Application Note: 387

Multi-residue Analysis of Pesticides in Food using GC/MS/MS with the TSQ Quantum GC

Kuniyo Sugitate, Michiko Kanai, Thermo Fisher Scientific, Yokohama, Japan
Masahiro Okihashi, Division of Food Chemistry, Osaka Prefectural Institute of Public Health, Osaka, Japan
Dipankar Ghosh, Thermo Fisher Scientific, San Jose, CA, USA

Key Words

- TSQ Quantum GC
- H-SRM
- Pesticide Residues in Food
- Positive List System
- QED
- SRM

Introduction

Food safety concerns are on the rise amongst consumers worldwide. In 2006, sweeping changes were made to the Food Hygiene Law in Japan regarding residues of agricultural chemicals, including pesticides, in foods. As a result, standard residue values were established for approximately 800 pesticides. All food items produced in or imported into Japan are required to meet the standards established by this law. If pesticide residues in any food items exceed these standards, then the distribution and sale of the food is prohibited. This Positive List System has had a significant effect not only on the Japanese domestic production, but also on much of the food exported to Japan from various foreign countries such as China, the United States, and Taiwan.

There are numerous types of pesticides regularly used in the agricultural industry, including insecticides, fungicides, herbicides, and growth regulators. Because each type has different physicochemical properties, there are limitations on simultaneous analysis. Among the pesticides for which standard values are currently set, GC/MS/MS can analyze approximately 300 compounds. The superior selectivity of this technique allows interference-free quantification, even with peak coelution, and provides positive confirmation of various pesticides in a single analytical run.

To accurately monitor pesticide residues, a high throughput multi-residue screening method that can quantitate a large number of pesticide residues during a single analytical run is needed.

Goal

To simultaneously analyze 103 pesticides using the TSQ Quantum™ GC system, using SRM and H-SRM. Additionally, to show the utility of QED MS/MS for structural confirmation of the analytes undergoing quantification.

Experimental Conditions

Sample Preparation

Green pepper, carrot, grapefruit and banana samples were prepared for analysis using a method based on the simple and quick QuEChERS approach.[1] A 10 g sample of food was homogenized in a food processor and placed in a polypropylene centrifuge tube. The sample was extracted with 20 mL of acetonitrile in a homogenizer. Then, 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride were added and the resulting mixture was centrifuged. After centrifugation, the supernatant was loaded onto a graphite carbon/PSA dual layer solid phase extraction column and eluted with 50 mL of acetonitrile/toluene (3:1). After the eluate was concentrated under reduced pressure, it was dissolved (1 g/mL) in 10 mL of acetonitrile/n-hexane to give the test solution.

GC

GC analysis was performed using the TRACE GC Ultra™ System (Thermo Fisher Scientific, Milan, Italy). The GC conditions were as follows:

Column: Rti-5MS 30 m x 0.25 mm I.D., 0.25 m df (Restek Corp., Bellefonte, PA)
Injection mode: Splitless with surge injection (200 kPa, 1 min)
Injection temperature: 240 °C
Oven temperature: 80 °C (1 min) 20 °C/min 180 °C 5 °C/min 280 °C (10 min)
Flow rate: Constant flow 1.2 mL/min
Transfer line temperature: 280 °C

AS

The samples were injected through the TriPlus™ autosampler (Thermo Fisher Scientific, Milan, Italy). The autosampler conditions were as follows:

Injection volume: 1 L
Injection mode: Hot needle
Syringe: 80 mm

MS

MS analysis was carried out on a TSQ Quantum™ GC triple stage quadrupole mass spectrometer. (Thermo Fisher Scientific, San Jose, CA). The MS conditions were as follows:

Ionization mode: EI positive ion
Ion volume: Closed EI
Emission current: 25 A
Ion source temperature: 220 °C
Scan type: SRM and H-SRM
Scan width: 0.002 a.m.u.
Scan time: 0.002 s, 0.005 s, 0.01 s
Peak width: Q1, 0.7 Da; Q3, 0.7 Da FWHM
Peak width for H-SRM: Q1, 0.4 Da; Q3, 0.7 Da FWHM
Collision gas (Ar) pressure: 1.2 mTorr

A total of 103 pesticides were analyzed to determine the product ion to be used for quantitation. Table 1 lists the SRM transitions and the optimum collision energy for each of the compounds and a summary of the calibration range, linearity, and the reproducibility of each individual compound at 5 ppb (ng/mL).

