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Application News

No. C171

Liquid Chromatograph Mass Spectrometry

Analysis of Pesticides Added to Appendix Method 20-2 for the Complementary Items for Water Quality Management in Japan Using a Triple Quadrupole LC/MS/MS

In March 2018, Notification Nos. 0328-1 to 4 were issued by the Director of the Water Supply Division, Pharmaceutical Safety and Environmental Health Bureau, MHLW and the number of pesticides listed in an inspection method for the Complementary Items for water quality management in Japan's water quality standards was greatly increased. The method is Appendix Method 20-2 which describes simultaneous analysis using a liquid chromatograph mass spectrometer system and the number of pesticides was increased to 181.

A total of 113 pesticides were added. Of these, 7 are newly added pesticides and the remaining 106 are those that can be analyzed according to the analytical conditions of Appendix Method 20-2 but were already listed in other appendix methods. The other methods are Appendix Method 5 and 5-2 for simultaneous analysis using a solid-phase extraction gas chromatograph mass spectrometer system, Appendix Method 18 for simultaneous analysis using a solid-phase extraction liquid chromatograph mass spectrometer system, Appendix Method 19 for analysis using a solid-phase extraction liquid chromatograph mass spectrometer system, and Appendix Method 20 for simultaneous analysis using a liquid chromatograph mass spectrometer.

Under the analytical conditions in the notification, 99 of the 113 added pesticides, excluding the 14 pesticides for which the method is referential, can be measured with good accuracy down to concentrations that are a hundredth of the target value or lower.

Based on the validation guidelines of the drinking water inspection method, this article introduces the results of using the LCMS-8050 liquid chromatograph mass spectrometer for evaluating 99 of the added pesticides. This includes the 7 newly added pesticides, pesticides with a relatively low concentration as the target value, and pesticides for which the target value was revised to be more strict.

H. Horiike

Analysis of a Solution Containing 113 Pesticide Standards

We measured the total ion current (TIC) chromatogram of a mixed standard solution containing the standards of the 113 added pesticides. The chromatogram is shown in Fig. 1. The measurement duration was 65 min.

The analytical conditions are listed in Table 1.

Since many of the pesticides added to the liquid chromatograph mass spectrometry method are also included in the method for simultaneous analysis using a solid-phase extraction GC-MS system, the properties of the pesticides are various. Therefore, taking into consideration the characteristic of some pesticides in which adsorption to the analytical column occurs, a metal-free ODS column was selected for use in analysis.

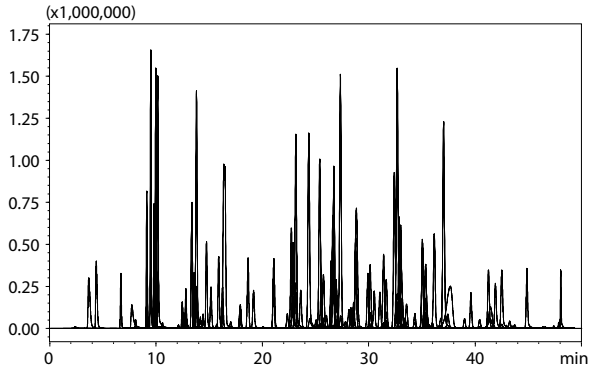


Fig. 1 TIC Chromatogram of a Mixed Standard Solution of 113 Pesticides (1 to 2 µg/L of each pesticide)

Table 1 Analytical Conditions

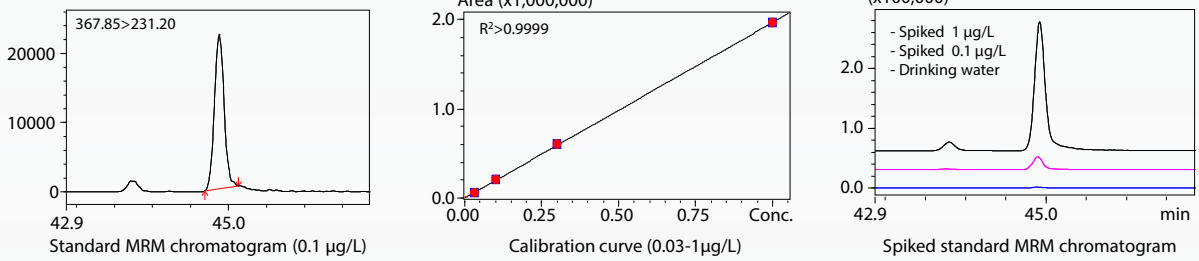
Column	:	L-column2 ODS Metal free (150 mm L. × 2.0 mm I.D., 3 µm, CERI)
Mobile phases	:	A 5 mmol/L Ammonium acetate-water
		B 5 mmol/L Ammonium acetate-methanol
Time schedule	:	B conc. 10 % (0 min) → 45 % (7 min) → 80 % (42 min) → 100% (46 - 51 min) → 10 % (51.01 - 65 min)
Flow rate	:	0.2 mL/min
Column temperature	:	40 °C
Injection volume	:	50 µL
Ionization	:	ESI (Positive / Negative)
DL temperature	:	300 °C
Block heater temperature	:	400 °C
Interface temperature	:	250 °C
Nebulizing gas flow	:	3 L/min
Drying gas flow	:	10 L/min
Heating gas flow	:	10 L/min

