SHIMADZU APPLICATION NEWS

LAAN-A-LM-E004

● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

No. C34

Simultaneous Analysis of Pesticides in Environment Water using LC-MS

On October 10, 2003, Japan's Ministry of Health, Labour and Welfare posted the "Enactment of the Ministerial Ordinance regarding Water Quality Standards and Amendments to the Enforcement Rules for the Water Works Law" (Health Water Notification No. 1010001). This publication indicates the inspection methods for the water quality control criteria items in Japan.
There are 101 pesticides listed in the water quality control criteria items. The additional methods 16, 17, 18 and 19 employ the LC-MS methods, among which method 16 is for simultaneous analysis of 27 compounds (28 including a decomposition compound), method 17 for analysis of acephate, method 18 for fosetyl, and 19 for polycarbamate.
Because the measurement results are evaluated using the "total pesticide method", measurements must be conducted down to 1/100 concentration of criteria values.
The additional method 16 specifies use of a reversed phase resin solid phase column and the method 17 specifies an activated carbon solid phase column for a 500-fold concentration. Since acephate is detected using the same LC-MS conditions as the 28 components shown in the method 16, introduced here is an example of a simultaneous LC-MS analysis using a divinyl benzene-*N* -vinyl pyrrolidone copolymer solid phase column and an activated carbon solid phase column linked to perform pretreatment concentration of the 29 pesticide compounds.
In this analysis, ion exchange water (milli-Q Gradient), river water and tap water were used as test water samples, each of the pesticides was added to the samples at a concentration 1/100 of the criteria value, and the addition recovery percent was obtained. Table 1 shows the ionization mode used for this measurement, the pesticide number, pesticide name, monitored ion (m/z) and the water quality management criteria value (mg/L).

Table 1 List of Pesticides for Attached Methods 16, 17

Ionization Mode	No.	Pesticide Name	Monitored Ion (m/z)	Criteria Value (mg/L)
Positive	1	Thiram	241	0.02
	18	Carbofuran	222	0.005
	21	Acephate	184	0.08
	26	Iprodione	330	0.3
	28	Oxine-Cu	146	0.04
	36	Asulam	231	0.2
	42	Bensulide	356	0.1
	48	Carbaryl	202	0.05
	55	Thiophanate-methyl	343	0.3
	58	Carpropamid	334	0.04
	68	Diuron	233	0.02
	74	Methomyl	163	0.03
	75	Benomyl	291	0.02
	76	Benfuracarb	411	0.04
	82	Probenazole	224	0.05
	86	Bensulfuron-methyl	411	0.4
	87	Tricyclazole	190	0.08
	90	Azoxystrobin	404	0.5
	95	Flazasulfuron	408	0.03
	96	Thiodicarb	355	0.08
	98	Siduron-A	233	0.3
	98	Siduron-B	233	0.3
	—	Methyl-benzimidazol-2-ylcarbamate (MBC) * Benomyl decomposition product	192	—
Negative	17	Bentazon	239	0.2
	19	2,4-D	219	0.03
	20	Triclopyr	196	0.006
	45	Mecoprop	213	0.005
	64	Dalapon	141	0.08
	84	Daimuron	313	0.8
	94	Halosulfuron-methyl	433	0.3

■ Analysis Outline

After adding 0.25g of EDTA-2Na·2H$_2$O to 500mL of test water, and then adding nitric acid (1+10) to adjust the pH to 3.5, the liquid was pumped through each of the conditioning divinyl benzene-*N* -vinyl pyrrolidone copolymer solid phase column and activated carbon solid phase column. The solid phase columns were dried, and the divinyl benzene-*N* -vinyl pyrrolidone copolymer solid phase column and activated carbon solid phase column were backflush-eluted with 5mL of acetonitrile and 5mL of methanol, respectively. Nitrogen was gently blown onto the eluate solution to concentrate it to less than 100µL, and the solution was brought to 1mL using purified water. 10µL was injected into the LC-MS, and analysis was conducted. The analysis flow chart is shown in Fig.1.

