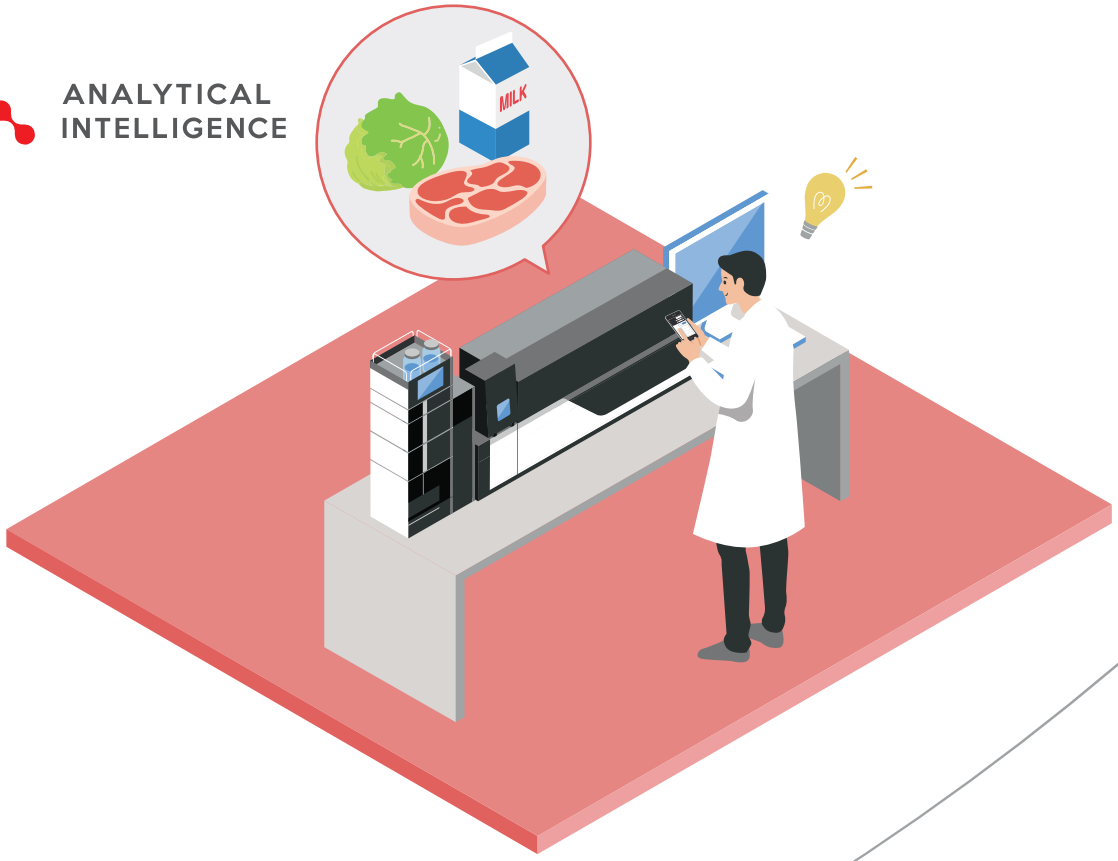
C146-E421

SHIMADZU
Excellence in Science

Solutions for Flexible Work Styles

LCMS
Food Safety Applications

ANALYTICAL
INTELLIGENCE



A new day of LC/MS analysis begins with "Analytical Intelligence"

Combining Shimazu LCMS™ with the Nexera™ Series, the "Analytical Intelligence" functions offer a flexible workflow from instrument preparation to analytical data processing.

ANALYTICAL INTELLIGENCE

Automated support functions utilizing digital technology, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher productivity and maximum reliability.

Start analysis right away with optimal conditions

The Nexera series ensures high-quality analysis with a variety of Analytical Intelligence functions, including smart startups, column-friendly FlowPilot, and mobile phase monitoring.

Automate skilled manual work: FlowPilot (movie)

Mobile phase monitoring to avoid running out of mobile phase during analysis

Simpler sample pretreatment

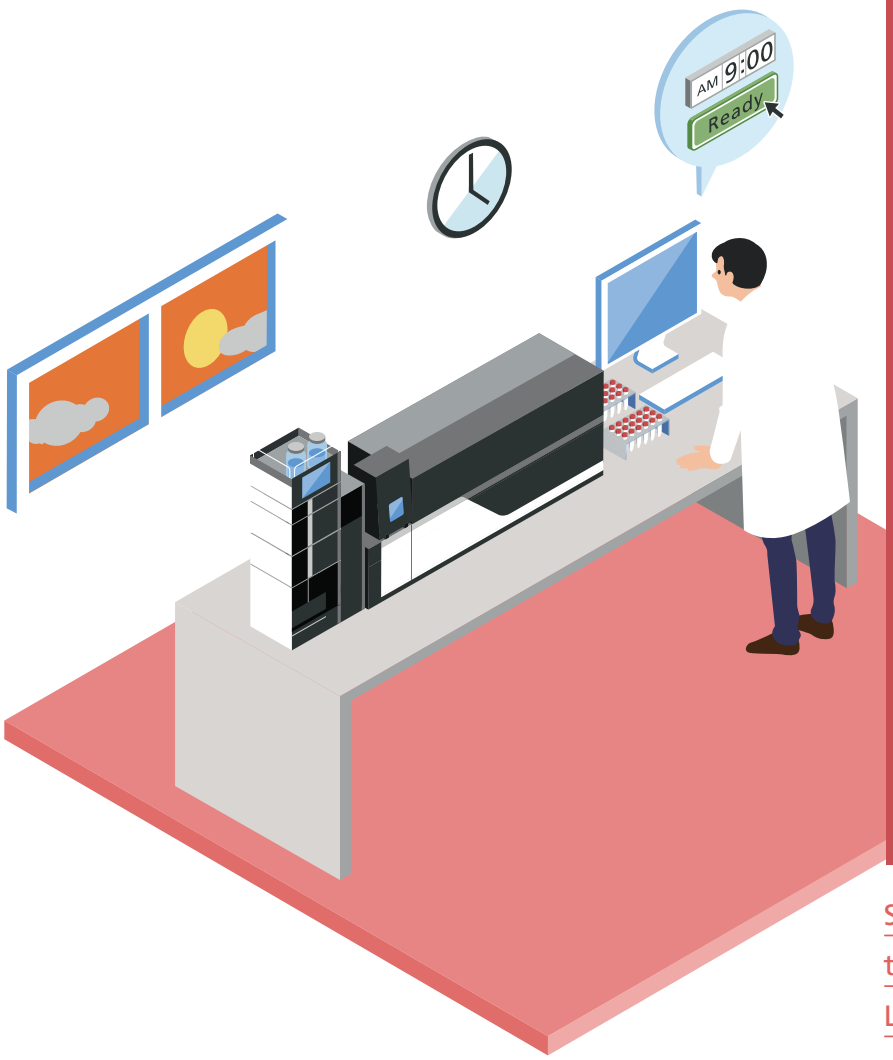
The features of the Nexera SIL-40 Series Autosampler reduce the time and labor required for sample pretreatment. Sample preparation workflow can be easily set on a graphical screen, simplifying pretreatment such as co-injection and derivatization and improving analytical repeatability.

SIL-40 Series Autosampler: Automatic Pretreatment Function (Co-Injection)

Problems in analysis are automatically detected and resolved

The system detects instrumental abnormalities during analysis, and restores itself to normal operating status if they occur.

Auto-diagnostics and auto-recovery (movie)



Software automates analytical runs and data analysis

The workflow from optimization of MS conditions to quantitative analysis is automated to maximize efficiency.

Seamless operation from MRM optimization through quantitative calculation: LabSolutions Connect™ MRM

Adaptable to diverse work styles

You can use a VPN connection from your home PC to access the LabSolutions™ CS servers in the laboratory for data analysis and report preparation.

Monitor laboratory status any time, anywhere

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Liquid Chromatography Mass Spectrometry

Shimadzu Pesticide MRM Library Support for LC/MS/MS

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Abstract

To help expand capabilities in LC/MS/MS pesticide monitoring programs we have created the Shimadzu Pesticide MRM Library. The Library has been created with 766 certified reference standards and has been verified for use with Shimadzu LCMS-8050 and 8060 systems.

The Library contains information that can be used to accelerate method development in LC/MS/MS pesticide analysis including;

An average of 8 MRM transitions for each reference standard (with optimized collision energies) are registered in the database including positive and negative ionization mode. In total, more than 6,000 MRM transitions are part of the Library.