Analysis of the Seven Newly Added Pesticides

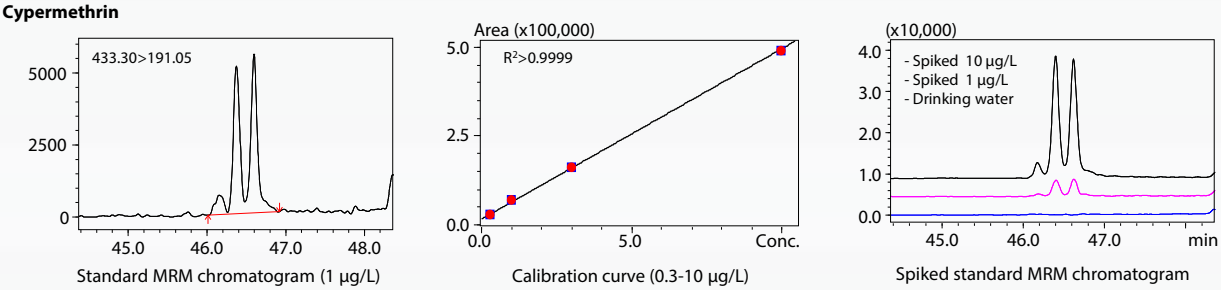
Among the pesticides added to Appendix Method 20-2, seven are new pesticides which until now did not have a designated inspection method. These seven[*1] are propargite, cypermethrin, ethiprole, cis-permethrin, trans-permethrin, fenoxanil, methamidophos, and tolfenpyrad. The following figures show the MRM chromatogram of each pesticide when the concentration is a hundredth of the target value or lower, and a four-point calibration curve when including a solution with a concentration that is a hundredth of the target value or lower. In addition, the figures include MRM chromatograms obtained when tap water dechlorinated using sodium ascorbate is spiked to a concentration of a tenth or a hundredth of the target value or lower.

*1 Counting isomers as individual items, there are eight items in all.