	R.T.	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy	R^2	Range	CV(%) n=5
Mevinphos	6.44	192	127	10	0.9999	0.1-100	4.03
XMC	7.52	122	107	10	0.9999	0.1-100	2.55
Tecnazene	8.03	261	203	15	0.9996	0.1-100	5.41
Ethoprpphos	8.22	200	114	10	0.9981	0.1-100	7.91
Ethalfluralin	8.42	316	276	10	0.9997	0.1-100	4.14
Benfluralin	8.62	292	264	10	0.9989	0.1-100	1.86
Monocrotophos	8.62	192	127	10	0.9754	5-100	19.47
α-BHC	9.03	219	183	15	0.9999	0.1-100	4.51
Dicloran	9.25	206	176	10	0.9994	0.1-100	2.30
Simazine	9.30	201	172	10	0.9999	0.1-100	4.33
Propazine	9.50	214	172	10	0.9998	0.1-100	1.99
β-BHC	9.57	219	183	15	1.0000	0.1-100	3.51
γ-BHC	9.73	219	183	15	0.9998	0.1-100	6.57
Cyanophos	9.78	243	109	10	0.9996	0.1-100	3.56
Pyroquilon	9.90	173	130	20	0.9996	0.1-100	2.95
Diazinon	4.94	304	179	15	0.9995	0.1-100	4.40
Phosphamidon-1	10.06	264	127	10	0.9989	0.1-100	10.31
Prohydrojasmon-1	10.12	184	83	20	0.9992	0.1-100	7.39
δ-BHC	10.26	219	183	15	0.9994	0.1-100	5.17
Prohydrojasmon-2	10.66	264	127	10	0.9972	0.1-100	17.11
Benoxacor	10.7	259	120	15	0.9999	0.1-100	3.30
Propanil	10.95	262	202	10	0.9993	0.1-100	3.65
Phosphamidon-2	10.97	264	127	10	0.9970	0.1-100	8.77
Dichlofenthion	10.99	279	223	15	0.9994	0.1-100	2.21
Dimethenamid	11.06	230	154	10	0.9996	0.1-100	2.51
Bromobutide	11.09	232	176	10	0.9990	0.1-100	5.91
Paration-methyl	11.24	263	109	10	0.9982	0.1-100	3.74
Tolclofos-methyl	11.38	265	250	15	0.9998	0.1-100	2.52
Ametryn	11.43	227	170	10	0.9999	0.1-100	0.90
Mefenoxam	11.57	249	190	10	0.9995	0.1-100	5.81
Bromacil	11.98	205	188	15	0.9988	0.1-100	3.87
Pirimiphos-methyl	12.00	305	276	10	0.9995	0.1-100	4.08
Quinoclamine	12.18	207	172	10	0.9989	0.1-100	4.24
Diethofencarb	12.34	225	125	15	0.9985	0.1-100	4.64
Cyanazine	12.52	225	189	10	0.9994	0.1-100	3.41
Chlorpyrifos	12.57	314	258	15	0.9991	0.1-100	3.37
Parathion	12.59	291	109	15	0.9962	0.1-100	9.76
Triadimefon	12.67	208	111	25	0.9986	0.1-100	6.10
Chlorthal-dimethyl	12.73	301	223	20	1.0000	0.1-100	1.23
Nitrothal-isopropyl	12.78	236	148	15	0.9974	0.1-100	5.53
Fthalide	13.04	272	243	10	0.9993	0.1-100	4.32
Fosthiazate	13.05 13.12	195	103	10	0.9956	5-100	6.29
Diphenamid	13.1	239	167	10	0.9997	0.1-100	4.67
Pyrifenox-Z	13.64	262	200	15	0.9979	0.2-100	4.54
Fipronil	13.79	123	81	10	0.9991	0.1-100	3.49
Allethrin	13.67	367	213	25	0.9991	5-100	3.79
Dimepiperate	13.87	145	112	10	0.9987	0.1-100	3.74
Quinalphos	13.87	274	121	10	0.9987	0.1-100	1.82
Phenthoate	13.88	146	118	10	0.9984	0.1-100	1.96
Paclobutrazol	14.45	236	125	15	0.9961	0.1-100	7.41
Endosulfan-α	14.67	241	206	15	0.9996	0.1-100	4.54
Butachlor	14.73	237	160	10	0.9998	0.1-100	5.26
Imazamethabenz-methyl	14.81	256	144	20	0.9932	2-100	12.09
Butamifos	15.00	286	202	15	0.9958	0.1-100	4.66
Flutlanil	15.06	173	145	15	0.9986	0.1-100	1.93