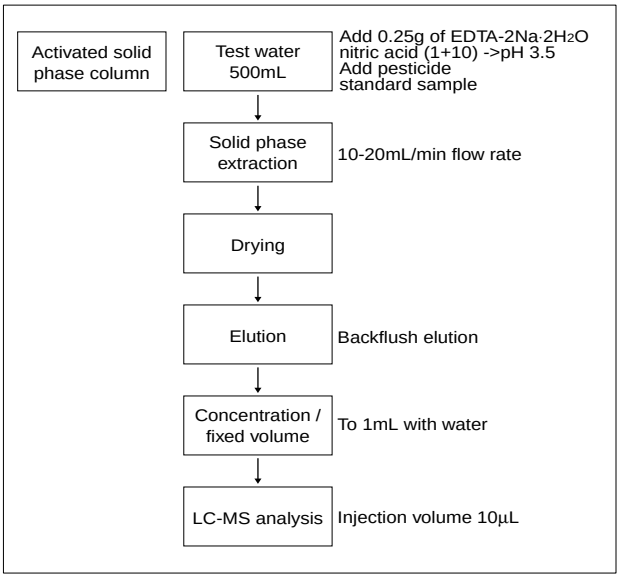

Fig.1 Flow Chart of the Analysis

■ Measurement Results

The LC-MS analytical conditions are shown in Table 3. Just as in the Shimadzu Application News No. C33, acetonitrile and 0.1% formic acid aqueous mobile phase is used, however, in order to sufficiently achieve separation from the contaminants contained in the sample following concentration via the solid phase column, the gradient conditions were changed slightly. In addition, considering the pesticide addition recovery percentage, the monitored ions for bensulide, flazasulfuron, siduron and triclopyr were changed from m/z 398($[M+H]^+$), m/z 406($[M-H]^-$), m/z 277($[M+HCOO]^-$) and m/z 254($[M-H]^-$) to m/z 356($[M-C(CH_3)_2+H]^+$), m/z 408($[M+H]^+$), m/z 233($[M+H]^+$) and m/z 196, respectively.

Fig.2 and 3 show the SIM chromatograms in positive mode, and Fig.4 and 5 show the SIM chromatograms in negative mode. The upper portion and lower portion of each figure show the ion exchange water and river water SIM chromatograms, respectively, to each of which was added the pesticide mixture standard sample at a concentration 1/100 of the criteria value, and where solid phase extraction (divinyl benzene-*N*-vinyl pyrrolidone copolymer solid phase column) was conducted to concentrate the samples by a factor of 500 times.

From the ion exchange water and river water results, it is clear that sufficient sensitivity was obtained to enable detection at concentrations 1/100 of the specified criteria values. In the river water mixture, the baseline is slightly raised and contaminant peaks can be seen, however, identification and quantitation for each pesticide peak was possible.

Table 2 shows the solid phase column addition recovery results. The table summarizes the average area value (As) of each pesticide when the standard sample was analyzed by LC-MS, as well as the area