Meta-data for each library entry such as CAS#, formula, activity, mono-isotopic mass and adduct masses, rank of MRM transitions, synonyms, InChI, InChIKey, compound names translation (Japanese and Chinese) and links to websites offering further information (alanwood.net, PAN pesticide database, Chemical Book, ChemSpider). The meta-data is intended not only to set up new methods quickly but to help search for compound properties.

Key words; Pesticide MRM Library,
766 compound library

Using the Shimadzu Pesticide MRM Library

Expanding pesticide monitoring programmes (or creating focused methods) can be quickly set up using the Library data base (Table 1) and create fully optimized MRM methods for LC/MS/MS analysis.

Users select the target pesticides and corresponding transitions from the Library and simply copy the list into a Shimadzu LabSolutions analytical method. The method will include optimized MRM transitions. Once the acquisition method is created users can start to acquire data for screening or quantitative LC/MS/MS analysis.

Table 1 The Shimadzu Pesticide MRM Library supports a list of over 766 compounds. Designed to build extended LC/MS/MS methods quickly and to review pesticide information easily.

Library entries	
Compound information	Compound Name Synonyms Japanese name Chinese name CAS Chemical Formula Mono-isotopic mass Theoretical *m/z* ([M+H]+, [M+Na]+, [M+K]+, [M+NH_4]+, [M-H]-) Activity InChI InChIKey
MS/MS parameters	Ionization mode Q1 (*m/z*) Q3 (*m/z*) Q1 Pre Bias CE Q3 Pre Bias
Web links	Alanwood.net PAN Pesticide Database Chemical Book ChemSpider

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
1	(*E*)-Fenpyroximate	134098-61-6	C24H27N3O4	421.2002	422.2075	420.1929	ESI+	6
2	(*E*)-Ferimzone	89269-64-7	C15H18N4	254.1531	255.1604	253.1458	ESI+	2
3	(*Z*)-Fenpyroximate	149054-53-5	C24H27N3O4	421.2002	422.2075	420.1929	ESI+	2
4	(*Z*)-Ferimzone	89269-64-7	C15H18N4	254.1531	255.1604	253.1458	ESI+	6
5	1-(3, 4-Dichlorophenyl)-3-methylurea	3567-62-2	C8H8Cl2N2O	218.0014	219.0087	216.9941	ESI+	19
6	1-(3, 4-Dichlorophenyl)urea	2327-02-8	C7H6Cl2N2O	203.9857	204.9930	202.9784	ESI+	17
7	1-(4-Isopropylphenyl)-3-methylurea	34123-57-4	C11H16N2O	192.1263	193.1336	191.1190	ESI+	6
8	1-(4-Isopropylphenyl)urea	56046-17-4	C10H14N2O	178.1106	179.1179	177.1033	ESI+	6
9	1-naphthaleneacetamide	86-86-2	C12H11NO	185.0841	186.0914	184.0768	ESI+	4
10	1-Naphthaleneacetic Acid	86-87-3	C12H10O2	186.0681	187.0754	185.0608	ESI-	1
11	2, 4, 5-T	93-76-5	C8H5Cl3O3	253.9304	254.9377	252.9231	ESI-	7
12	2, 4, 6-Tribromophenol	118-79-6	C6H3Br3O	327.7734	328.7807	326.7661	ESI+	10
13	2, 4, 6-Trichlorophenol	88-06-2	C6H3Cl3O	195.9249	196.9322	194.9176	ESI-	3
14	2, 4-D (2, 4-PA)	94-75-7	C8H6Cl2O3	219.9694	220.9767	218.9621	ESI-	7
15	2, 4-DB	94-82-6	C10H10Cl2O3	248.0007	249.0080	246.9934	ESI-	5
16	2, 4-dimethylaniline	95-68-1	C8H11N	121.0891	122.0964	120.0818	ESI+	5
17	2, 6-Dichlorobenzamide	2008-58-4	C7H5Cl2NO	188.9748	189.9821	187.9675	ESI+	13
18	2-Naphthoxy acetic acid	120-23-0	C12H10O3	202.0630	203.0703	201.0557	ESI-	2
19	2-Phenylphenol	90-43-7	C12H10O	170.0732	171.0805	169.0659	ESI-	2
20	3-(3-Indolyl)-propionic acid	830-96-6	C11H11NO2	189.0790	190.0863	188.0717	ESI+	6
21	3, 4, 5-Trimethacarb	2686-99-9	C11H15NO2	193.1103	194.1176	192.1030	ESI+	12
22	3-Indolyl-acetic acid	87-51-4	C10H9NO2	175.0633	176.0706	174.0560	ESI+	12
23	3-Methylphosphinicopropionic acid	15090-23-0	C4H9O4P	152.0238	153.0311	151.0165	ESI+	12
24	4-(3-Indolyl)-butyric acid	133-32-4	C12H13NO2	203.0946	204.1019	202.0873	ESI+	14
25	4-Chlorophenoxyacetic acid	122-88-3	C8H7ClO3	186.0084	187.0157	185.0011	ESI-	4
26	6-chloro-3-phenylpyridazin-4-ol	40020-01-7	C10H7ClN2O	206.0247	207.0320	205.0174	ESI+	6
27	6-Furfurylaminopurine	525-79-1	C10H9N5O	215.0807	216.0880	214.0734	ESI+	9
28	Acephate	30560-19-1	C4H10NO3PS	183.0119	184.0192	182.0046	ESI+	6
29	Acequinocyl	57960-19-7	C24H32O4	384.2301	385.2374	383.2228	ESI+	6
30	Acetamiprid	135410-20-7	C10H11ClN4	222.0672	223.0745	221.0599	ESI+	10
31	Acibenzolar-S-methyl	135158-54-2	C8H6N2OS2	209.9922	210.9995	208.9849	ESI+	6
32	Acifluorfen	50594-66-6	C14H7ClF3NO5	360.9965	362.0038	359.9892	ESI-	12
33	Aclonifen	74070-46-5	C12H9ClN2O3	264.0302	265.0375	263.0229	ESI+	2
34	Acrinathrin	101007-06-1	C26H21F6NO5	541.1324	542.1397	540.1251	ESI+	12
35	Alachlor	15972-60-8	C14H20ClNO2	269.1183	270.1256	268.1110	ESI+	12
36	Alanycarb	83130-01-2	C17H25N3O4S2	399.1286	400.1359	398.1213	ESI+	6
37	Aldicarb	116-06-3	C7H14N2O2S	190.0776	191.0849	189.0703	ESI+	5
38	Aldicarb-sulfone (Aldoxycarb)	1646-88-4	C7H14N2O4S	222.0674	223.0747	221.0601	ESI+	5
39	Aldicarb-sulfoxide	1646-87-3	C7H14N2O3S	206.0725	207.0798	205.0652	ESI+	8
40	Allethrin	584-79-2	C19H26O3	302.1882	303.1955	301.1809	ESI+	12
41	Allidochlor	93-71-0	C8H12ClNO	173.0607	174.0680	172.0534	ESI+	12
42	Ametoctradin	865318-97-4	C15H25N5	275.2110	276.2183	274.2037	ESI+	6
43	Ametryn	834-12-8	C9H17N5S	227.1205	228.1278	226.1132	ESI+	6
44	Amidosulfuron	120923-37-7	C9H15N5O7S2	369.0413	370.0486	368.0340	ESI+	8
45	Aminocarb	2032-59-9	C11H16N2O2	208.1212	209.1285	207.1139	ESI+	6
46	Aminopyralid	150114-71-9	C6H4Cl2N2O2	205.9650	206.9723	204.9577	ESI+	7
47	Amisulbrom	348635-87-0	C13H13BrFN5O4S2	464.9576	465.9649	463.9503	ESI+	10
48	Amitraz	33089-61-1	C19H23N3	293.1892	294.1965	292.1819	ESI+	2
49	Amitrole	61-82-5	C2H4N4	84.0436	85.0509	83.0363	ESI+	5
50	AMPA	1066-51-9	CH6NO3P	111.0085	112.0158	110.0012	ESI-	3
51	Ancymidol	12771-68-5	C15H16N2O2	256.1212	257.1285	255.1139	ESI+	6
52	Anilazine	101-05-3	C9H5Cl3N4	273.9580	274.9653	272.9507	ESI+	12
53	Anilofos	64249-01-0	C13H19ClNO3PS2	367.0232	368.0305	366.0159	ESI+	12
54	Aramite	140-57-8	C15H23ClO4S	334.1006	335.1079	333.0933	ESI+	12
55	Asulam	3337-71-1	C8H10N2O4S	230.0361	231.0434	229.0288	ESI+	9
56	Atraton	1610-17-9	C9H17N5O	211.1433	212.1506	210.1360	ESI+	6
57	Atrazine	1912-24-9	C8H14ClN5	215.0938	216.1011	214.0865	ESI+	8
58	Atrazine-2-hydroxy	2163-68-0	C8H15N5O	197.1277	198.1350	196.1204	ESI+	6
59	Atrazine-desethyl	6190-65-4	C6H10ClN5	187.0625	188.0698	186.0552	ESI+	9
60	Atrazine-desethyl-2-hydroxy	19988-24-0	C6H11N5O	169.0964	170.1037	168.0891	ESI+	5
61	Atrazine-desisopropyl	1007-28-9	C5H8ClN5	173.0468	174.0541	172.0395	ESI+	10
62	Avermectin B1a	65195-55-3	C48H72O14	872.4922	873.4995	871.4849	ESI+	4
63	Avermectin B1b	65195-56-4	C47H70O14	858.4766	859.4839	857.4693	ESI+	3
64	Azaconazole	60207-31-0	C12H11Cl2N3O2	299.0228	300.0301	298.0155	ESI+	8
65	Azadirachtin	11141-17-6	C35H44O16	720.2629	721.2702	719.2556	ESI+	8
66	Azamethiphos	35575-96-3	C9H10ClN2O5PS	323.9737	324.9810	322.9664	ESI+	11
67	Azimsulfuron	120162-55-2	C13H16N10O5S	424.1026	425.1099	423.0953	ESI+	5
68	Azinphos-ethyl	2642-71-9	C12H16N3O3PS2	345.0371	346.0444	344.0298	ESI+	5
69	Azinphos-methyl	86-50-0	C10H12N3O3PS2	317.0058	318.0131	315.9985	ESI+	6
70	Aziprotryne	4658-28-0	C7H11N7S	225.0797	226.0870	224.0724	ESI+	4
71	Azobenzene	103-33-3	C12H10N2	182.0844	183.0917	181.0771	ESI+	2
72	Azoxystrobin	131860-33-8	C22H17N3O5	403.1168	404.1241	402.1095	ESI+	5
73	Barban	101-27-9	C11H9Cl2NO2	257.0010	258.0083	255.9937	ESI+	11
74	Beflubutamid	113614-08-7	C18H17F4NO2	355.1195	356.1268	354.1122	ESI+	10
75	Benalaxyl	71626-11-4	C20H23NO3	325.1678	326.1751	324.1605	ESI+	6