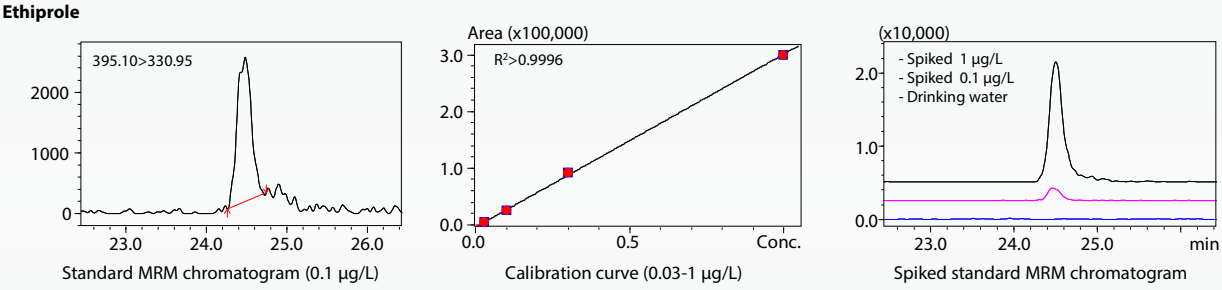
Propargite



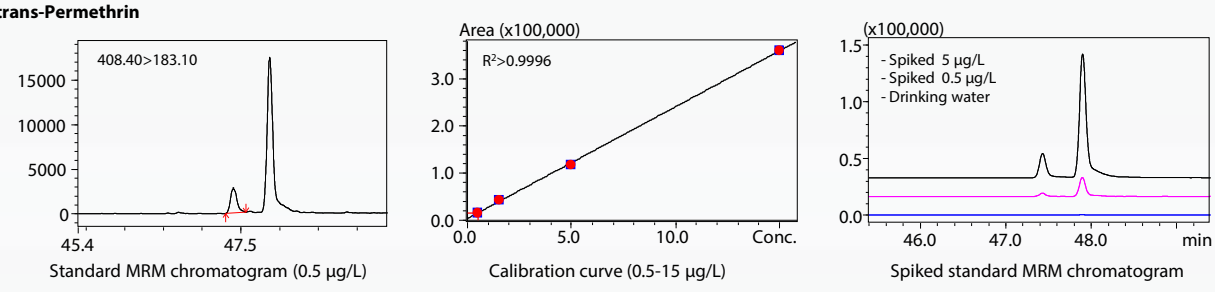
Cypermethrin



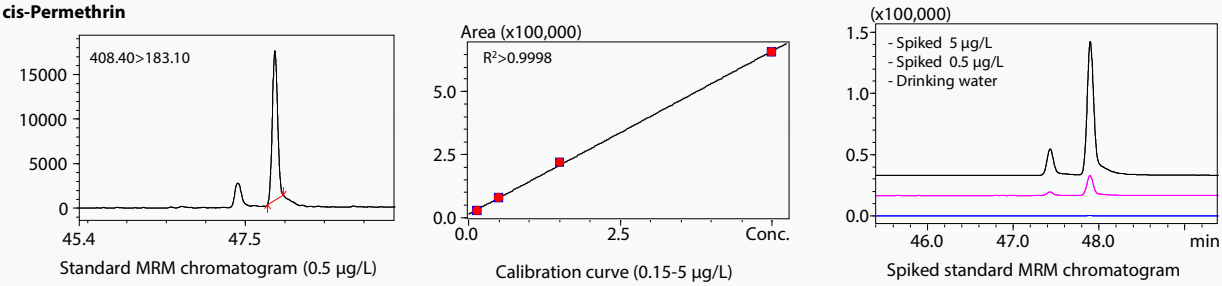
Ethiprole

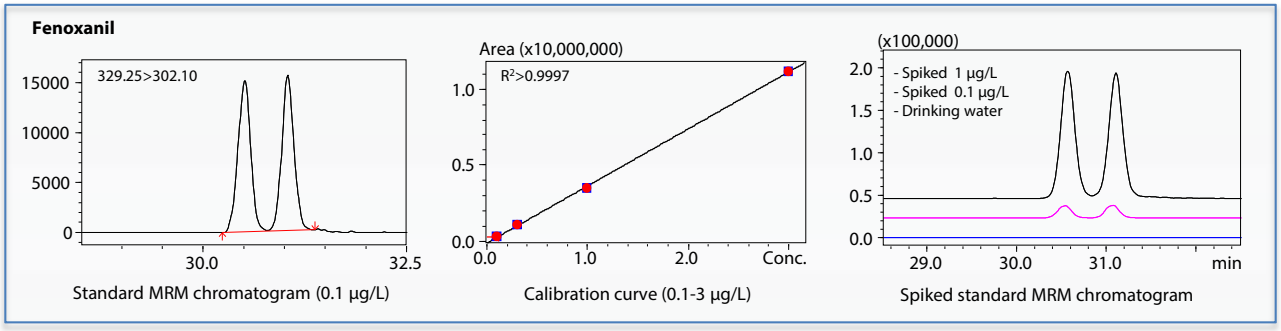


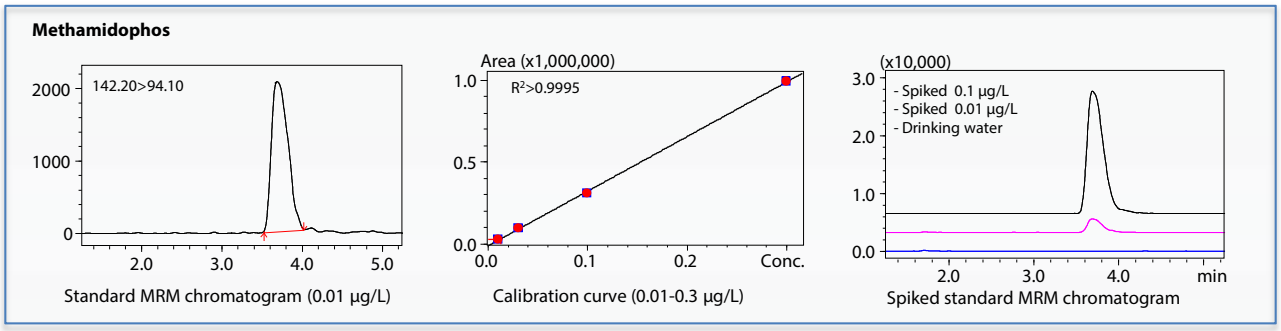
trans-Permethrin

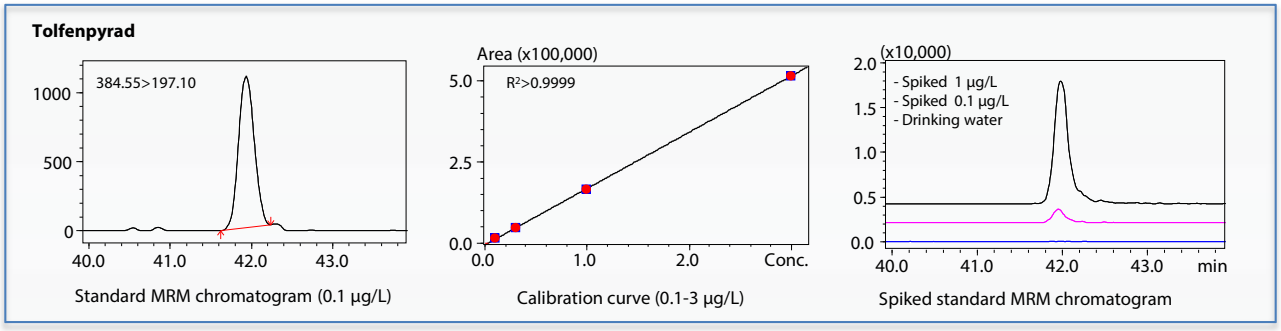


cis-Permethrin



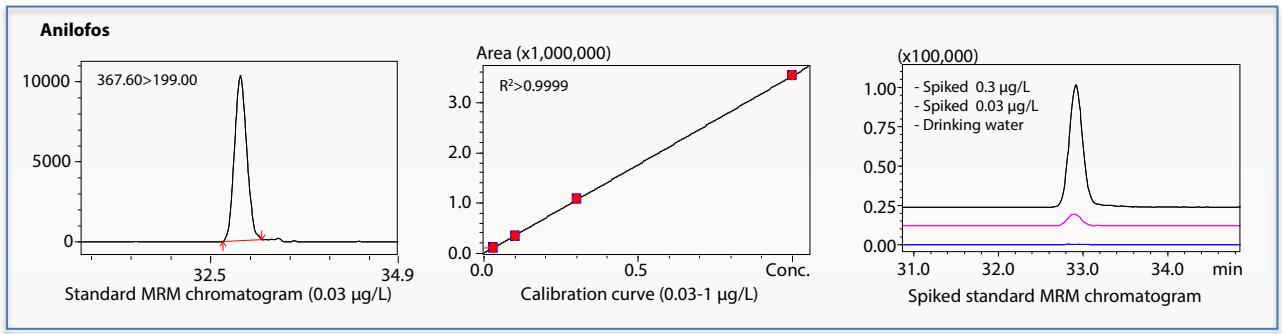


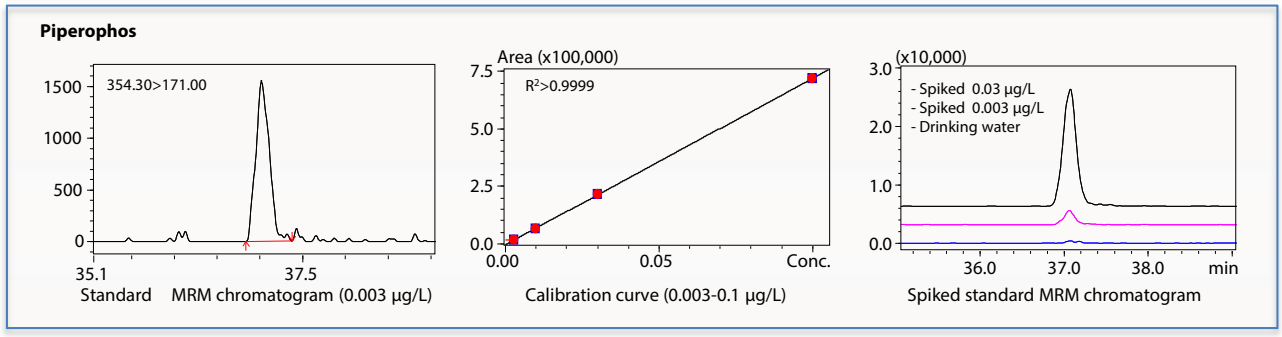




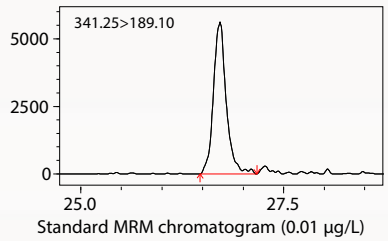
Analysis of Pesticides with a Low Concentration as the Target Value

From among the pesticides included in Appendix Method 20-2 that have a low concentration as the target value, we selected seven pesticides for analysis: anilofos, piperophos, pyridaphenthion, cadusafos, propaphos, simazine, and fipronil. The following figures show for each pesticide an MRM chromatogram and a four-point calibration curve including a solution with a concentration that is a hundredth or lower than the target value. In addition, the figures include MRM chromatograms obtained when tap water dechlorinated using sodium ascorbate is spiked to a tenth or a hundredth of the target value.