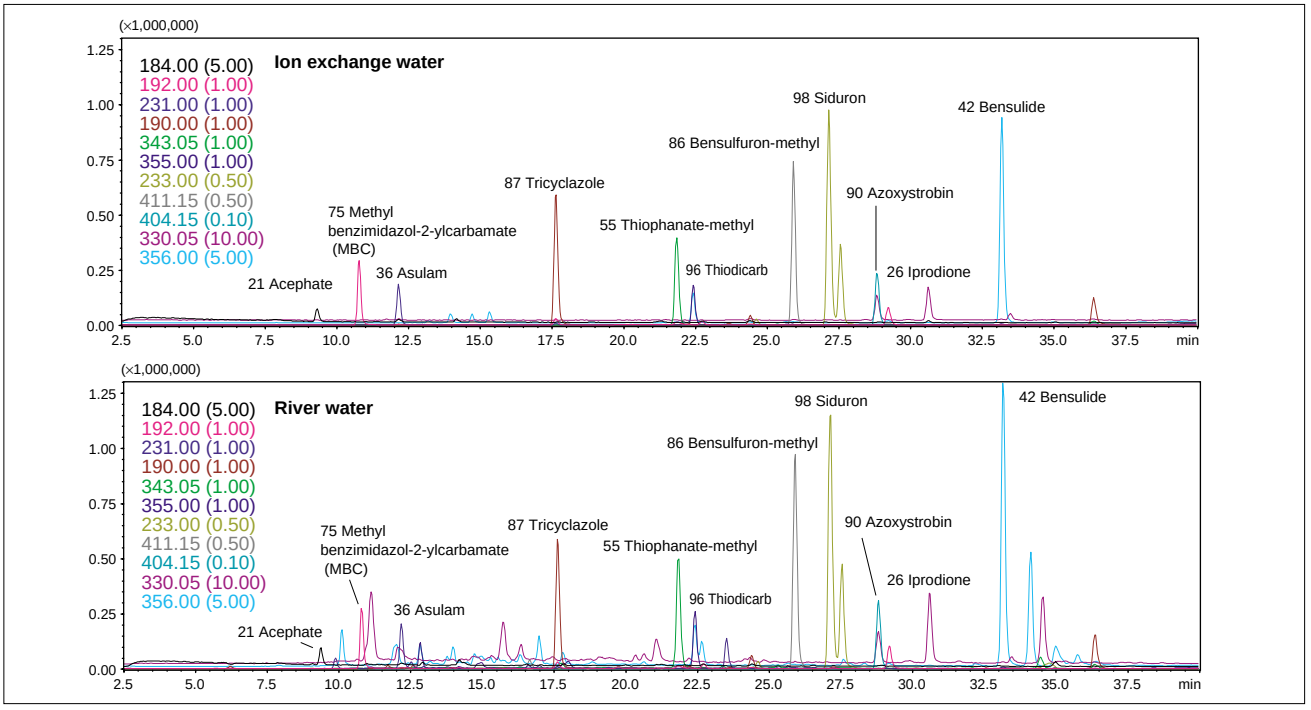

Fig.2 SIM Chromatograms of Pesticides (ESI-positive) (Criteria value: 0.08mg/L minimum)
(Upper: Pesticides added to ion exchange water, Lower: Pesticides added to river water)

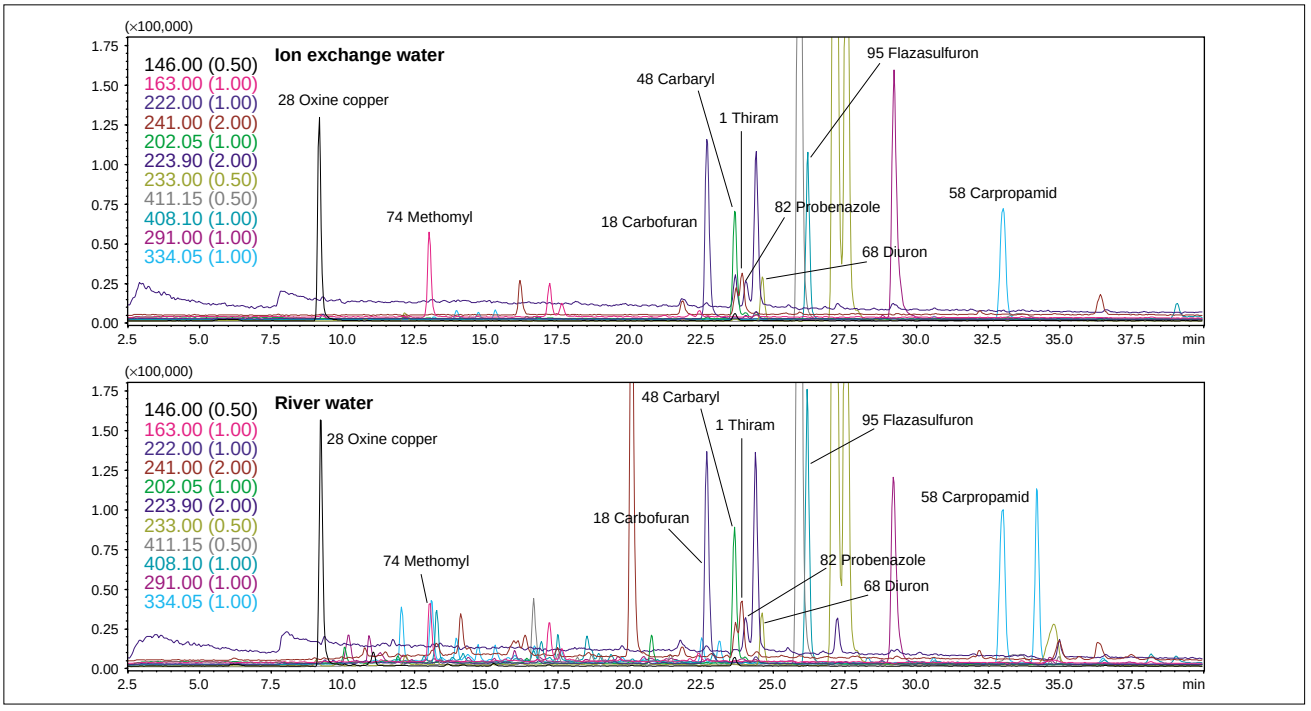

Fig.3 SIM Chromatograms of Pesticides (ESI-positive) (Criteria value: up to 0.08mg/L)
(Upper: Pesticides added to ion exchange water, Lower: Pesticides added to river water)

values (Ax) of the pesticides added to the ion exchange water, river water and tap water samples concentrated 500 times via the divinyl benzene-*N*-vinyl pyrrolidone copolymer solid phase column and activated carbon column, and the corresponding recoveries (Ax/As × 100). Benomyl (m/z 291) and benfuracarb (m/z 411) decompose quickly in aqueous solution, so benomyl and benfuracarb are actually detected as MBC (m/z 192) and carbofuran (m/z 222), respectively.