Hexaconazole	15.06	214	172	15	0.9924	0.1-100	8.98
Profenofos	15.28	337	267	15	0.9968	0.1-100	6.61
Uniconazole-P	15.38	234	137	15	0.9966	0.1-100	11.37
Pretilachlor	15.37	162	132	15	0.9982	0.1-100	6.72
Flamprop-methyl	15.66	276	105	10	0.9986	0.1-100	3.93
Oxyfluorfen	15.69	361	300	10	0.9980	0.5-100	6.07
Azaconazole	15.79	217	173	15	0.9981	0.1-100	7.07
Bupirimate	15.82	316	208	10	0.9982	0.1-100	4.65
Thifluzamide	15.84	449	429	10	0.9972	0.1-100	2.75
Fenoxanil	16.25	293	155	20	0.9989	0.1-100	3.73
Chlorbenzilate	16.43	251	139	15	0.9976	0.1-100	0.81
Pyriminobac-methyl-Z	16.76	302	256	15	0.9986	0.1-100	2.70
Oxadixyl	16.86	163	132	10	0.9998	0.1-100	3.72
Triazophos	17.30	257	162	10	0.9941	0.2-100	6.72
Fluacrypyrim	17.38	189	129	10	0.9988	0.1-100	2.15
Edifenphos	17.72	310	173	10	0.9927	0.1-100	7.95
Quinoxyfen	17.74	272	237	10	0.9993	0.1-100	4.50
Lenacil	17.78	153	136	15	0.9979	0.1-100	5.19
Trifloxystrobin	18.01	222	162	10	0.9966	0.1-100	8.47
Pyriminobac-methyl-E	18.19	302	256	15	0.9982	0.1-100	2.12
Tebuconazole	18.39	250	125	20	0.9907	0.2-100	13.03
Diclofop-methyl	18.51	253	162	15	0.9991	0.1-100	2.14
Mefenpyr-diethyl	19.15	253	189	20	0.9992	0.1-100	3.35
Pyributicarb	19.24	165	108	10	0.9973	0.1-100	2.00
Pyridafenthion	19.46	152	116	20	0.9940	0.2-100	4.71
Acetamiprid	19.39	340	199	10	1.0000	50-100	–
Bromopropylate	19.64	341	185	15	0.9956	0.1-100	3.72
Piperophos	19.84	320	122	10	0.9939	0.2-100	7.51
Fenpropathrin	19.98	265	210	10	0.9973	0.1-100	6.87
Etoxazole	20.06	300	270	20	0.9969	0.1-100	8.84
Tebufenpyrad	20.10	333	171	20	0.9978	0.5-100	13.35
Anilofos	20.31	226	157	15	0.9948	0.2-100	5.56
Phenothrin-1	20.49	183	165	10	0.9967	5-100	16.13
Tetradifon	20.54	356	229	10	0.9998	0.2-100	4.17
Phenothrin-2	20.66	183	165	10	0.9968	0.1-100	3.79
Mefenacet	21.22	192	136	15	0.9955	0.1-100	4.90
Cyhalofop-buthyl	21.23	357	229	10	0.9967	0.1-100	5.52
Cyhalothrin-1	21.30	181	152	20	0.9975	0.2-100	3.21
Cyhalothrin-2	21.66	181	152	20	0.9984	0.2-100	6.67
Pyrazophos	22.06	373	232	10	0.9963	0.1-100	10.46
Bitertanol	22.80 22.97	170	141	20	0.9873	0.1-100	6.76
Pyridaben	23.18	147	117	20	0.9958	0.1-100	1.29
Cafenstrole	24.03	100	72	5	0.9958	0.1-100	9.77
Cypermethrin-1	24.72	181	152	20	0.9983	2-100	9.29
Halfenprox	24.79	263	235	15	0.9979	0.1-100	10.25
Cypermethrin-2	24.92	181	152	20	0.9982	2-100	6.91
Cypermethrin-3	25.06	181	152	20	0.9985	2-100	16.27
Cypermethrin-4	25.13	181	152	20	0.9948	2-100	13.79
Fenvalerate-1	26.47	167	125	10	0.9977	0.1-100	3.11
Flumioxazin	26.50	354	176	20	0.9937	0.1-100	9.66
Fenvarelate-2	26.91	167	125	10	0.9979	0.1-100	3.26
Deltamethrin+ Tralomethrin	28.15	181	152	20	0.9967	0.2-100	8.20
Tolfenpyrad	29.11	383	171	20	0.9968	2-100	4.84
Imibenconazole	30.35	375	260	15	1.0000	50-100	–