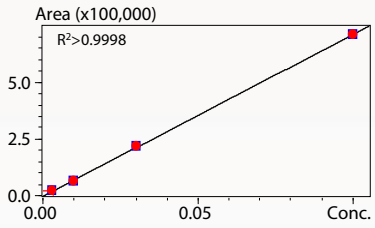




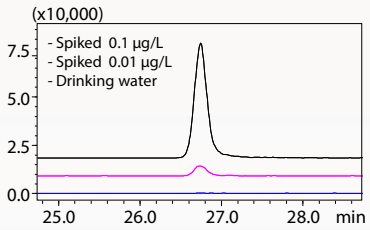
Pyridaphenthion



Standard MRM chromatogram (0.01 µg/L)

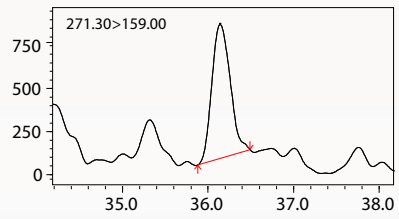


Calibration curve (0.003-0.1 µg/L)

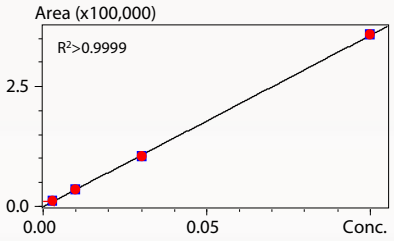


Spiked standard MRM chromatogram

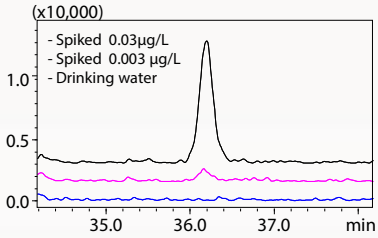
Cadusafos



Standard MRM chromatogram (0.003 µg/L)

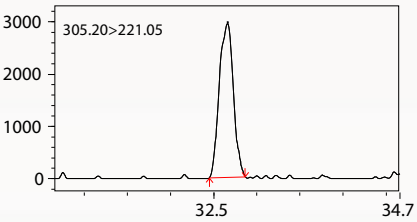


Calibration curve (0.003-0.1 µg/L)

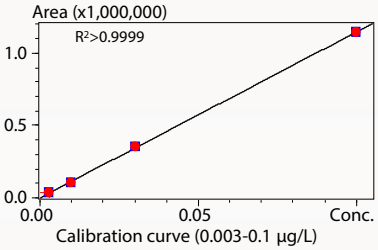


Spiked standard MRM chromatogram

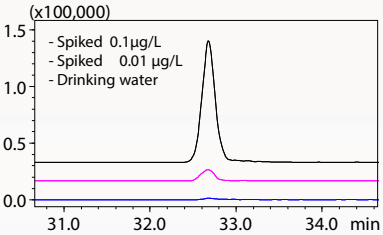
Propaphos



Standard MRM chromatogram (0.01 µg/L)

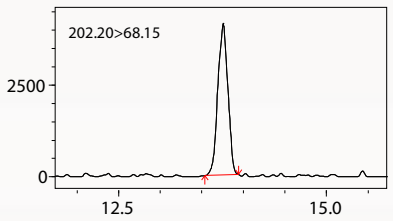


Calibration curve (0.003-0.1 µg/L)

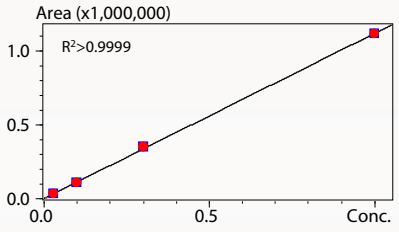


Spiked standard MRM chromatogram

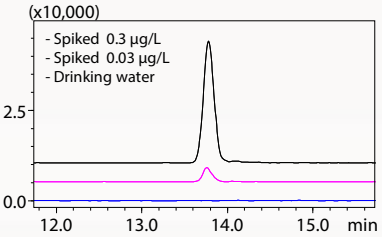
Simazine (CAT)



Standard MRM chromatogram (0.03 µg/L)

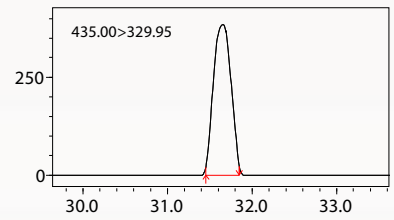


Calibration curve (0.03-1µg/L)

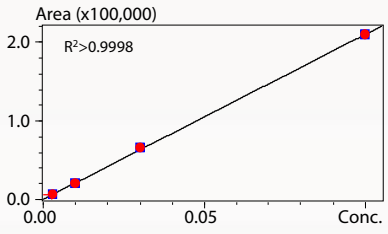


Spiked standard MRM chromatogram

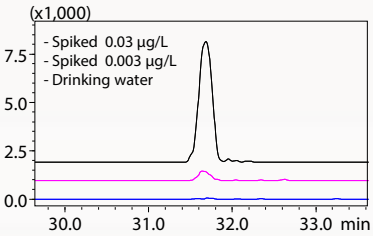
Fipronil



Standard MRM chromatogram (0.003 µg/L)

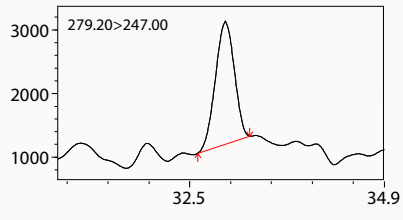


Calibration curve (0.003-0.1 µg/L)

