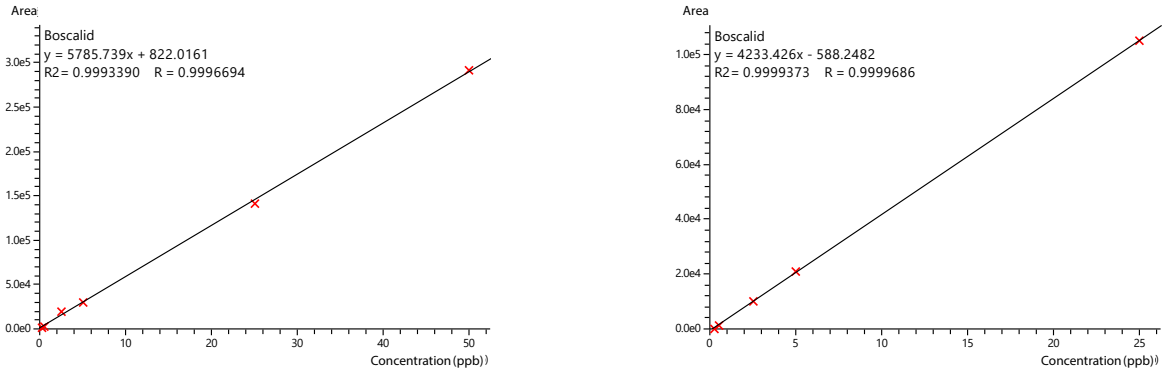

Fig. 5 Calibration Curve of Boscalid (Left: in Solvent, Right: in Strawberry Extract)

Table 3 Linear Range of 54 Pesticides

Compound	Calibration Range (ppb)		Compound	Calibration Range (ppb)	
	in solvent	in strawberry extract		in solvent	in strawberry extract
(E)-Fenpyroximate	0.25-50	0.25-25	Flufenoxuron	0.25-50	0.25-25
(Z)-Fenpyroximate	0.25-50	0.25-25	Fluridone	0.25-50	0.25-25
Acibenzolar-S-methyl	2.5-50	2.5-25	Hexythiazox	0.25-50	0.25-25
Aldicarb-sulfone (Aldoxycarb)	0.25-50	0.25-25	Imazalil	0.5-50	2.5-25
Anilofos	0.25-50	0.25-25	Imidacloprid	0.25-50	0.25-25
Azamethiphos	0.25-50	0.25-25	Indanofan	2.5-50	5-25
Azinphos-methyl	2.5-50	2.5-25	Iprovalicarb	0.25-50	0.25-25
Azoxystrobin	0.25-50	0.25-25	Lactofen	0.5-50	0.25-25
Benzofenap	5-50	2.5-25	Mepanipyrim	2.5-50	2.5-25
Boscalid	0.25-50	0.25-25	Methabenzthiazuron	0.5-50	0.25-25
Carbaryl (NAC)	0.5-50	2.5-25	Methomyl	2.5-50	2.5-25
Carpropamid	0.25-50	0.25-25	Monolinuron	0.25-50	0.25-25
Chloridazon	0.25-50	0.25-25	Novaluron	2.5-50	2.5-25
Chloroxuron	0.25-50	0.25-25	Oxaziclomefone	0.25-50	0.25-25
Clofentezine	0.5-50	0.5-25	Oxycarboxin	0.25-50	0.25-25
Cloquintocet-mexyl	0.25-50	0.25-25	Pirimicarb	0.25-50	0.25-25
Clothianidin	0.5-50	2.5-25	Pyraclostrobin	5-50	2.5-25
Cumyluron	0.25-50	0.25-25	Pyrazolynate	0.25-50	0.25-25
Cyazofamid	0.25-50	0.5-25	Pyriftalid	0.25-50	0.25-25
Cyprodinil	0.25-50	0.25-25	Simeconazole	0.25-50	0.25-25
Dimethomorph (E, Z)	0.25-50	0.25-25	Spinosyn A	0.25-50	2.5-25
Diuron (DCMU)	0.25-50	0.25-25	Spinosyn D	0.25-50	2.5-25
Epoxiconazole	0.25-50	0.25-25	Tebuthiuron	0.25-50	0.25-25
Fenamidone	0.25-50	0.25-25	Thiacloprid	0.25-50	0.25-25
Fenobucarb	0.25-50	0.25-25	Thiamethoxam	0.25-50	0.5-25
Fenoxaprop-ethyl	0.25-50	0.25-25	Thiodicarb	0.25-50	0.25-25
Flufenacet	0.25-50	0.25-25	Triflumuron	0.25-50	0.5-25

■ Spike and Recovery Test

A spike and recovery test was performed using strawberry extract to which 54 pesticides mixture standard solution was spiked at 10 ppb per sample (concentration in pretreated sample solution was 2.5 ppb), and the recovery rate and mass error (n=4) were evaluated. The results of recovery rate, reproducibility (%RSD), and mass error are shown in Table 4, and the breakdown of recovery rate is shown in Fig. 6.