In the ion exchange water addition preparation, except for acephate and dalapon, recovery rates of 81~139% were obtained for 25 components with the divinyl benzene-*N*-vinyl pyrrolidone copolymer solid phase column, demonstrating that detection of 1/100 of the water quality management criteria values is possible with this method. Among the attached method 16, dalapon only yielded low recoveries, 5% with the reversed phase resin solid phase column and 28% with the activate carbon solid phase column.

Since extraction of acephate is by the activated carbon solid phase column according to the attached method 17, the 8% recovery rate with the reversed phase resin solid phase column is low, however, the recovery from the activated column attached to the solid phase column was 66%. Since this analysis is an experiment, separate analyses were conducted for each eluate solution, however, by combining the final eluates, it is possible to perform simultaneous quantitation for the 28 compounds (excluding dalopon) in the attached methods 16 and 17.

In the tap water addition preparation, it is thought that the poor recoveries of thiram and asulam, and other compounds may be due to the effect of chlorine, etc. in the tap water.

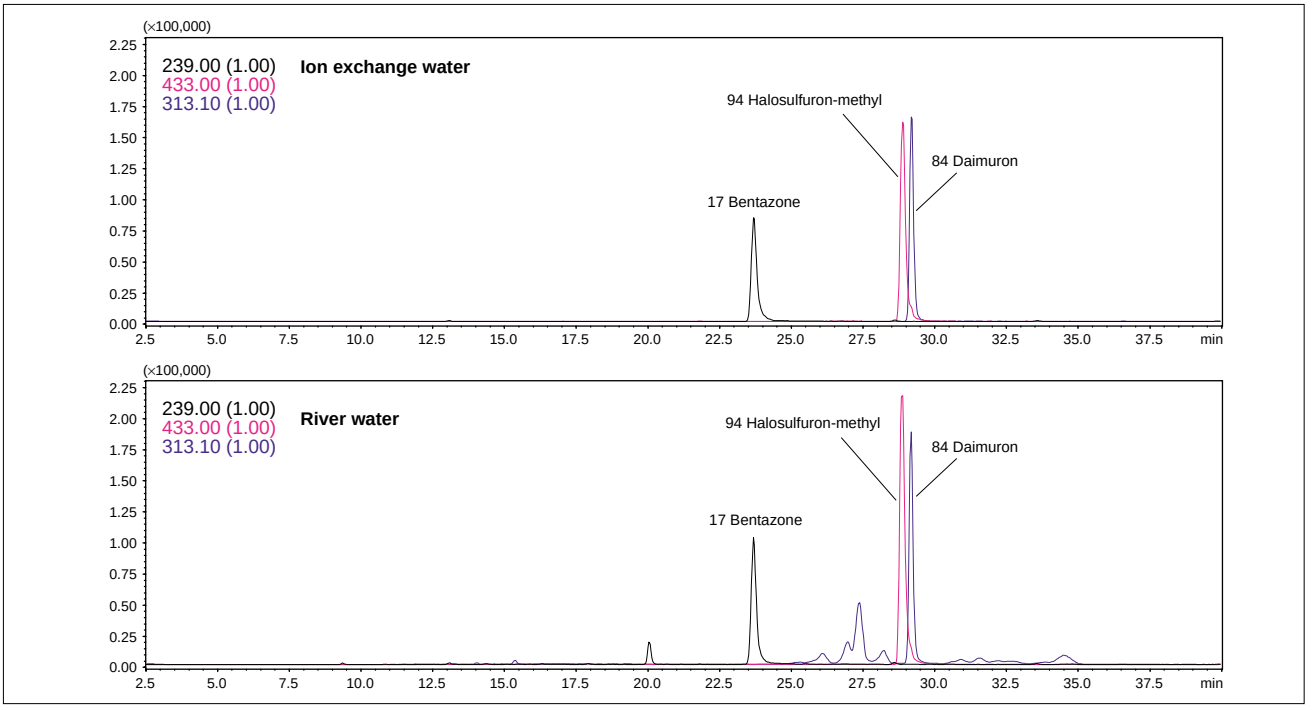

Fig.4 SIM Chromatograms of Pesticides (ESI-negative) (Criteria value: 0.1mg/L minimum)
(Upper: Pesticides added to ion exchange water, Lower: Pesticides added to river water)