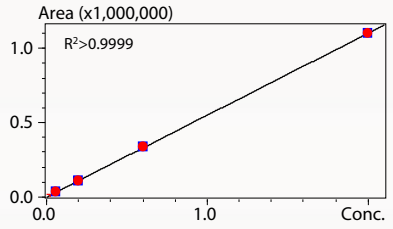


Spiked standard MRM chromatogram

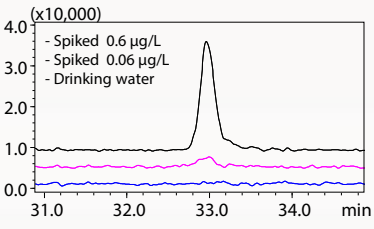
MPP (Fenthion)



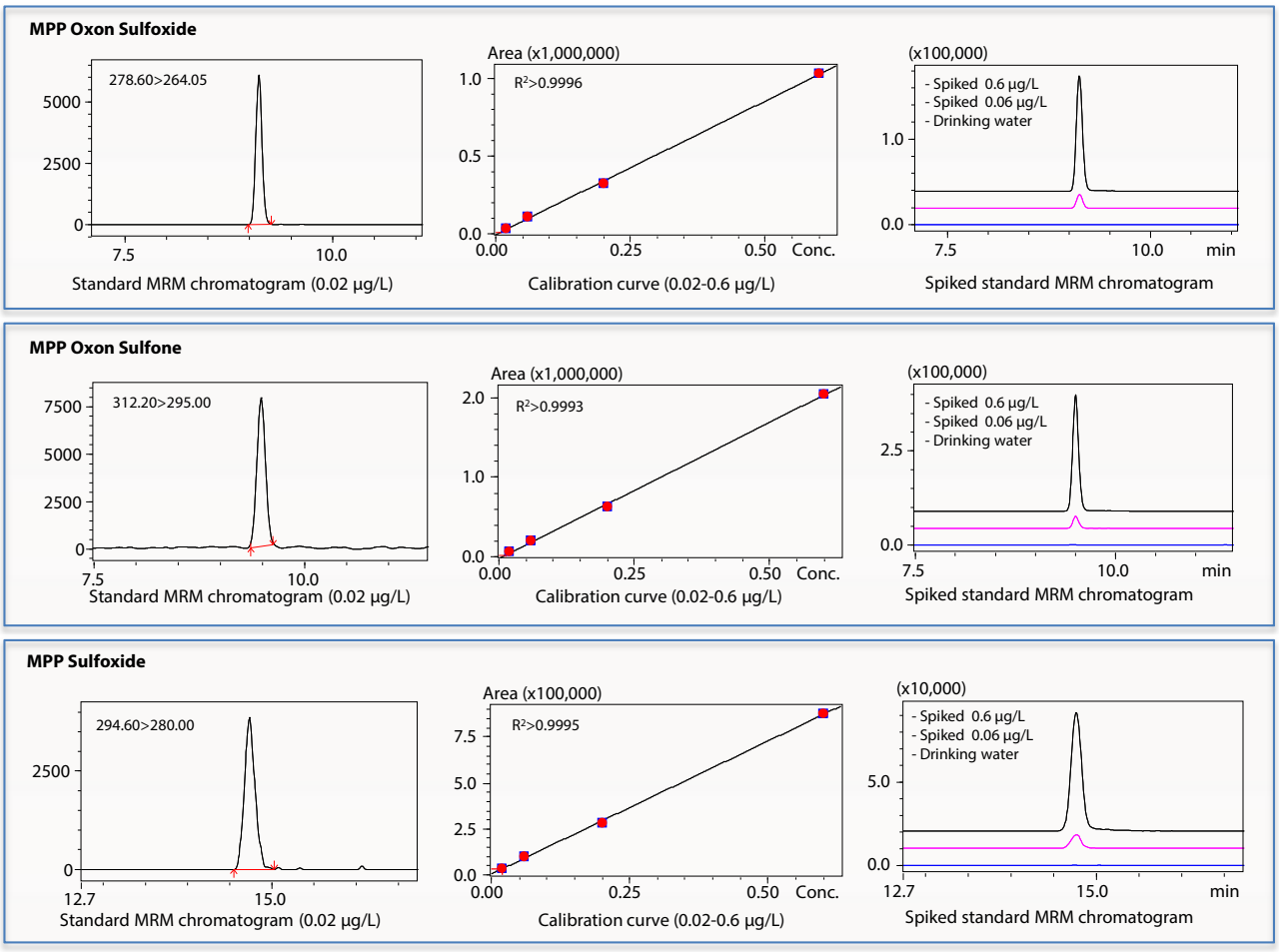
Standard MRM chromatogram (0.06 µg/L)



Calibration curve (0.06-2 µg/L)