Table 1: Retention times, SRM conditions, calibration range, linearity, and the reproducibility of each individual pesticide residue compound

Results and Discussion

Figure 1 shows an example calibration curve for Propazine at 0.1-100 ppb with a corresponding chromatogram at 1 ppb, showing excellent reproducibility (r^2 = 0.9998).

Figure 2 shows examples of GC/MS/MS chromatograms of various pesticides in which 1 ppb of each pesticide was added to green pepper. Even at this extremely low concentration (1/10 of the uniform standard value for pesticides), it was possible to make measurements with remarkably high sensitivity with the TSQ Quantum GC.

Figure 3 shows the chromatograms for cypermethrin, fenvalerate and deltamethrin (+ tralomethrin). Cypermethrin is a synthetic pyrethroid compound with a high detection ratio in agricultural produce. In addition to having a slow elution time in the GC, it has 4 peaks that are due to different isomers that must be resolved. As the chromatograms show, measurements with good sensitivity were obtained even at the low concentration of 5 ppb.

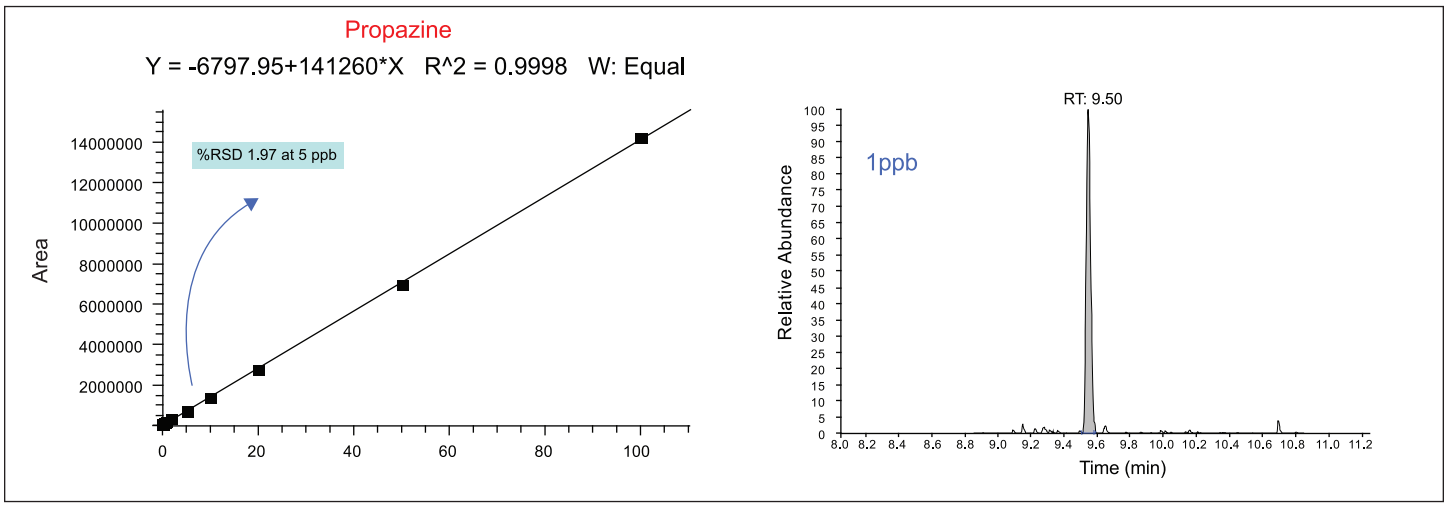

Figure 1: Calibration curve (0.1-100 ppb) and SRM chromatogram (1 ppb) for Propazine