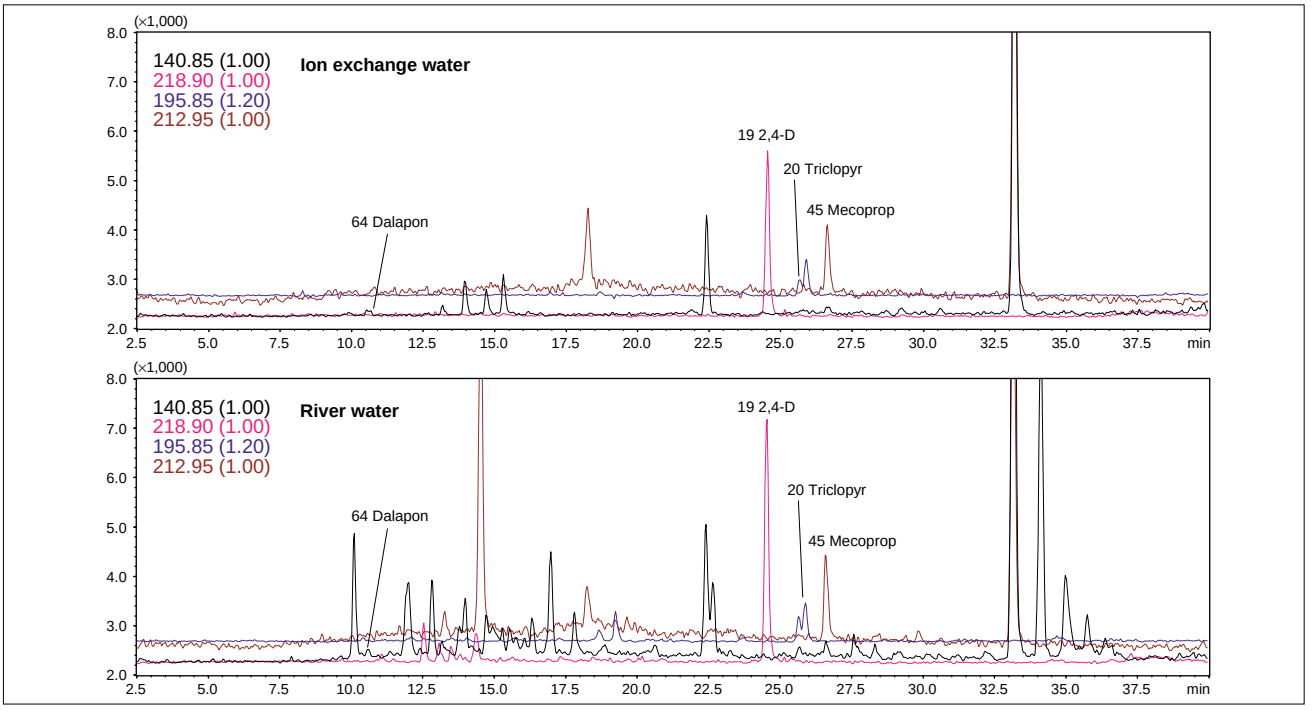

Fig.5 SIM Chromatograms of Pesticides (ESI-negative) (Criteria value: up to 0.1mg/L)
(Upper: Pesticides added to ion exchange water, Lower: Pesticides added to river water)