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
76	Benazolin	3813-05-6	C9H6ClNO3S	242.9757	243.9830	241.9684	ESI+	6
77	Benazolin-ethyl	25059-80-7	C11H10ClNO3S	271.0070	272.0143	269.9997	ESI+	18
78	Bendiocarb	22781-23-3	C11H13NO4	223.0845	224.0918	222.0772	ESI+	6
79	Benfuracarb	82560-54-1	C20H30N2O5S	410.1875	411.1948	409.1802	ESI+	5
80	Benfuresate	68505-69-1	C12H16O4S	256.0769	257.0842	255.0696	ESI+	2
81	Benodanil	15310-01-7	C13H10INO	322.9807	323.9880	321.9734	ESI+	6
82	Benoxacor	98730-04-2	C11H11Cl2NO2	259.0167	260.0240	258.0094	ESI+	17
83	Bensulfuron-methyl	83055-99-6	C16H18N4O7S	410.0896	411.0969	409.0823	ESI+	6
84	Bensulide	741-58-2	C14H24NO4PS3	397.0605	398.0678	396.0532	ESI+	9
85	Bentazone	25057-89-0	C10H12N2O3S	240.0569	241.0642	239.0496	ESI-	5
86	Benthiavalicarb-isopropyl	177406-68-7	C18H24FN3O3S	381.1522	382.1595	380.1449	ESI+	5
87	Benthiazole	21564-17-0	C9H6N2S3	237.9693	238.9766	236.9620	ESI+	6
88	Benzanilide	93-98-1	C13H11NO	197.0841	198.0914	196.0768	ESI+	4
89	Benzofenap	82692-44-2	C22H20Cl2N2O3	430.0851	431.0924	429.0778	ESI+	2
90	Benzoximate	29104-30-1	C18H18ClNO5	363.0874	364.0947	362.0801	ESI+	12
91	Benzoylprop-ethyl	22212-55-1	C18H17Cl2NO3	365.0585	366.0658	364.0512	ESI+	6
92	Benzthiazuron	1929-88-0	C9H9N3OS	207.0466	208.0539	206.0393	ESI+	9
93	Benzyldimethyldodecylammonium	139-07-1	C21H37N	303.2926	304.2999	302.2853	ESI+	4
94	Benzyldimethylhexadecylammonium	122-18-9	C25H45N	359.3552	360.3625	358.3479	ESI+	3
95	Benzyldimethyltetradecylammonium	139-08-2	C23H41N	331.3239	332.3312	330.3166	ESI+	3
96	Bifenazate	149877-41-8	C17H20N2O3	300.1474	301.1547	299.1401	ESI+	6
97	Bifenox	42576-02-3	C14H9Cl2NO5	340.9858	341.9931	339.9785	ESI+	8
98	Bifenthrin	82657-04-3	C23H22ClF3O2	422.1260	423.1333	421.1187	ESI+	5
99	Bioresmethrin	28434-01-7	C22H26O3	338.1882	339.1955	337.1809	ESI+	6
100	Bispyribac-sodium	125401-92-5	C19H17N4NaO8	452.0944	453.1017	451.0871	ESI+	8
101	Bitertanol	55179-31-2	C20H23N3O2	337.1790	338.1863	336.1717	ESI+	6
102	Bixafen	581809-46-3	C18H12Cl2F3N3O	413.0310	414.0383	412.0237	ESI+	12
103	Boscalid	188425-85-6	C18H12Cl2N2O	342.0327	343.0400	341.0254	ESI+	12
104	Brodifacoum	56073-10-0	C31H23BrO3	522.0831	523.0904	521.0758	ESI+	12
105	Bromacil	314-40-9	C9H13BrN2O2	260.0160	261.0233	259.0087	ESI+	9
106	Bromadiolone	28772-56-7	C30H23BrO4	526.0780	527.0853	525.0707	ESI-	12
107	Bromfenvinfos	33399-00-7	C12H14BrCl2O4P	401.9190	402.9263	400.9117	ESI+	17
108	Bromobutide	74712-19-9	C15H22BrNO	311.0885	312.0958	310.0812	ESI+	10
109	Bromophos-ethyl	4824-78-6	C10H12BrCl2O3PS	391.8805	392.8878	390.8732	ESI+	3
110	Bromophos-methyl	2104-96-3	C8H8BrCl2O3PS	363.8492	364.8565	362.8419	ESI+	6
111	Bromoxynil	1689-84-5	C7H3Br2NO	274.8581	275.8654	273.8508	ESI-	11
112	Bromuconazole	116255-48-2	C13H12BrCl2N3O	374.9541	375.9614	373.9468	ESI+	11
113	Bupirimate	41483-43-6	C13H24N4O3S	316.1569	317.1642	315.1496	ESI+	6
114	Buprofezin	69327-76-0	C16H23N3OS	305.1562	306.1635	304.1489	ESI+	6
115	Butachlor	23184-66-9	C17H26ClNO2	311.1652	312.1725	310.1579	ESI+	12
116	Butafenacil	134605-64-4	C20H18ClF3N2O6	474.0805	475.0878	473.0732	ESI+	10
117	Butamifos	36335-67-8	C13H21N2O4PS	332.0960	333.1033	331.0887	ESI+	12
118	Butocarboxim	34681-10-2	C7H14N2O2S	190.0776	191.0849	189.0703	ESI+	3
119	Butocarboxim-sulfone	34681-23-7	C7H14N2O4S	222.0674	223.0747	221.0601	ESI+	14
120	Butocarboxim-sulfoxide	34681-24-8	C7H14N2O3S	206.0725	207.0798	205.0652	ESI+	6
121	Butralin	33629-47-9	C14H21N3O4	295.1532	296.1605	294.1459	ESI+	6
122	Buturon	3766-60-7	C12H13ClN2O	236.0716	237.0789	235.0643	ESI+	9
123	Butylate	2008-41-5	C11H23NOS	217.1500	218.1573	216.1427	ESI+	3
124	Cadusafos	95465-99-9	C10H23O2PS2	270.0877	271.0950	269.0804	ESI+	5
125	Cafenstrole	125306-83-4	C16H22N4O3S	350.1413	351.1486	349.1340	ESI+	3
126	Captafol	2425-06-1	C10H9Cl4NO2S	346.9108	347.9181	345.9035	ESI+	1
127	Carbaryl (NAC)	63-25-2	C12H11NO2	201.0790	202.0863	200.0717	ESI+	6
128	Carbendazim	10605-21-7	C9H9N3O2	191.0695	192.0768	190.0622	ESI+	5
129	Carbetamide	16118-49-3	C12H16N2O3	236.1161	237.1234	235.1088	ESI+	6
130	Carbofuran	1563-66-2	C12H15NO3	221.1052	222.1125	220.0979	ESI+	6
131	Carbofuran-3-hydroxy (3-Hydroxycarbofuran)	16655-82-6	C12H15NO4	237.1001	238.1074	236.0928	ESI+	12
132	Carbofuran-3-keto	16709-30-1	C12H13NO4	235.0845	236.0918	234.0772	ESI+	12
133	Carbophenothion	786-19-6	C11H16ClO2PS3	341.9739	342.9812	340.9666	ESI+	9
134	Carbosulfan	55285-14-8	C20H32N2O3S	380.2134	381.2207	379.2061	ESI+	6
135	Carboxin	5234-68-4	C12H13NO2S	235.0667	236.0740	234.0594	ESI+	6
136	Carfentrazone-ethyl	128639-02-1	C15H14Cl2F3N3O3	411.0364	412.0437	410.0291	ESI+	5
137	Carpropamid	104030-54-8	C15H18Cl3NO	333.0454	334.0527	332.0381	ESI+	18
138	Cartap	15263-53-3	C7H15N3O2S2	237.0606	238.0679	236.0533	ESI+	3
139	Chinomethionat	2439-01-2	C10H6N2OS2	233.9922	234.9995	232.9849	ESI+	6
140	Chloramphenicol	56-75-7	C11H12Cl2N2O5	322.0123	323.0196	321.0050	ESI-	17
141	Chlorantraniliprole	500008-45-7	C18H14BrCl2N5O2	480.9708	481.9781	479.9635	ESI+	28
142	Chlorbromuron	13360-45-7	C9H10BrClN2O2	291.9614	292.9687	290.9541	ESI+	12
143	Chlorbufam	1967-16-4	C11H10ClNO2	223.0400	224.0473	222.0327	ESI+	4
144	Chlordimeform	6164-98-3	C10H13ClN2	196.0767	197.0840	195.0694	ESI+	12
145	Chlorfenvinphos	470-90-6	C12H14Cl3O4P	357.9695	358.9768	356.9622	ESI+	12
146	Chlorfluazuron	71422-67-8	C20H9Cl3F5N3O3	538.9630	539.9703	537.9557	ESI+	17
147	Chloridazon	1698-60-8	C10H8ClN3O	221.0356	222.0429	220.0283	ESI+	11
148	Chlorimuron-ethyl	90982-32-4	C15H15ClN4O6S	414.0401	415.0474	413.0328	ESI+	12
149	Chlormequat-chloride	999-81-5	C5H13Cl2N	157.0425	158.0498	156.0352	ESI+	6
150	Chlorophacinone	3691-35-8	C23H15ClO3	374.0710	375.0783	373.0637	ESI-	15