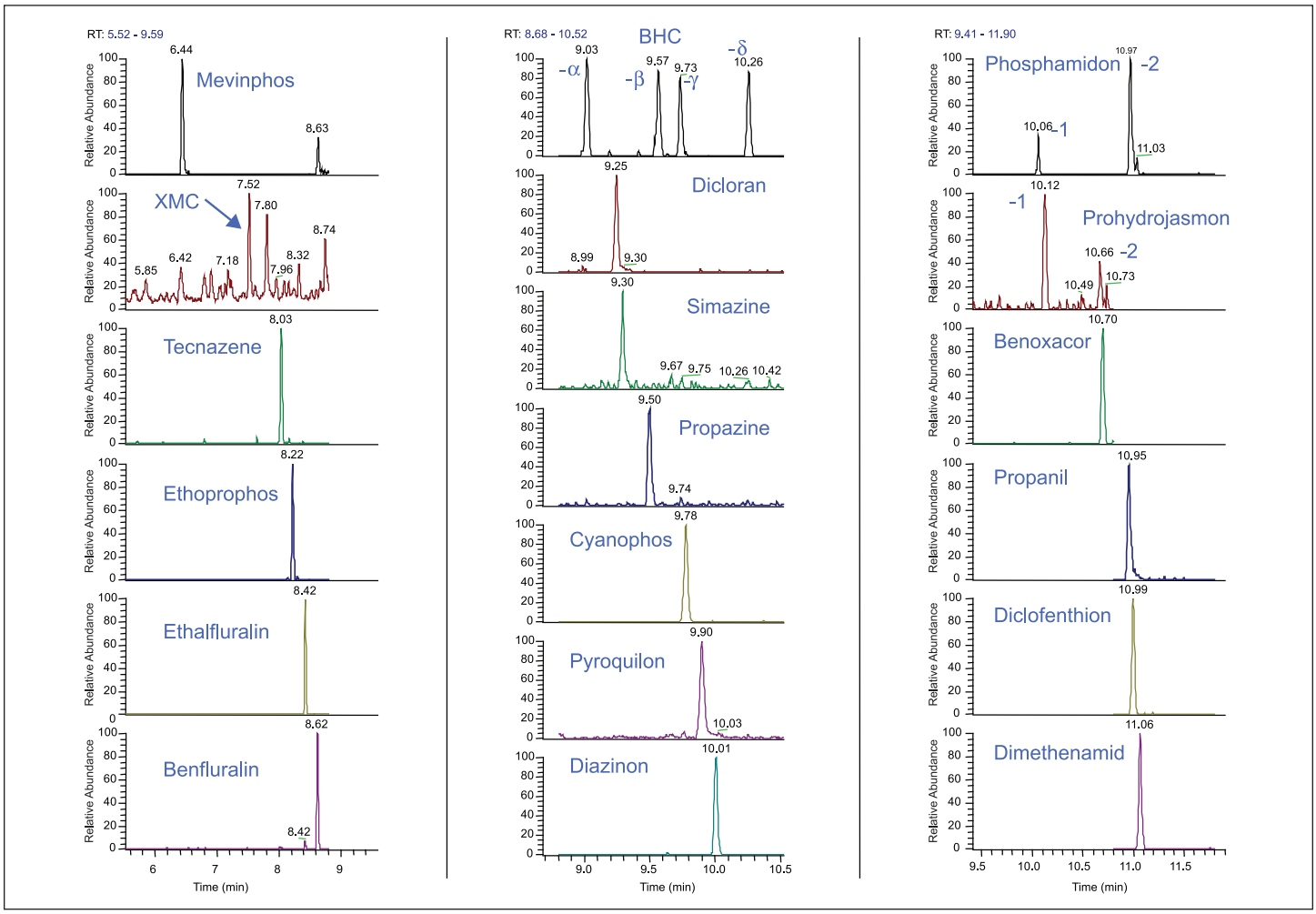

Figure 2: GC/MS/MS chromatograms of various pesticides at 1 ppb in green pepper samples