Table 2 Recoveries of Pesticides

No.	Pesticide Name	Ionization Mode	Mass Number (m/z)	Retention Time (min)	Std / Pesticide Mixture	Resin Solid Phase Column Ion Exchange Water		Resin Solid Phase Column River Water		Resin Solid Phase Column Tap Water	
					Average Area	Average Area	Recovery (%)	Average Area	Recovery (%)	Average Area	Recovery (%)
28	Oxine-Cu	ESI(+)	146	9.1640	2536161	2270502	90	2590196	102	1264508	50
21	Acephate	ESI(+)	184	9.3113	1281560	100248	8	115380	9	103954	8
64	Dalapon	ESI(−)	141	10.5560	22646	1233	5	1054	5	1798	8
-	MBC	ESI(+)	192	10.7413	1836985	2544503	139	2446088	133	2410520	131
36	Asulam	ESI(+)	231	12.1390	1901601	1705211	90	1814171	95	157573	8
74	Methomyl	ESI(+)	163	12.9933	598218	487014	81	372044	62	182442	30
87	Tricyclazole	ESI(+)	190	17.5980	6315401	5723195	91	5568845	88	6107171	97
55	Thiophanate-methyl	ESI(+)	343	21.8070	4895682	4002006	82	5172645	106	0	0
96	Thiodicarb	ESI(+)	355	22.3850	1860440	1648123	89	2445425	131	788158	42
18	Carbofuran	ESI(+)	222	22.6577	1116075	1044080	94	1218048	109	1284624	115
48	Carbaryl	ESI(+)	202	23.6203	784720	710779	91	927849	118	815183	104
17	Bentazon	ESI(−)	239	23.6610	1227639	1184164	96	1296595	106	747034	61
1	Thiram	ESI(+)	241	23.8747	177080	147462	83	195679	111	2089	1
82	Probenazole	ESI(+)	224	24.0103	107633	86891	81	121214	113	103624	96
19	2,4-D	ESI(−)	219	24.5107	39066	33385	85	48273	124	38298	98
68	Diuron	ESI(+)	233	24.5823	581798	534267	92	695376	120	541103	93
20	Tryclopyr	ESI(−)	196	25.6430	3369	2718	81	3687	109	3076	91
86	Bensulfuron-methyl	ESI(+)	411	25.8767	15922945	14051070	88	19794965	124	5007981	31
95	Flazasulfuron	ESI(+)	408	26.1623	1111154	977366	88	1621623	146	136324	12
45	Mecoprop	ESI(−)	213	26.5920	14079	12741	90	17483	124	13776	98
98	Siduron-A	ESI(+)	233	27.1050	21448903	19895942	93	24980380	116	16965517	79
98	Siduron-B	ESI(+)	233	27.5097	8366253	7875350	94	9929777	119	6421904	77
90	Azoxystrobin	ESI(+)	404	28.7813	27617530	25759400	93	33437455	121	25147575	91
94	Halosulfuron-methyl	ESI(−)	433	28.8403	2435565	2179959	90	2820816	116	219475	9
84	Daimuron	ESI(−)	313	29.1487	1586780	1658620	105	1840805	116	1190543	75
26	Iprodione	ESI(+)	330	30.5663	168173	147034	87	318492	189	177725	106
75	Benomyl	ESI(+)	291	30.8053	39904	3374	8	1223	3	1867	5
58	Carpropamid	ESI(+)	334	32.9563	1128933	987225	87	1358701	120	715769	63
42	Bensulide	ESI(+)	356	33.1297	2256143	1828860	81	2624467	116	0	0
76	Benfuracarb	ESI(+)	411	36.6470	364295	0	0	0	0	0	0

No.	Pesticide Name	Ionization Mode	Mass Number (m/z)	Retention Time (min)	Std / Pesticide Mixture	Activated Carbon Solid Phase Column Ion Exchange Water		Activated Carbon Solid Phase Column River Water		Activated Carbon Solid Phase Column Tap Water	
					Average Area	Average Area	Recovery (%)	Average Area	Recovery (%)	Average Area	Recovery (%)
21	Acephate	ESI(+)	184	9.3113	1281560	843333	66	602590	47	733333	57
64	Dalapon	ESI(−)	141	10.5560	22646	6346	28	4966	22	4230	19

Table 3 Analytical Conditions

Column	: L-column ODS (150mmL. × 2.1mmI.D.)
Mobile phase A	: 0.1%(w/v) Formic Acid-Water
Mobile phase B	: Acetonitrile
Gradient program	: 0%B (0min)→ 95%B (40min) → 0%B (40.01min)→STOP(50min)
Flow rate	: 0.2 mL/min
Injection volume	: 10μL
Column temperature	: 40˚C
Probe voltage	: +4.5 / -3.5kV (ESI-Positive Mode/ ESI-Negative Mode)
CDL temperature	: 250˚C
Block Heater temperature	: 200˚C
Nebulizing gas flow	: 1.5L/min
Drying gas pressure	: 0.2MPa
CDL, Q-array voltages	: Default Values
SIM	: see Table 1

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710

Cable Add.:SHIMADZU TOKYO

Printed in Japan 3100-08501-10A-IK