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
151	Chlorotoluron	15545-48-9	C10H13ClN2O	212.0716	213.0789	211.0643	ESI+	8
152	Chloroxuron	1982-47-4	C15H15ClN2O2	290.0822	291.0895	289.0749	ESI+	12
153	Chloroxynil	1891-95-8	C7H3Cl2NO	186.9592	187.9665	185.9519	ESI-	6
154	Chlorpropham	101-21-3	C10H12ClNO2	213.0557	214.0630	212.0484	ESI+	2
155	Chlorpyrifos	2921-88-2	C9H11Cl3NO3PS	348.9263	349.9336	347.9190	ESI+	16
156	Chlorpyrifos-methyl	5598-13-0	C7H7Cl3NO3PS	320.8950	321.9023	319.8877	ESI+	12
157	Chlorpyrifos-oxon	5598-15-2	C9H11Cl3NO4P	332.9491	333.9564	331.9418	ESI+	24
158	Chlorsulfuron	64902-72-3	C12H12ClN5O4S	357.0299	358.0372	356.0226	ESI+	18
159	Chlorthiamid	1918-13-4	C7H5Cl2NS	204.9520	205.9593	203.9447	ESI+	16
160	Chromafenozide	143807-66-3	C24H30N2O3	394.2256	395.2329	393.2183	ESI+	6
161	Cinidon-ethyl	142891-20-1	C19H17Cl2NO4	393.0535	394.0608	392.0462	ESI+	24
162	Cinosulfuron	94593-91-6	C15H19N5O7S	413.1005	414.1078	412.0932	ESI+	6
163	Clethodim	99129-21-2	C17H26ClNO3S	359.1322	360.1395	358.1249	ESI+	10
164	Climbazole	38083-17-9	C15H17ClN2O2	292.0979	293.1052	291.0906	ESI+	9
165	Clodinafop (free acid)	114420-56-3	C14H11ClFNO4	311.0361	312.0434	310.0288	ESI+	8
166	Clodinafop-propargyl	105512-06-9	C17H13ClFNO4	349.0517	350.0590	348.0444	ESI+	12
167	Clofentezine	74115-24-5	C14H8Cl2N4	302.0126	303.0199	301.0053	ESI+	10
168	Clomazone	81777-89-1	C12H14ClNO2	239.0713	240.0786	238.0640	ESI+	8
169	Clomeprop	84496-56-0	C16H15Cl2NO2	323.0480	324.0553	322.0407	ESI+	21
170	Cloprop	101-10-0	C9H9ClO3	200.0240	201.0313	199.0167	ESI-	2
171	Clopyralid	1702-17-6	C6H3Cl2NO2	190.9541	191.9614	189.9468	ESI-	2
172	Cloquintocet-mexyl	99607-70-2	C18H22ClNO3	335.1288	336.1361	334.1215	ESI+	9
173	Cloransulam-methyl	147150-35-4	C15H13ClFN5O5S	429.0310	430.0383	428.0237	ESI+	12
174	Clothianidin	210880-92-5	C6H8ClN5O2S	249.0087	250.0160	248.0014	ESI+	7
175	Coumachlor	81-82-3	C19H15ClO4	342.0659	343.0732	341.0586	ESI+	18
176	Coumaphos	56-72-4	C14H16ClO5PS	362.0145	363.0218	361.0072	ESI+	12
177	Coumatetralyl	5836-29-3	C19H16O3	292.1099	293.1172	291.1026	ESI+	6
178	Crimidine	535-89-7	C7H10ClN3	171.0563	172.0636	170.0490	ESI+	12
179	Crotoxyphos	7700-17-6	C14H19O6P	314.0919	315.0992	313.0846	ESI+	6
180	Crufomate	299-86-5	C12H19ClNO3P	291.0791	292.0864	290.0718	ESI+	12
181	Cumyluron	99485-76-4	C17H19ClN2O	302.1186	303.1259	301.1113	ESI+	2
182	Cyanazine	21725-46-2	C9H13ClN6	240.0890	241.0963	239.0817	ESI+	6
183	Cyanofenphos	13067-93-1	C15H14NO2PS	303.0483	304.0556	302.0410	ESI+	6
184	Cyazofamid	120116-88-3	C13H13ClN4O2S	324.0448	325.0521	323.0375	ESI+	5
185	Cyclanilide	113136-77-9	C11H9Cl2NO3	272.9959	274.0032	271.9886	ESI-	20
186	Cycloate	1134-23-2	C11H21NOS	215.1344	216.1417	214.1271	ESI+	5
187	Cycloheximide	66-81-9	C15H23NO4	281.1627	282.1700	280.1554	ESI+	12
188	Cycloprothrin	63935-38-6	C26H21Cl2NO4	481.0848	482.0921	480.0775	ESI+	2
189	Cyclosulfamuron	136849-15-5	C17H19N5O6S	421.1056	422.1129	420.0983	ESI+	6
190	Cycloxydim	101205-02-1	C17H27NO3S	325.1712	326.1785	324.1639	ESI+	10
191	Cycluron	2163-69-1	C11H22N2O	198.1732	199.1805	197.1659	ESI+	5
192	Cyflufenamid	180409-60-3	C20H17F5N2O2	412.1210	413.1283	411.1137	ESI+	6
193	Cyflumetofen	400882-07-7	C24H24F3NO4	447.1657	448.1730	446.1584	ESI+	8
194	Cyhalofop-butyl	122008-85-9	C20H20FNO4	357.1376	358.1449	356.1303	ESI+	3
195	Cymiazole	61676-87-7	C12H14N2S	218.0878	219.0951	217.0805	ESI+	6
196	Cymoxanil	57966-95-7	C7H10N4O3	198.0753	199.0826	197.0680	ESI+	4
197	Cypermethrin	52315-07-8	C22H19Cl2NO3	415.0742	416.0815	414.0669	ESI+	10
198	Cyphenothrin	39515-40-7	C24H25NO3	375.1834	376.1907	374.1761	ESI+	12
199	Cyproconazole	94361-06-5	C15H18ClN3O	291.1138	292.1211	290.1065	ESI+	10
200	Cyprodinil	121552-61-2	C14H15N3	225.1266	226.1339	224.1193	ESI+	6
201	Cyromazine	66215-27-8	C6H10N6	166.0967	167.1040	165.0894	ESI+	6
202	Daimuron (Dymron)	42609-52-9	C17H20N2O	268.1576	269.1649	267.1503	ESI+	6
203	Dalapon	75-99-0	C3H4Cl2O2	141.9588	142.9661	140.9515	ESI-	10
204	Daminozide	1596-84-5	C6H12N2O3	160.0848	161.0921	159.0775	ESI+	6
205	Dazomet	533-74-4	C5H10N2S2	162.0285	163.0358	161.0212	ESI+	6
206	Deet	134-62-3	C12H17NO	191.1310	192.1383	190.1237	ESI+	2
207	Deltamethrin	52918-63-5	C22H19Br2NO3	502.9732	503.9805	501.9659	ESI+	12
208	Demeton-O	298-03-3	C8H19O3PS2	258.0513	259.0586	257.0440	ESI+	2
209	Demeton-S	126-75-0	C8H19O3PS2	258.0513	259.0586	257.0440	ESI+	3
210	Demeton-S-methyl	919-86-8	C6H15O3PS2	230.0200	231.0273	229.0127	ESI+	2
211	Demeton-S-methyl-sulfone	17040-19-6	C6H15O5PS2	262.0099	263.0172	261.0026	ESI+	6
212	Desmedipham	13684-56-5	C16H16N2O4	300.1110	301.1183	299.1037	ESI+	6
213	Desmetryn	1014-69-3	C8H15N5S	213.1048	214.1121	212.0975	ESI+	4
214	Diafenthiuron	80060-09-9	C23H32N2OS	384.2235	385.2308	383.2162	ESI+	12
215	Dialifos	10311-84-9	C14H17ClNO4PS2	393.0025	394.0098	391.9952	ESI+	12
216	Diallate	2303-16-4	C10H17Cl2NOS	269.0408	270.0481	268.0335	ESI+	12
217	Diazinon	333-41-5	C12H21N2O3PS	304.1010	305.1083	303.0937	ESI+	6
218	Dicamba	1918-00-9	C8H6Cl2O3	219.9694	220.9767	218.9621	ESI-	2
219	Dichlofenthion	97-17-6	C10H13Cl2O3PS	313.9700	314.9773	312.9627	ESI+	8
220	Dichlofluanid	1085-98-9	C9H11Cl2FN2O2S2	331.9623	332.9696	330.9550	ESI+	11
221	Dichlormid	37764-25-3	C8H11Cl2NO	207.0218	208.0291	206.0145	ESI+	19
222	Dichlorprop	120-36-5	C9H8Cl2O3	233.9850	234.9923	232.9777	ESI-	8
223	Dichlorvos	62-73-7	C4H7Cl2O4P	219.9459	220.9532	218.9386	ESI+	17
224	Diclobutrazol	75736-33-3	C15H19Cl2N3O	327.0905	328.0978	326.0832	ESI+	4
225	Diclofop	40843-25-2	C15H12Cl2O4	326.0113	327.0186	325.0040	ESI-	4