Spiked standard MRM chromatogram

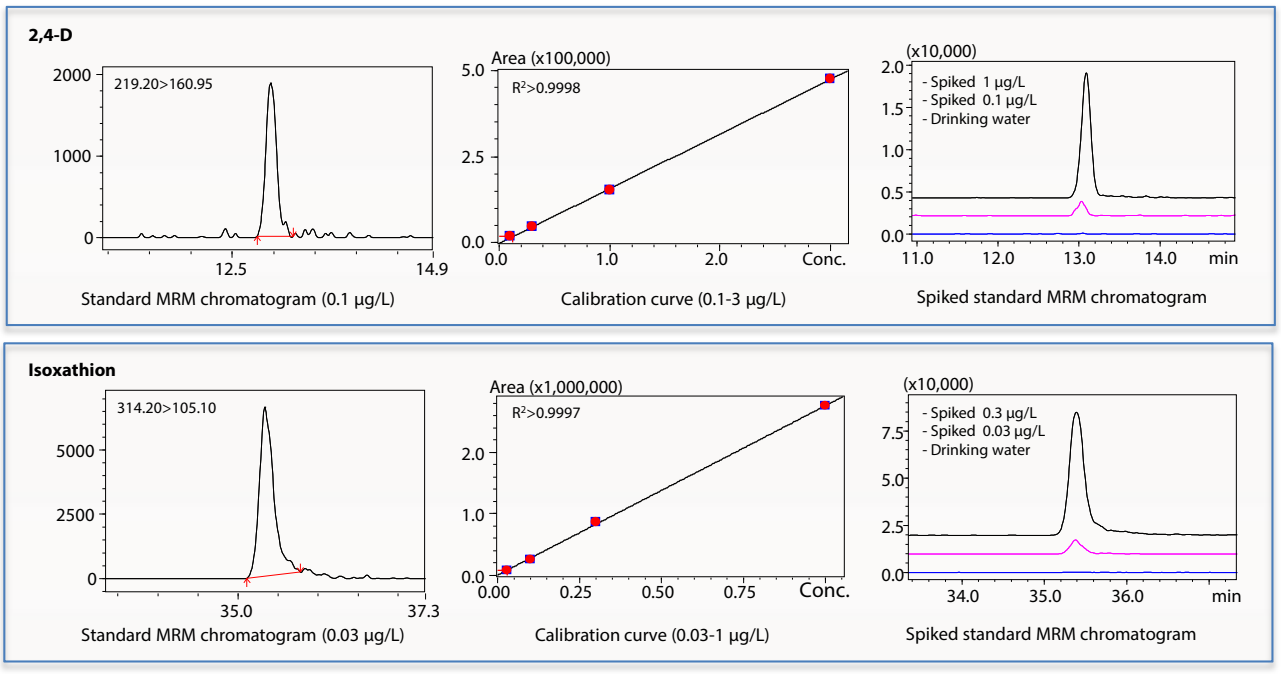


Analysis of Pesticides with a Revised Target Value

Of the pesticides added to Appendix Method 20-2, the criterion for 2,4-D and isoxathion were revised and made stricter. For 2,4-D, the target value was revised from 0.03 mg/L to 0.02 mg/L, and for isoxathion from 0.008 mg/L to 0.005 mg/L.

Regarding 2,4-D, the following figures show the MRM chromatogram when the standard concentration is 0.1 µg/L, which is lower than a hundredth of the target value, and a four-point calibration curve including a 0.1 µg/L solution. For isoxathion, the MRM chromatogram when the standard concentration is 0.03 µg/L, which is lower than a hundredth of the target value, and a four-point calibration curve including a 0.03 µg/L solution are indicated.

In addition, the figures include MRM chromatograms for each of the two pesticides obtained when tap water dechlorinated using sodium ascorbate is spiked to a concentration lower than a tenth or a hundredth of the target value.



Repeatability and Accuracy Results of Spike-and-Recovery Tests Using Tap Water (99 Pesticides)

Excluding the 14 pesticides deemed to be referential, spike-and-recovery tests were conducted for the 99 pesticides added to Appendix Method 20-2. Tap water was spiked to a concentration of a hundredth of the target value or lower for each pesticide and the sample solution was measured (n=5). The results are shown below.

The tap water used in the evaluations was dechlorinated using sodium ascorbate. However, regarding flazasulfuron and bensulfuron methyl, sodium thiosulfate was used instead since decomposition occurs when sodium ascorbate is used.

1) Pesticides included in Appendix Method 5 (51 pesticides)

	Pesticide	R.T. (min)	+/–	Precursor Ion	Product Ion	1/100 of Target Value (μg/L)	Spiked Concentration (μg/L)	Recovery Rate (n=5, %)	Repeatability (n=5, %RSD)	Calibration Curve Range (μg/L)	Coefficient of Determination (R^2)
1	Alachlor	41.5	+	270	238	0.3	0.3	110	7.8	0.3-10	0.9999
2	Anilofos	32.9	+	368	199	0.03	0.03	83	3.3	0.03-1	0.9999
3	Atrazine	17.9	+	216	174	0.1	0.1	97	3.3	0.1-3	0.9999
4	Bifenox	36.3	+	359	310	2	1	99	3.9	0.3-10	0.9999
5	Bromobutide	28.3	+	314	196	1	1	88	3.1	0.03-1	0.9999
6	Buprofezin	41.2	+	306	201	0.2	0.1	89	4.8	0.03-1	0.9997
7	Butamifos	35.3	+	333	180	0.2	0.1	90	6.1	0.01-0.3	0.9997
	Butamifos Oxon	26.5	+	317	244	0.2	0.1	92	3.0	0.01-0.3	0.9994
8	Cafenstrole	26.7	+	351	100	0.08	0.03	85	17.9	0.03-1	0.9978
9	Chlorpyrifos	43.2	+	350	198	0.03	0.03	94	8.7	0.03-1	0.9998
	Chlorpyrifos Oxon	29.9	+	336	280	0.03	0.03	83	8.6	0.01-1	0.9999
10	Dithiopyr	39.0	+	402	354	0.09	0.03	110	6.4	0.03-1	0.9999
11	Diazinon	33.4	+	305	169	0.03	0.03	110	16.6	0.03-1	0.9999
	Diazinon Oxon	21.1	+	289	153	0.03	0.03	87	2.1	0.03-1	0.9997
12	Dimepiperate	36.9	+	146	69	0.03	0.03	83	17.4	0.03-1	0.9999
13	Dimethametryn	31.4	+	256	186	0.2	0.1	83	4.9	0.03-1	0.9997
14	Dimethoate	9.2	+	230	199	0.5	0.3	93	6.3	0.01-0.3	0.9995
15	Edifenphos (EDDP)	32.4	+	311	283	0.06	0.03	93	4.0	0.01-1	0.9999
16	EPN	37.4	+	324	296	0.04	0.03	100	9.1	0.01-1	0.9999
	EPN Oxon	22.7	+	308	280	0.04	0.03	97	2.9	0.03-3	0.9999
17	Esprocarb	40.4	+	266	91	0.1	0.1	88	2.5	0.1-3	0.9999
18	Ethofenprox	48.1	+	394	177	0.8	0.3	93	10.1	0.1-3	0.9999
19	Fenobucarb (BPMC)	22.3	+	208	95	0.3	0.3	81	2.8	0.03-1	0.9998
20	Flutolanil	25.7	+	324	262	2	1	82	2.0	0.1-3	0.9998
21	Fthalide	27.1	–	271	243	1	1	110	7.1	1-30	0.9997
22	Iprobenfos (IBP)	31.4	+	289	91	0.9	0.3	95	4.1	0.1-3	0.9999
	Isofenphos Oxon	27.3	+	330	201	0.01	0.01	80	5.7	0.003-0.1	0.9997
23	Isoprocarb (MIPC)	17.9	+	194	95	0.1	0.1	83	5.8	0.1-3	0.9999
24	Isoprothiolane (IPT)	25.4	+	291	231	3	3	93	1.0	0.1-3	0.9998
25	Isoxathion	35.3	+	314	105	0.05	0.03	86	3.4	0.03-1	0.9997
	Isoxathion Oxon	24.4	+	298	242	0.08	0.03	86	4.9	0.03-1	0.9997
26	Malathion	25.5	+	348	331	7	3	86	3.3	0.1-3	0.9999
	Malaoxon	14.2	+	315	99	7	3	83	2.3	0.1-3	0.9999
27	Mefenacet	26.6	+	299	148	0.2	0.1	83	2.1	0.03-1	0.9999
-	MEP Oxon	14.4	+	262	104	0.1	0.1	93	11.5	0.03-1	0.9999
28	Mepronil	25.4	+	270	119	1	1	88	3.7	0.03-1	0.9999
29	Metalaxyl	18.7	+	280	220	0.6	0.3	81	1.6	0.1-3	0.9999
30	Methidathion (DMTP)	20.1	+	320	145	0.04	0.03	86	15.1	0.03-1	0.9999
31	Methyldymron	26.8	+	269	151	0.3	0.3	103	5.9	0.03-1	0.9995
32	Napropamide	28.6	+	272	171	0.3	0.3	83	4.4	0.1-3	0.9999
33	Pencycron	36.0	+	329	125	1	1	87	5.6	0.1-3	0.9999
34	Pendimethalin	43.5	+	282	212	3	3	88	2.7	3-100	0.9999
35	Phenthoate (PAP)	31.7	+	321	247	0.07	0.03	107	14.5	0.03-3	0.9998
36	Piperophos	37.0	+	354	171	0.009	0.003	100	13.9	0.003-0.1	0.9999