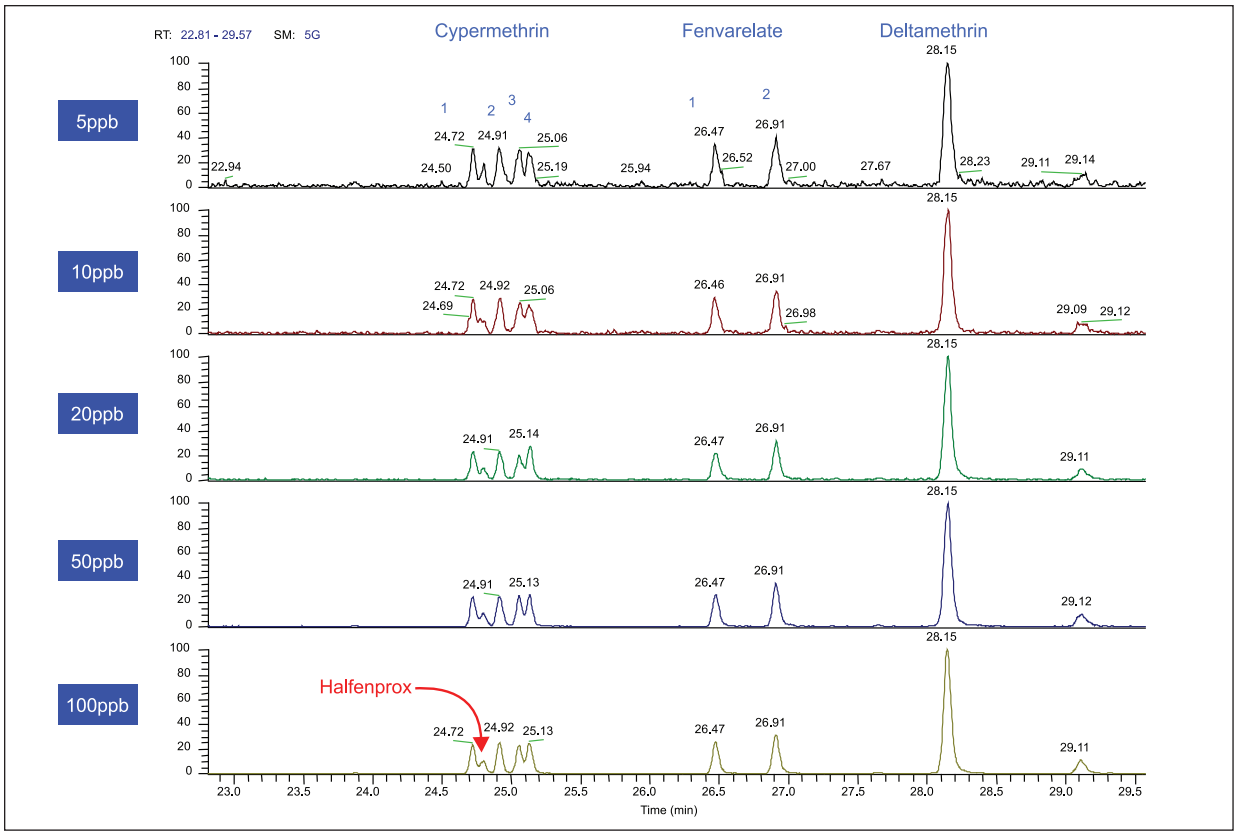

Figure 3: Chromatograms for cypermethrin, fenvalerate and deltamethrin

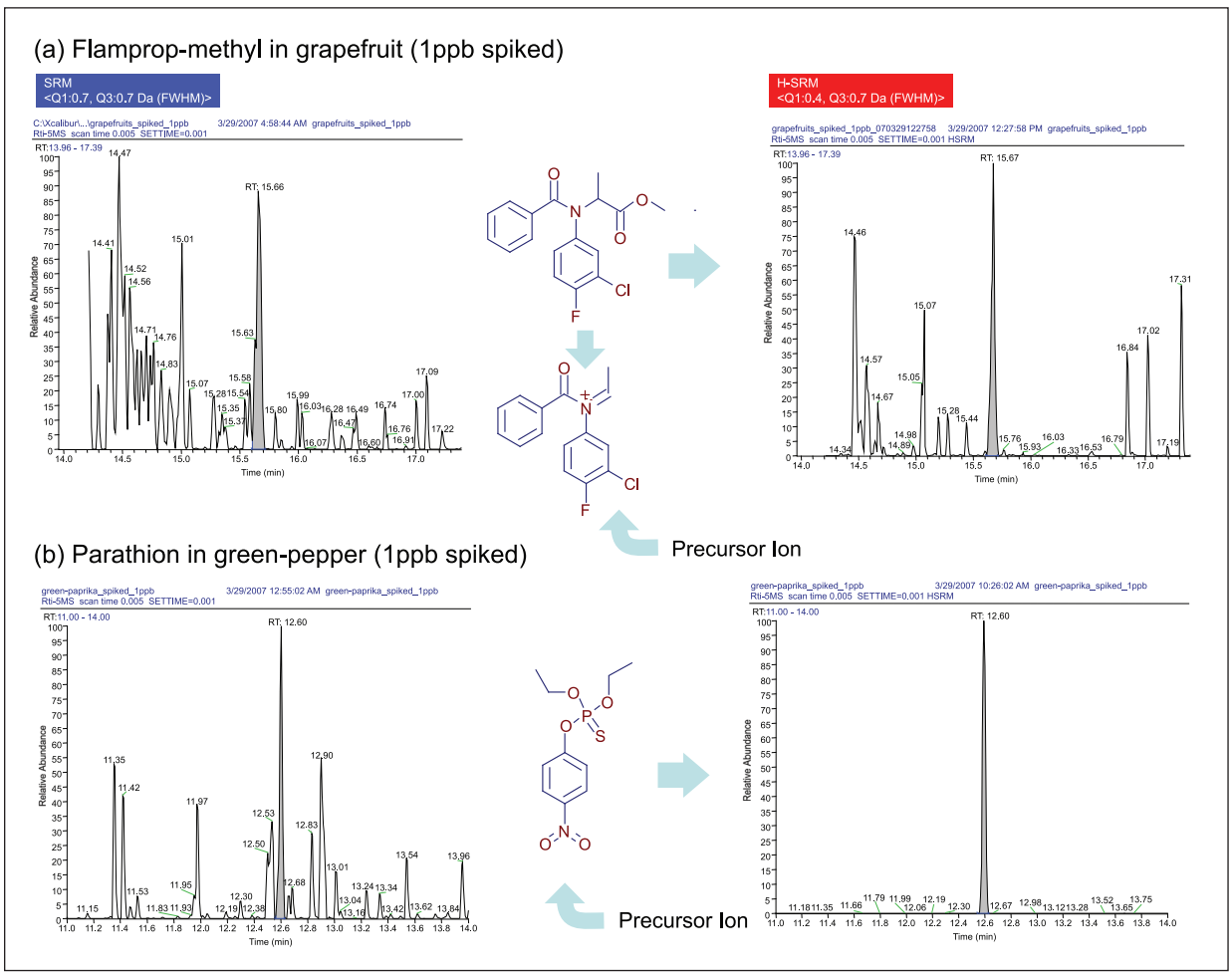

Figure 4: Comparison of SRM Mode with H-SRM Mode. (a) Flamprop-methyl in grapefruit (1 ppb). (b) Parathion in green-pepper (1 ppb).

Advantages of H-SRM

H-SRM is an acronym for Highly-Selective Reaction Monitoring, which is a more advanced form of Selective Reaction Monitoring (SRM). H-SRM can eliminate chemical noise, lower detection limits, and reduce the likelihood of generating false positives. For many pesticides that are subject to matrix-dependent interference, the measurements can be successfully carried out using the H-SRM mode. With H-SRM, the precursor ion is selected with a smaller peak width. The more stringent tolerance accounts for the higher selectivity, which can lower LOQs and increase precision and accuracy at the limits of detection. The effects of H-SRM over SRM are illustrated for flamprop-methyl in grapefruit and parathion in green-pepper in Figure 4.

Structural Confirmation with QED

QED MS/MS stands for Quantification Enhanced by Data Dependant™ MS/MS. A QED scan on a triple quadrupole instrument delivers an information rich mass spectrum that can be used for structural confirmation of analytes while undergoing quantification by SRM (or H-SRM). The specificity provided by H-SRM followed by QED MS/MS provides uncompromised quantitation performance at low levels followed by a fast, highly-specific full MS/MS scan for confirmation. Figure 5 shows the QED scan results obtained from a carrot test sample spiked with 10 ppb diazinon.