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
226	Diclofop-methyl	51338-27-3	C16H14Cl2O4	340.0269	341.0342	339.0196	ESI+	12
227	Dicloran	99-30-9	C6H4Cl2N2O2	205.9650	206.9723	204.9577	ESI+	4
228	Diclosulam	145701-21-9	C13H10Cl2FN5O3S	404.9865	405.9938	403.9792	ESI+	9
229	Dicrotophos	141-66-2	C8H16NO5P	237.0766	238.0839	236.0693	ESI+	6
230	Dicyclanil	112636-83-6	C8H10N6	190.0967	191.1040	189.0894	ESI+	6
231	Didecyldimethylammonium	7173-51-5	C22H47N	325.3709	326.3782	324.3636	ESI+	6
232	Diethanolamine	111-42-2	C4H11NO2	105.0790	106.0863	104.0717	ESI+	6
233	Diethofencarb	87130-20-9	C14H21NO4	267.1471	268.1544	266.1398	ESI+	6
234	Difenacoum	56073-07-5	C31H24O3	444.1725	445.1798	443.1652	ESI+	12
235	Difenoconazole	119446-68-3	C19H17Cl2N3O3	405.0647	406.0720	404.0574	ESI+	12
236	Difenoxuron	14214-32-5	C16H18N2O3	286.1317	287.1390	285.1244	ESI+	6
237	Difenzoquat-methyl-sulfate	43222-48-6	C17H16N2	248.1313	249.1386	247.1240	ESI+	6
238	Diflubenzuron	35367-38-5	C14H9ClF2N2O2	310.0321	311.0394	309.0248	ESI+	9
239	Diflufenican	83164-33-4	C19H11F5N2O2	394.0741	395.0814	393.0668	ESI+	12
240	Dimefuron	34205-21-5	C15H19ClN4O3	338.1146	339.1219	337.1073	ESI+	5
241	Dimepiperate	61432-55-1	C15H21NOS	263.1344	264.1417	262.1271	ESI+	6
242	Dimethachlon	24096-53-5	C10H7Cl2NO2	242.9854	243.9927	241.9781	ESI-	2
243	Dimethachlor	50563-36-5	C13H18ClNO2	255.1026	256.1099	254.0953	ESI+	12
244	Dimethametryn	22936-75-0	C11H21N5S	255.1518	256.1591	254.1445	ESI+	6
245	Dimethenamid	87674-68-8	C12H18ClNO2S	275.0747	276.0820	274.0674	ESI+	12
246	Dimethirimol	5221-53-4	C11H19N3O	209.1528	210.1601	208.1455	ESI+	3
247	Dimethoate	60-51-5	C5H12NO3PS2	228.9996	230.0069	227.9923	ESI+	6
248	Dimethomorph	110488-70-5	C21H22ClNO4	387.1237	388.1310	386.1164	ESI+	12
249	Dimetilan	644-64-4	C10H16N4O3	240.1222	241.1295	239.1149	ESI+	6
250	Dimoxystrobin	149961-52-4	C19H22N2O3	326.1630	327.1703	325.1557	ESI+	6
251	Diniconazole	83657-24-3	C15H17Cl2N3O	325.0749	326.0822	324.0676	ESI+	7
252	Dinocap	39300-45-3	C18H24N2O6	364.1634	365.1707	363.1561	ESI+	6
253	Dinoseb	88-85-7	C10H12N2O5	240.0746	241.0819	239.0673	ESI-	4
254	Dinotefuran	165252-70-0	C7H14N4O3	202.1066	203.1139	201.0993	ESI+	6
255	Dinoterb	1420-07-1	C10H12N2O5	240.0746	241.0819	239.0673	ESI-	4
256	Dioxacarb	6988-21-2	C11H13NO4	223.0845	224.0918	222.0772	ESI+	6
257	Dioxathion	78-34-2	C12H26O6P2S4	456.0087	457.0160	455.0014	ESI+	6
258	Diphenamid	957-51-7	C16H17NO	239.1310	240.1383	238.1237	ESI+	6
259	Diphenylamine	122-39-4	C12H11N	169.0891	170.0964	168.0818	ESI+	4
260	Dipropetryn	4147-51-7	C11H21N5S	255.1518	256.1591	254.1445	ESI+	6
261	Diquat	6385-62-2	C12H12N2	184.1000	185.1073	183.0927	ESI+	3
262	Disulfoton	298-04-4	C8H19O2PS3	274.0285	275.0358	273.0212	ESI+	3
263	Disulfoton-sulfone	2497-06-5	C8H19O4PS3	306.0183	307.0256	305.0110	ESI+	6
264	Disulfoton-sulfoxide	2497-07-6	C8H19O3PS3	290.0234	291.0307	289.0161	ESI+	6
265	Ditalimfos	5131-24-8	C12H14NO4PS	299.0381	300.0454	298.0308	ESI+	6
266	Dithianon	3347-22-6	C14H4N2O2S2	295.9714	296.9787	294.9641	ESI-	4
267	Dithiopyr	97886-45-8	C15H16F5NO2S2	401.0543	402.0616	400.0470	ESI+	6
268	Diuron (DCMU)	330-54-1	C9H10Cl2N2O	232.0170	233.0243	231.0097	ESI+	7
269	DMST	66840-71-9	C9H14N2O2S	214.0776	215.0849	213.0703	ESI+	4
270	DNOC	534-52-1	C7H6N2O5	198.0277	199.0350	197.0204	ESI-	6
271	Dodemorph	1593-77-7	C18H35NO	281.2719	282.2792	280.2646	ESI+	6
272	Dodine	2439-10-3	C15H33N3O2	287.2573	288.2646	286.2500	ESI+	6
273	Doramectin	117704-25-3	C50H74O14	898.5079	899.5152	897.5006	ESI+	10
274	Edifenphos	17109-49-8	C14H15O2PS2	310.0251	311.0324	309.0178	ESI+	6
275	Emamectin B1a	119791-41-2	C49H75NO13	885.5238	886.5311	884.5165	ESI+	5
276	Emamectin B1b	137335-79-6	C55H79NO15	871.5082	872.5155	870.5009	ESI+	3
277	Endosulfan-sulfate	1031-07-8	C9H6Cl6O4S	419.8118	420.8191	418.8045	ESI-	3
278	EPN	2104-64-5	C14H14NO4PS	323.0381	324.0454	322.0308	ESI+	6
279	Epoxiconazole	133855-98-8	C17H13ClFN3O	329.0731	330.0804	328.0658	ESI+	9
280	EPTC	759-94-4	C9H19NOS	189.1187	190.1260	188.1114	ESI+	5
281	Esfenvalerate	66230-04-4	C25H22ClNO3	419.1288	420.1361	418.1215	ESI+	2
282	Esprocarb	85785-20-2	C15H23NOS	265.1500	266.1573	264.1427	ESI+	5
283	Etaconazole	60207-93-4	C14H15Cl2N3O2	327.0541	328.0614	326.0468	ESI+	12
284	Ethametsulfuron-methyl	97780-06-8	C15H18N6O6S	410.1009	411.1082	409.0936	ESI+	6
285	Ethephon	16672-87-0	C2H6ClO3P	143.9743	144.9816	142.9670	ESI-	6
286	Ethidimuron	30043-49-3	C7H12N4O3S2	264.0351	265.0424	263.0278	ESI+	11
287	Ethiofencarb	29973-13-5	C11H15NO2S	225.0823	226.0896	224.0750	ESI+	10
288	Ethiofencarb-sulfone	53380-23-7	C11H15NO4S	257.0722	258.0795	256.0649	ESI+	8
289	Ethiofencarb-sulfoxide	53380-22-6	C11H15NO3S	241.0773	242.0846	240.0700	ESI+	4
290	Ethion	563-12-2	C9H22O4P2S4	383.9876	384.9949	382.9803	ESI+	6
291	Ethiprole	181587-01-9	C13H9Cl2F3N4OS	395.9826	396.9899	394.9753	ESI+	30
292	Ethirimol	23947-60-6	C11H19N3O	209.1528	210.1601	208.1455	ESI+	6
293	Ethofumesate	26225-79-6	C13H18O5S	286.0875	287.0948	285.0802	ESI+	11
294	Ethoprophos	13194-48-4	C8H19O2PS2	242.0564	243.0637	241.0491	ESI+	6
295	Ethoxyquin	91-53-2	C14H19NO	217.1467	218.1540	216.1394	ESI+	4
296	Ethoxysulfuron	126801-58-9	C15H18N4O7S	398.0896	399.0969	397.0823	ESI+	6
297	Ethylenethiourea	96-45-7	C3H6N2S	102.0252	103.0325	101.0179	ESI+	6
298	Etofenprox	80844-07-1	C25H28O3	376.2038	377.2111	375.1965	ESI+	6
299	Etoxazole	153233-91-1	C21H23F2NO2	359.1697	360.1770	358.1624	ESI+	6
300	Etrimfos	38260-54-7	C10H17N2O4PS	292.0647	293.0720	291.0574	ESI+	6