Recovery rates were 70-120% for 50 of the 54 compounds. Good recovery rate and reproducibility were obtained without significant matrix inhibition, even in solutions containing high sample concentration.

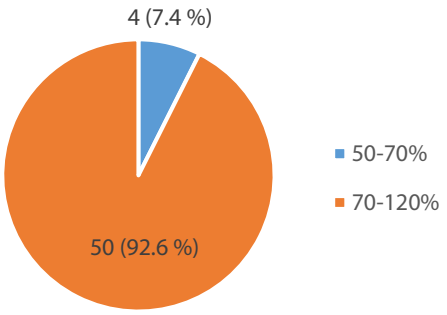

Fig. 6 Breakdown of Recovery Rate

Table 4 Recovery Rate, Reproducibility (%RSD) and Mass Error (n=4)

Compound	Recovery Rate (%)	%RSD	Mass Error (mDa)	Compound	Recovery Rate (%)	%RSD	Mass Error (mDa)
(E)-Fenpyroximate	93.2	8.5	-0.6	Flufenoxuron	108.1	4.7	-0.6
(Z)-Fenpyroximate	91.5	5.4	-0.6	Fluridone	96.4	6.5	-0.6
Acibenzolar-S-methyl	91.4	1.6	-0.6	Hexythiazox	90.9	5.8	-0.7
Aldicarb-sulfone (Aldoxycarb)	54.3	4.7	-0.5	Imazalil	81.9	9.4	-0.7
Anilofos	89.6	2.6	-0.6	Imidacloprid	97.1	2.5	-0.4
Azamethiphos	94.4	1.5	-0.5	Indanofan	92.5	20.3	-0.7
Azinphos-methyl	96.5	8.0	-0.9	Iprovalicarb	94.9	5.2	-0.9
Azoxystrobin	95.1	1.4	-0.6	Lactofen	100.2	3.5	-0.3
Benzofenap	85.3	2.8	-0.5	Mepanipyrim	85.0	4.5	-0.1
Boscalid	92.5	5.3	-0.6	Methabenzthiazuron	87.6	5.8	-0.6
Carbaryl (NAC)	92.5	7.2	-0.8	Methomyl	89.8	6.1	0.1
Carpropamid	96.7	2.2	-0.8	Monolinuron	89.2	6.5	-0.7
Chloridazon	64.4	1.4	-0.6	Novaluron	102.6	3.5	-0.7
Chloroxuron	100.8	1.4	-0.7	Oxaziclomefone	92.7	2.3	-0.6
Clofentezine	83.8	6.9	-0.4	Oxycarboxin	78.2	5.2	-0.6
Cloquintocet-mexyl	86.7	6.7	-0.5	Pirimicarb	73.7	3.3	-0.6
Clothianidin	53.6	2.2	-0.5	Pyraclostrobin	84.0	5.4	-0.7
Cumyluron	96.0	1.2	-0.5	Pyrazolynate	112.7	5.2	-0.7
Cyazofamid	96.4	5.9	-0.7	Pyriftalid	94.2	1.8	-0.5
Cyprodinil	82.7	6.5	-0.7	Simeconazole	104.6	1.3	-0.6
Dimethomorph (E, Z)	96.9	2.5	-0.5	Spinosyn A	88.7	4.8	-1.2
Diuron (DCMU)	89.9	4.3	-0.6	Spinosyn D	97.7	3.1	-1.2
Epoxiconazole	100.5	2.4	-0.5	Tebuthiuron	88.4	1.4	-0.7
Fenamidone	93.0	0.6	-0.7	Thiacloprid	92.1	4.7	-0.7
Fenobucarb	109.8	9.0	-0.5	Thiamethoxam	63.1	4.2	-0.5
Fenoxaprop-ethyl	90.6	2.3	-0.7	Thiodicarb	83.4	1.4	-0.4
Flufenacet	91.1	5.6	-0.5	Triflumuron	94.5	6.0	-0.5

■ Conclusion

The sample preparation method combining the QuEChERS (EN 15662) and SPEEDIA made it possible to speed up and simplify the preparation process. Full scan analysis of pretreated strawberry samples using LCMS-9030 provided good results for spike recovery rate, reproducibility, and linearity. It was demonstrated that the analytical method introduced in this article enables “rapid, simple, and highly precise” analysis, and is useful for the analysis of residual pesticides in food.

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