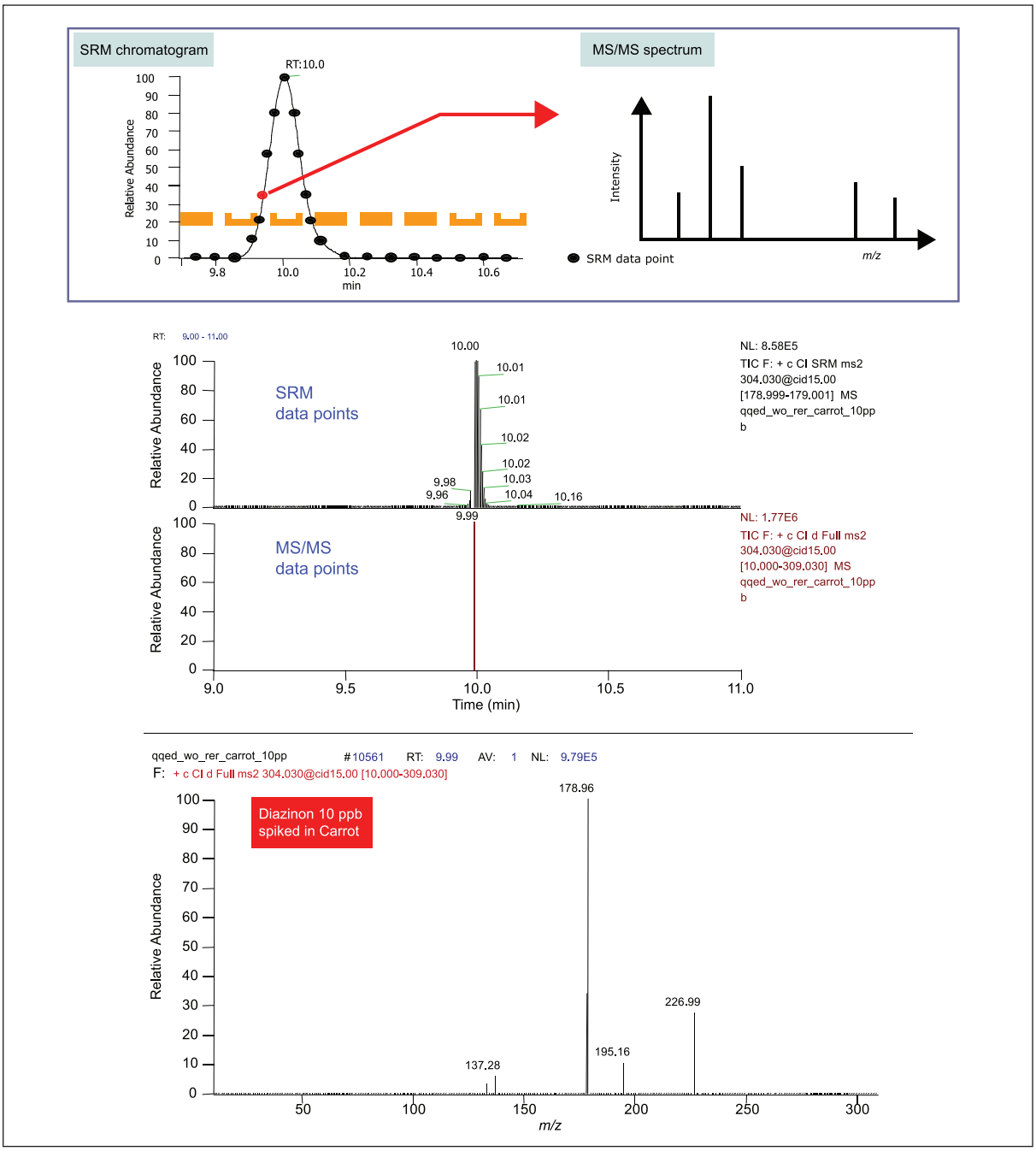

Figure 5: Chromatogram from a carrot test sample (upper row) and the MS/MS spectrum obtained with QED (lower row)

Zero Cross-talk

Cross-talk can potentially occur when fragment ions from one SRM transition remain in the collision cell while a second SRM transition takes place. This can cause signal artifacts in the second SRM transition's chromatogram. It can be especially problematic when different SRM events have the same product ions formed from different precursor ions. However, the orthogonal design of the collision cell in the TSQ Quantum eliminates cross-talk. Figure 6 shows the absence of cross-talk between two different SRM transitions of paclobutrazol and thifluzamide. Both yield a product ion of *m/z* 125, but no artifacts are seen in either chromatogram with a scan time of 10 ms. Similarly, the SRM transitions of triszophos and diclofop-methyl 5 also show no evidence of cross talk, even though they both yield product ions at *m/z* 162.

Conclusion

Simultaneous analysis was carried out on multi-component pesticide residues in food products using a quadrupole GC/MS/MS system, the TSQ Quantum GC. Results obtained indicated excellent sensitivity (0.1 ppb), reproducibility (10% at 5 ppb) and linearity (R2 > 0.995) in the range of 0.1-100 ppb. No cross-talk was observed for the analysis of closely eluting multi-component mixtures. Using H-SRM, interferences from the sample matrix background were substantially reduced, leading to improved LOQs. In addition, QED provided MS/MS structural confirmation of the analytes undergoing quantification.