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
301	Famoxadone	131807-57-3	C22H18N2O4	374.1267	375.1340	373.1194	ESI+	6
302	Famphur	52-85-7	C10H16NO5PS2	325.0208	326.0281	324.0135	ESI+	12
303	Fenamidone	161326-34-7	C17H17N3OS	311.1092	312.1165	310.1019	ESI+	6
304	Fenaminosulf	140-56-7	C8H10N3NaO3S	251.0341	252.0414	250.0268	ESI+	2
305	Fenamiphos	22224-92-6	C13H22NO3PS	303.1058	304.1131	302.0985	ESI+	6
306	Fenamiphos-sulfone	31972-44-8	C13H22NO5PS	335.0956	336.1029	334.0883	ESI+	6
307	Fenamiphos-sulfoxide	31972-43-7	C13H22NO4PS	319.1007	320.1080	318.0934	ESI+	6
308	Fenarimol	60168-88-9	C17H12Cl2N2O	330.0327	331.0400	329.0254	ESI+	12
309	Fenazaquin	120928-09-8	C20H22N2O	306.1732	307.1805	305.1659	ESI+	6
310	Fenazox	495-48-7	C12H10N2O	198.0793	199.0866	197.0720	ESI+	6
311	Fenbuconazole	114369-43-6	C19H17ClN4	336.1142	337.1215	335.1069	ESI+	8
312	Fenbutatin-oxide	13356-08-6	C60H78OSn2	1054.4121	1055.4194	1053.4048	ESI+	11
313	Fenchlorazol-ethyl	103112-35-2	C12H8Cl5N3O2	400.9059	401.9132	399.8986	ESI+	118
314	Fenfuram	24691-80-3	C12H11NO2	201.0790	202.0863	200.0717	ESI+	6
315	Fenhexamid	126833-17-8	C14H17Cl2NO2	301.0636	302.0709	300.0563	ESI+	23
316	Fenitrothion	122-14-5	C9H12NO5PS	277.0174	278.0247	276.0101	ESI+	2
317	Fenobucarb	3766-81-2	C12H17NO2	207.1259	208.1332	206.1186	ESI+	6
318	Fenoprop	93-72-1	C9H7Cl3O3	267.9461	268.9534	266.9388	ESI-	8
319	Fenothiocarb	62850-32-2	C13H19NO2S	253.1136	254.1209	252.1063	ESI+	4
320	Fenoxanil	115852-48-7	C15H18Cl2N2O2	328.0745	329.0818	327.0672	ESI+	29
321	Fenoxaprop	95617-09-7	C16H12ClNO5	333.0404	334.0477	332.0331	ESI+	23
322	Fenoxaprop-ethyl	66441-23-4	C18H16ClNO5	361.0717	362.0790	360.0644	ESI+	12
323	Fenoxaprop-P-ethyl	71283-80-2	C18H16ClNO5	361.0717	362.0790	360.0644	ESI+	12
324	Fenoxycarb	79127-80-3	C17H19NO4	301.1314	302.1387	300.1241	ESI+	6
325	Fenpropathrin	64257-84-7	C22H23NO3	349.1678	350.1751	348.1605	ESI+	11
326	Fenpropidin	67306-00-7	C19H31N	273.2457	274.2530	272.2384	ESI+	6
327	Fenpropimorph	67564-91-4	C20H33NO	303.2562	304.2635	302.2489	ESI+	6
328	Fensulfothion	115-90-2	C11H17O4PS2	308.0306	309.0379	307.0233	ESI+	6
329	Fensulfothion-oxon	6552-21-2	C11H17O5PS	292.0534	293.0607	291.0461	ESI+	6
330	Fensulfothion-oxon-sulfone	6132-17-8	C11H17O6PS	308.0483	309.0556	307.0410	ESI+	4
331	Fensulfothion-sulfone	14255-72-2	C11H17O5PS2	324.0255	325.0328	323.0182	ESI+	6
332	Fenthion	55-38-9	C10H15O3PS2	278.0200	279.0273	277.0127	ESI+	6
333	Fenthion-oxon	6552-12-1	C10H15O4PS	262.0429	263.0502	261.0356	ESI+	6
334	Fenthion-oxon-sulfone	14086-35-2	C10H15O6PS	294.0327	295.0400	293.0254	ESI+	12
335	Fenthion-oxon-sulfoxide	6552-13-2	C10H15O5PS	278.0378	279.0451	277.0305	ESI+	3
336	Fenthion-sulfone	3761-42-0	C10H15O5PS2	310.0099	311.0172	309.0026	ESI+	4
337	Fenthion-sulfoxide	3761-41-9	C10H15O4PS2	294.0149	295.0222	293.0076	ESI+	6
338	Fenuron	101-42-8	C9H12N2O	164.0950	165.1023	163.0877	ESI+	6
339	Fenvalerate	51630-58-1	C25H22ClNO3	419.1288	420.1361	418.1215	ESI+	6
340	Fipronil	120068-37-3	C12H4Cl2F6N4OS	435.9387	436.9460	434.9314	ESI-	12
341	Fipronil-desulfinyl	205650-65-3	C12H4Cl2F6N4	387.9717	388.9790	386.9644	ESI-	12
342	Fipronil-sulfide	120067-83-6	C12H4Cl2F6N4S	419.9438	420.9511	418.9365	ESI-	12
343	Fipronil-sulfone	120068-36-2	C12H4Cl2F6N4O2S	451.9336	452.9409	450.9263	ESI-	12
344	Flamprop-isopropyl	52756-22-6	C19H19ClFNO3	363.1037	364.1110	362.0964	ESI+	10
345	Flamprop-methyl	52756-25-9	C17H15ClFNO3	335.0724	336.0797	334.0651	ESI+	4
346	Flamprop-M-isopropyl	63782-90-1	C19H19ClFNO3	363.1037	364.1110	362.0964	ESI+	10
347	Flazasulfuron	104040-78-0	C13H12F3N5O5S	407.0511	408.0584	406.0438	ESI+	6
348	Flocoumafen	90035-08-8	C33H25F3O4	542.1705	543.1778	541.1632	ESI+	12
349	Flonicamid	158062-67-0	C9H6F3N3O	229.0463	230.0536	228.0390	ESI+	8
350	Florasulam	145701-23-1	C12H8F3N5O3S	359.0300	360.0373	358.0227	ESI+	2
351	Fluacrypyrim	229977-93-9	C20H21F3N2O5	426.1403	427.1476	425.1330	ESI+	6
352	Fluazifop	69335-91-7	C15H12F3NO4	327.0718	328.0791	326.0645	ESI+	12
353	Fluazifop-butyl	69806-50-4	C19H20F3NO4	383.1344	384.1417	382.1271	ESI+	6
354	Fluazifop-P (free acid)	83066-88-0	C15H12F3NO4	327.0718	328.0791	326.0645	ESI+	12
355	Fluazifop-P-butyl	79241-46-6	C19H20F3NO4	383.1344	384.1417	382.1271	ESI+	6
356	Fluazinam	79622-59-6	C13H4Cl2F6N4O4	463.9514	464.9587	462.9441	ESI-	12
357	Fluazuron	86811-58-7	C20H10Cl2F5N3O3	505.0019	506.0092	503.9946	ESI+	17
358	Flubendiamide	272451-65-7	C23H22F7IN2O4S	682.0233	683.0306	681.0160	ESI+	5
359	Flucycloxuron	94050-52-9	C25H20ClF2N3O3	483.1161	484.1234	482.1088	ESI+	10
360	Flucythrinate	70124-77-5	C26H23F2NO4	451.1595	452.1668	450.1522	ESI+	4
361	Fludioxonil	131341-86-1	C12H6F2N2O2	248.0397	249.0470	247.0324	ESI-	6
362	Flufenacet	142459-58-3	C14H13F4N3O2S	363.0665	364.0738	362.0592	ESI+	6
363	Flufenoxuron	101463-69-8	C21H11ClF6N2O3	488.0362	489.0435	487.0289	ESI+	8
364	Flumetralin	62924-70-3	C16H12ClF4N3O4	421.0452	422.0525	420.0379	ESI+	3
365	Flumetsulam	98967-40-9	C12H9F2N5O2S	325.0445	326.0518	324.0372	ESI+	2
366	Flumioxazin	103361-09-7	C19H15FN2O4	354.1016	355.1089	353.0943	ESI+	2
367	Fluometuron	2164-17-2	C10H11F3N2O	232.0823	233.0896	231.0750	ESI+	4
368	Fluopicolide	239110-15-7	C14H8Cl3F3N2O	381.9654	382.9727	380.9581	ESI+	11
369	Fluopyram	658066-35-4	C16H11ClF6N2O	396.0464	397.0537	395.0391	ESI+	12
370	Fluoroglycofen-ethyl	77501-90-7	C18H13ClF3NO7	447.0333	448.0406	446.0260	ESI+	12
371	Fluoxastrobin	361377-29-9	C21H16ClFN4O5	458.0793	459.0866	457.0720	ESI+	12
372	Flupyrsulfuron-methyl	144740-54-5	C15H14F3N5O7S	465.0566	466.0639	464.0493	ESI+	12
373	Fluquinconazole	136426-54-5	C16H8Cl2FN5O	375.0090	376.0163	374.0017	ESI+	10
374	Fluridone	59756-60-4	C19H14F3NO	329.1027	330.1100	328.0954	ESI+	4
375	Flurochloridone	61213-25-0	C12H10Cl2F3NO	311.0092	312.0165	310.0019	ESI+	18