	Pesticide	R.T. (min)	+/–	Precursor Ion	Product Ion	1/100 of Target Value (μg/L)	Spiked Concentration (μg/L)	Recovery Rate (n=5, %)	Repeatability (n=5, %RSD)	Calibration Curve Range (μg/L)	Coefficient of Determination (R^2)
37	Pretilachlor	37.6	+	312	252	0.5	0.3	93	3.2	0.03-1	0.9999
38	Procymidone	27.0	–	314	282	0.9	0.3	88	16.9	0.3-10	0.9998
39	Propiconazole	33.1	+	342	159	0.5	0.3	83	3.6	0.03-3	0.9999
40	Propyzamide	25.0	–	254	228	0.5	0.3	108	3.7	0.1-3	0.9993
41	Pyributicarb	42.5	+	331	181	0.2	0.1	81	3.7	0.03-1	0.9999
42	Pyridaphenthion	26.7	+	341	189	0.02	0.01	80	6.5	0.003-0.1	0.9998
43	Pyriproxyfen	43.0	+	322	96	3	3	94	1.5	0.3-10	0.9999
44	Pyroquilon	12.8	+	174	117	0.5	0.3	87	2.2	0.1-3	0.9999
45	Simazine (CAT)	13.7	+	202	68	0.03	0.03	80	11.4	0.03-1	0.9999
46	Simetryn	17.9	+	214	68	0.3	0.3	100	2.9	0.1-3	0.9998
47	Terbucarb (MBPMC)	37.0	+	295	222	0.2	0.1	100	12.3	0.1-3	0.9999
48	Thenylchlor	28.2	+	324	127	2	1	95	2.2	0.1-3	0.9996
49	Thobencarb	35.5	+	258	125	0.2	0.1	113	0.6	0.1-3	0.9999
50	Tolclofos-methyl	35.9	+	301	269	2	1	103	5.6	1-30	0.9999
	Tolclofos-methyl Oxon	23.1	+	285	109	2	1	98	1.0	0.1-3	0.9998
51	Trichorfon (DEP)	9.2	+	259	109	0.05	0.03	98	6.3	0.01-0.3	0.9999

2) Pesticides included in Appendix Method 5-2 (18 pesticides)

	Pesticide	R.T. (min)	+/–	Precursor Ion	Product Ion	1/100 of Target Value (μg/L)	Spiked Concentration (μg/L)	Recovery Rate (n=5,%)	Repeatability (n=5,%RSD)	Calibration Curve Range (μg/L)	Coefficient of Determination (R^2)
52	Benfuresate	19.8	+	274	18	0.7	0.3	116	10.5	0.3-10	0.9997
53	Butachlor	41.4	+	312	238	0.3	0.3	109	5.2	0.1-3	0.9999
54	Cadusafos	36.2	+	271	159	0.006	0.003	100	16.1	0.003-0.1	0.9999
55	Cinmethylin	40.5	+	292	105	1	1	103	9.7	1-100	0.9999
56	Dichlofenthion (ECP)	42.5	+	315	259	0.06	0.03	104	14.5	0.03-1	0.9999
57	Chlorpyrifos-methyl	36.7	+	322	125	0.3	0.3	103	7.7	0.1-3	0.9999
58	Dimethylvinphos_E	26.9	+	331	127	0.1	0.1	94	1.0	0.03-1	0.9999
	Dimethylvinphos_Z	25.4	+	331	127	0.1	0.1	96	5.7	0.03-1	0.9999
59	Fosthiazate	16.4	+	284	104	0.03	0.03	87	3.2	0.03-1	0.9999
60	Metrachlor	28.8	+	284	252	2	1	93	1.6	0.03-1	0.9999
61	Orysastrobin	26.0	+	392	205	1	1	88	1.0	0.1-3	0.9999
62	Paclobutrazol	28.7	+	294	70	0.5	0.3	90	1.6	0.01-1	0.9998
63	Phosalone	35.1	+	368	182	0.05	0.03	93	5.8	0.01-0.3	0.9999
64	Propaphos	32.7	+	305	221	0.01	0.01	90	9.0	0.003-0.1	0.9998
65	Propoxur (PHC)	13.6	+	210	111	2	1	84	2.9	0.1-3	0.9999
66	Pyraclofos	35.0	+	361	257	-	0.01	110	7.3	0.01-1	0.9999
67	Pyrazoxyfen	33.0	+	403	91	0.04	0.03	107	2.8	0.003-0.1	0.9999
68	Quinoclamine (ACN)	12.6	+	208	105	0.05	0.03	100	9.7	0.03-1	0.9999
69	Uniconazole-P	30.1	+	292	70	0.4	0.3	90	0.4	0.03-1	0.9998