References

1. Okihashi, M.; Kitagawa, Y.; Akutsu, K.; Obana, H.; Tanaka, Y. *"Rapid method for the determination of 180 pesticide residues in foods by gas chromatography/mass spectrometry and flame photometric detection"*; J Pestic Sci **2005**, *30*(4), 368-77.
2. The Japanese Ministry of Health, Labour, and Welfare: *www.mhlw.go.jp/english/topics/foodsafety/positivelist060228/index.html* (English) *www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/zanryu2/index.html* (Japanese)

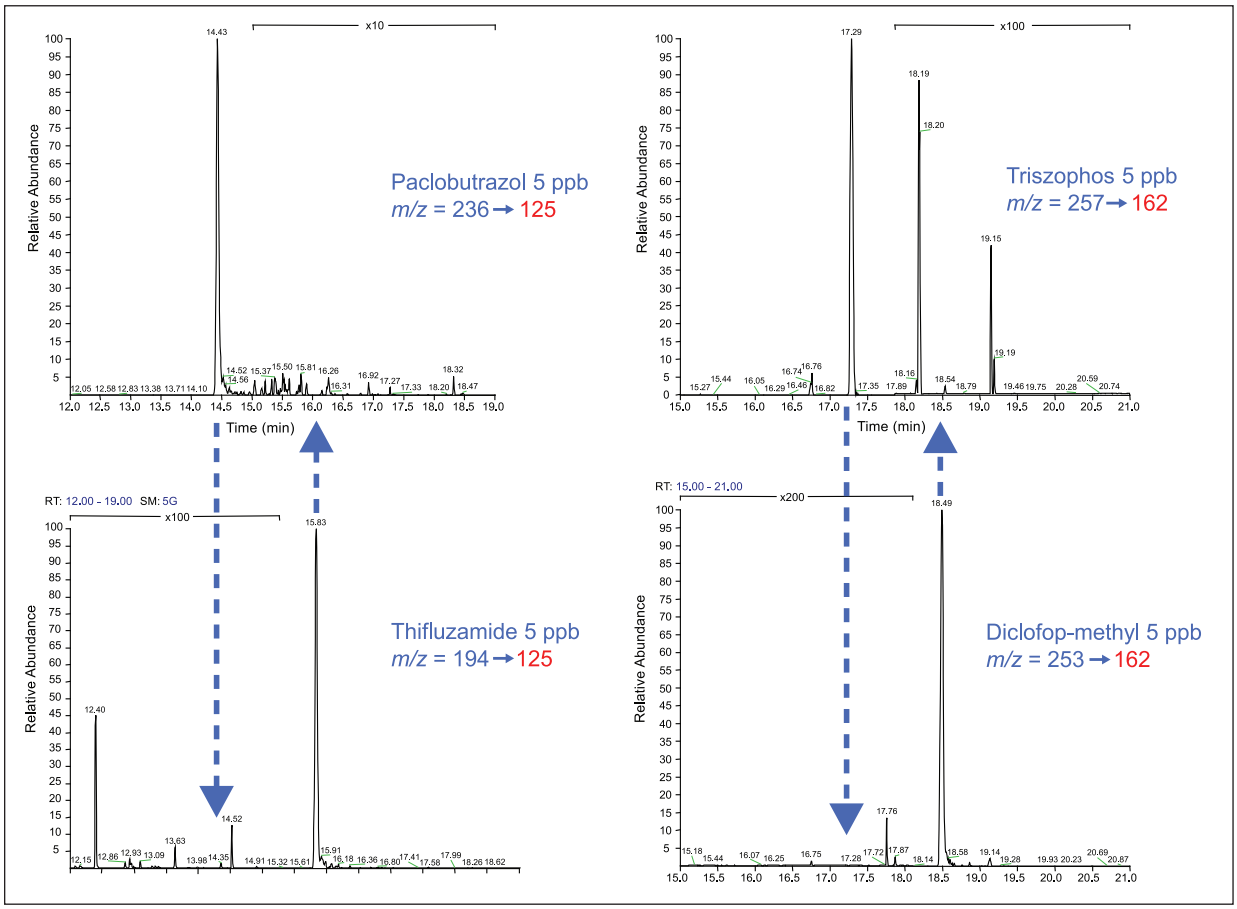

Figure 6: No cross-talk was observed in the SRM transitions of paclobutrazol and thifluzamide or in the SRM transitions of triszophos and diclofop-methyl.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Australia +61 2 8844 9500
Austria +43 1 333 50340
Belgium +32 2 482 30 30
Canada +1 800 532 4752
China +86 10 5850 3588
Denmark +45 70 23 62 60
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9434
Italy +39 02 950 591
Japan +81 45 453 9100
Latin America +1 608 276 5659
Netherlands +31 76 587 98 88
South Africa +27 11 570 1840
Spain +34 91 657 4930
Sweden/Norway/ Finland +46 8 556 468 00
Switzerland +41 61 48784 00
UK +44 1442 233555
USA +1 800 532 4752

www.thermo.com

ISO 9001 REGISTERED COMPANY

Thermo Finnigan LLC, San Jose, CA USA is ISO Certified.

AN387_E 05/07M