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
376	Fluroxypyr	69377-81-7	C7H5Cl2FN2O3	253.9661	254.9734	252.9588	ESI+	15
377	Fluroxypyr-1-methylheptylester	81406-37-3	C15H21Cl2FN2O3	366.0913	367.0986	365.0840	ESI+	12
378	Flurprimidol	56425-91-3	C15H15F3N2O2	312.1086	313.1159	311.1013	ESI+	6
379	Flurtamone	96525-23-4	C18H14F3NO2	333.0977	334.1050	332.0904	ESI+	6
380	Flusilazole	85509-19-9	C16H15F2N3Si	315.1003	316.1076	314.0930	ESI+	6
381	Fluthiacet-methyl	117337-19-6	C15H15ClFN3O3S2	403.0227	404.0300	402.0154	ESI+	9
382	Flutolanil	66332-96-5	C17H16F3NO2	323.1133	324.1206	322.1060	ESI+	12
383	Flutriafol	76674-21-0	C16H13F2N3O	301.1027	302.1100	300.0954	ESI+	5
384	Fluxapyroxad	907204-31-3	C18H12F5N3O	381.0901	382.0974	380.0828	ESI+	11
385	Fomesafen	72178-02-0	C15H10ClF3N2O6S	437.9900	438.9973	436.9827	ESI+	21
386	Fonofos	944-22-9	C10H15OPS2	246.0302	247.0375	245.0229	ESI+	5
387	Foramsulfuron	173159-57-4	C17H20N6O7S	452.1114	453.1187	451.1041	ESI+	6
388	Forchlorfenuron	68157-60-8	C12H10ClN3O	247.0512	248.0585	246.0439	ESI+	12
389	Fosetyl-aluminium	39148-24-8	C2H7O3P	110.0133	111.0206	109.0060	ESI-	3
390	Fosthiazate	98886-44-3	C9H18NO3PS2	283.0466	284.0539	282.0393	ESI+	6
391	Fuberidazole	3878-19-1	C11H8N2O	184.0637	185.0710	183.0564	ESI+	6
392	Furalaxyl	57646-30-7	C17H19NO4	301.1314	302.1387	300.1241	ESI+	3
393	Furametpyr	123572-88-3	C17H20ClN3O2	333.1244	334.1317	332.1171	ESI+	12
394	Furathiocarb	65907-30-4	C18H26N2O5S	382.1562	383.1635	381.1489	ESI+	6
395	Furmecyclox	60568-05-0	C14H21NO3	251.1521	252.1594	250.1448	ESI+	6
396	Gibberellic acid (Gibberellin)	77-06-5	C19H22O6	346.1416	347.1489	345.1343	ESI-	11
397	Gluphosinate	77182-82-2	C5H12NO4P	181.0504	182.0577	180.0431	ESI+	10
398	Glyphosate	1071-83-6	C3H8NO5P	169.0140	170.0213	168.0067	ESI+	8
399	Halofenozide	112226-61-6	C18H19ClN2O2	330.1135	331.1208	329.1062	ESI+	12
400	Halosulfuron-methyl	100784-20-1	C13H15ClN6O7S	434.0411	435.0484	433.0338	ESI+	11
401	Haloxyfop	69806-34-4	C15H11ClF3NO4	361.0329	362.0402	360.0256	ESI+	9
402	Haloxyfop-2-ethoxyethyl	87237-48-7	C19H19ClF3NO5	433.0904	434.0977	432.0831	ESI+	12
403	Haloxyfop-methyl	69806-40-2	C16H13ClF3NO4	375.0485	376.0558	374.0412	ESI+	12
404	Haloxyfop-R-methyl	72619-32-0	C16H13ClF3NO4	375.0485	376.0558	374.0412	ESI+	12
405	Heptenophos	23560-59-0	C9H12ClO4P	250.0162	251.0235	249.0089	ESI+	9
406	Hexaconazole	79983-71-4	C14H17Cl2N3O	313.0749	314.0822	312.0676	ESI+	10
407	Hexaflumuron	86479-06-3	C16H8Cl2F6N2O3	459.9816	460.9889	458.9743	ESI-	12
408	Hexazinone	51235-04-2	C12H20N4O2	252.1586	253.1659	251.1513	ESI+	3
409	Hexythiazox	78587-05-0	C17H21ClN2O2S	352.1012	353.1085	351.0939	ESI+	11
410	Hydramethylnon	67485-29-4	C25H24F6N4	494.1905	495.1978	493.1832	ESI+	12
411	Hymexazol	10004-44-1	C4H5NO2	99.0320	100.0393	98.0247	ESI+	3
412	Imazalil	35554-44-0	C14H14Cl2N2O	296.0483	297.0556	295.0410	ESI+	12
413	Imazamethabenz-methyl	81405-85-8	C16H20N2O3	288.1474	289.1547	287.1401	ESI+	12
414	Imazamox	114311-32-9	C15H19N3O4	305.1376	306.1449	304.1303	ESI+	10
415	Imazapic	104098-48-8	C14H17N3O3	275.1270	276.1343	274.1197	ESI+	11
416	Imazapyr	81334-34-1	C13H15N3O3	261.1113	262.1186	260.1040	ESI+	11
417	Imazaquin	81335-37-7	C17H17N3O3	311.1270	312.1343	310.1197	ESI+	6
418	Imazethapyr	81335-77-5	C15H19N3O3	289.1426	290.1499	288.1353	ESI+	12
419	Imazosulfuron	122548-33-8	C14H13ClN6O5S	412.0357	413.0430	411.0284	ESI+	13
420	Imibenconazole	86598-92-7	C17H13Cl3N4S	409.9927	411.0000	408.9854	ESI+	22
421	Imidacloprid	138261-41-3	C9H10ClN5O2	255.0523	256.0596	254.0450	ESI+	8
422	Indanofan	133220-30-1	C20H17ClO3	340.0866	341.0939	339.0793	ESI+	6
423	Indoxacarb	173584-44-6	C22H17ClF3N3O7	527.0707	528.0780	526.0634	ESI+	12
424	Iodosulfuron-methyl	144550-36-7	C14H14IN5O6S	506.9710	507.9783	505.9637	ESI+	8
425	Ioxynil	1689-83-4	C7H3I2NO	370.8304	371.8377	369.8231	ESI-	4
426	Ipconazole	125225-28-7	C18H24ClN3O	333.1608	334.1681	332.1535	ESI+	5
427	Iprobenfos	26087-47-8	C13H21O3PS	288.0949	289.1022	287.0876	ESI+	3
428	Iprodione	36734-19-7	C13H13Cl2N3O3	329.0334	330.0407	328.0261	ESI+	4
429	Iprovalicarb	140923-17-7	C18H28N2O3	320.2100	321.2173	319.2027	ESI+	6
430	Irgarol 1051	28159-98-0	C11H19N5S	253.1361	254.1434	252.1288	ESI+	6
431	Isazofos	42509-80-8	C9H17ClN3O3PS	313.0417	314.0490	312.0344	ESI+	12
432	Isocarbamid	30979-48-7	C8H15N3O2	185.1164	186.1237	184.1091	ESI+	6
433	Isocarbofos	24353-61-5	C11H16NO4PS	289.0538	290.0611	288.0465	ESI+	6
434	Isofenphos	25311-71-1	C15H24NO4PS	345.1164	346.1237	344.1091	ESI+	6
435	Isofenphos-methyl	99675-03-3	C14H22NO4PS	331.1007	332.1080	330.0934	ESI+	6
436	Isofenphos-oxon	31120-85-1	C15H24NO5P	329.1392	330.1465	328.1319	ESI+	3
437	Isomethiozin	57052-04-7	C12H20N4OS	268.1358	269.1431	267.1285	ESI+	6
438	Isonoruron	28805-78-9	C13H22N2O	222.1732	223.1805	221.1659	ESI+	6
439	Isoprocarb	2631-40-5	C11H15NO2	193.1103	194.1176	192.1030	ESI+	3
440	Isopropalin	33820-53-0	C15H23N3O4	309.1689	310.1762	308.1616	ESI+	6
441	Isoprothiolane	50512-35-1	C12H18O4S2	290.0647	291.0720	289.0574	ESI+	6
442	Isoproturon	34123-59-6	C12H18N2O	206.1419	207.1492	205.1346	ESI+	6
443	Isopyrazam	881685-58-1	C20H23F2N3O	359.1809	360.1882	358.1736	ESI+	9
444	Isoxaben	82558-50-7	C18H24N2O4	332.1736	333.1809	331.1663	ESI+	6
445	Isoxadifen-ethyl	163520-33-0	C18H17NO3	295.1208	296.1281	294.1135	ESI+	12
446	Isoxaflutole	141112-29-0	C15H12F3NO4S	359.0439	360.0512	358.0366	ESI+	5
447	Isoxathion	18854-01-8	C13H16NO4PS	313.0538	314.0611	312.0465	ESI+	6
448	Ivermectine	70288-86-7	C48H74O14	874.5079	875.5152	873.5006	ESI+	6
449	Karbutilate	4849-32-5	C14H21N3O3	279.1583	280.1656	278.1510	ESI+	16
450	Kasugamycin	6980-18-3	C14H25N3O9	379.1591	380.1664	378.1518	ESI+	3