3) Newly added pesticides (7 pesticides)

	Pesticide	R.T. (min)	+/–	Precursor Ion	Product Ion	1/100 of Target Value (μg/L)	Spiked Concentration (μg/L)	Recovery Rate (n=5, %)	Repeatability (n=5, %RSD)	Calibration Curve Range (μg/L)	Coefficient of Determination (R^2)
70	Cypermethrin	46.5	+	433	191	1	1	90	10.1	0.3-10	0.9999
71	Ethiprole	24.4	–	395	331	0.1	0.1	86	3.9	0.03-1	0.9996
72	Fenoxanil	30.7	+	329	302	0.2	0.1	112	1.7	0.1-3	0.9997
73	Methamidophos	3.7	+	142	94	0.01	0.01	83	0.8	0.01-0.3	0.9995
74	Propargite (BPPS)	44.8	+	368	231	0.2	0.1	91	6.1	0.03-1	0.9999
75	Permethrin_trans	47.8	+	408	183	1	0.5	101	8.0	0.5-15	0.9996
	Permethrin_cis	48.1	+	408	183	1	0.5	106	4.3	0.05-1.5	0.9998
76	Tolfenpyrad	41.9	+	385	197	0.1	0.1	89	8.6	0.1-3	0.9999

4) Pesticides included in Appendix Method 18 (20 pesticides)

	Pesticide	R.T. (min)	+/–	Precursor Ion	Product Ion	1/100 of Target Value (µg/L)	Spiked Concentration (µg/L)	Recovery Rate (n=5, %)	Repeatability (n=5, %RSD)	Calibration Curve Range (µg/L)	Coefficient of Determination (R^2)
77	2,4-D	13.1	–	219	161	0.2	0.1	103	5.1	0.1-3	0.9998
78	Azoxystrobin	23.1	+	404	372	5	3	80	0.8	0.3-10	0.9996
79	Bensulfuron-methyl	17.0	+	411	149	5	3	82	4.7	0.1-3	0.9997
80	Bensulide (SAP)	31.5	+	398	158	1	1	82	3.5	0.1-3	0.9999
81	Bentazone	8.1	–	239	132	2	1	113	0.9	0.03-1	0.9996
82	Carbaryl (NAC)	15.2	+	202	145	0.5	0.3	95	2.8	0.1-3	0.9998
83	Carbendazim (MBC)	10.2	+	192	160	0.2	0.1	95	1.4	0.1-3	0.9997
84	Carbofuran	13.8	+	222	165	0.05	0.03	97	1.9	0.03-3	0.9998
85	Carpropamid	32.7	+	334	139	0.4	0.3	95	1.5	0.1-3	0.9999
86	Diuron (DCMU)	19.2	+	233	72	0.2	0.1	94	3.4	0.03-1	0.9999
87	Dymron	26.2	+	269	151	8	3	99	1.8	0.1--3	0.9999
88	Fipronil	31.6	–	435	330	0.005	0.003	100	15.9	0.003-0.1	0.9998
89	Flazasulfuron	9.8	+	408	182	0.3	0.3	86	7.2	0.003-0.1	0.9993
90	Halosulfuron-methyl	12.1	+	435	182	3	3	100	10.5	0.1-3	0.9999
91	Mecoprop (MCPP)	15.7	–	213	141	0.5	0.3	120	2.1	0.3-10	0.9998
92	Methomyl	6.7	+	163	88	0.3	0.3	97	0.3	0.01-0.3	0.9995
93	MPP (Fenthion)	32.9	+	279	247	0.06	0.06	100	4.4	0.06-2	0.9999
	MPP Oxon	21.1	+	263	231	0.06	0.06	100	4.4	0.06-2	0.9999
	MPP Oxon Sulfoxide	9.1	+	279	264	0.06	0.06	103	4.7	0.02-0.6	0.9996
	MPP Oxon Sulfone	9.5	+	312	295	0.06	0.06	90	3.0	0.02-06	0.9993
	MPP Sulfoxide	14.7	+	295	280	0.06	0.06	100	7.4	0.02-0.6	0.9995
	MPP Sulfone	15.9	+	328	311	0.06	0.06	96	4.7	0.02-0.6	0.9998
94	Siduron	23.0	+	233	137	3	3	96	0.7	0.1-3	0.9999
95	Thiodicarb	16.3	+	377	64	0.8	0.3	83	3.8	0.3-10	0.9997
96	Tricyclazole	10.6	+	190	163	1	1	84	6.4	0.1-3	0.9999

5) Pesticides included in Appendix Methods 19 and 20 (3 pesticides)

	Pesticide	R.T. (min)	+/–	Precursor Ion	Product Ion	1/100 of Target Value (µg/L)	Spiked Concentration (µg/L)	Recovery Rate (n=5, %)	Repeatability (n=5, %RSD)	Calibration Curve Range (µg/L)	Coefficient of Determination (R^2)
97	2,2-DPA (Dalapon)	4.2	–	141	97	0.8	0.3	107	3.4	0.3-10	0.9999
98	Acephate	4.4	+	184	143	0.06	0.03	110	1.6	0.03-3	0.9997
99	Benfuracarb	39.6	+	411	195	0.4	0.3	89	4.8	0.1-3	0.9999

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