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
451	Kresoxim-methyl	143390-89-0	C18H19NO4	313.1314	314.1387	312.1241	ESI+	6
452	Lactofen	77501-63-4	C19H15ClF3NO7	461.0489	462.0562	460.0416	ESI+	12
453	Lambda-Cyhalothrin	91465-08-6	C23H19ClF3NO3	449.1006	450.1079	448.0933	ESI+	4
454	Lenacil	2164-08-1	C13H18N2O2	234.1368	235.1441	233.1295	ESI+	6
455	Linuron	330-55-2	C9H10Cl2N2O2	248.0119	249.0192	247.0046	ESI+	12
456	Lufenuron	103055-07-8	C17H8Cl2F8N2O3	509.9784	510.9857	508.9711	ESI-	10
457	Malaoxon	1634-78-2	C10H19O7PS	314.0589	315.0662	313.0516	ESI+	6
458	Malathion	121-75-5	C10H19O6PS2	330.0361	331.0434	329.0288	ESI+	12
459	Maleic-hydrazide	123-33-1	C4H4N2O2	112.0273	113.0346	111.0200	ESI+	3
460	Mandipropamid	374726-62-2	C23H22ClNO4	411.1237	412.1310	410.1164	ESI+	12
461	MCPA (MCP)	94-74-6	C9H9ClO3	200.0240	201.0313	199.0167	ESI-	3
462	MCPA-butoxyethyl ester	19480-43-4	C15H21ClO4	300.1128	301.1201	299.1055	ESI+	12
463	MCPB	94-81-5	C11H13ClO3	228.0553	229.0626	227.0480	ESI-	3
464	Mecarbam	2595-54-2	C10H20NO5PS2	329.0521	330.0594	328.0448	ESI+	6
465	Mecoprop (MCPP)	93-65-2	C10H11ClO3	214.0397	215.0470	213.0324	ESI-	2
466	Mecoprop-P	16484-77-8	C10H11ClO3	214.0397	215.0470	213.0324	ESI-	4
467	Mefenacet	73250-68-7	C16H14N2O2S	298.0776	299.0849	297.0703	ESI+	6
468	Mefenpyr-diethyl	135590-91-9	C16H18Cl2N2O4	372.0644	373.0717	371.0571	ESI+	24
469	Mefluidide	53780-34-0	C11H13F3N2O3S	310.0599	311.0672	309.0526	ESI+	10
470	Mepanipyrim	110235-47-7	C14H13N3	223.1109	224.1182	222.1036	ESI+	6
471	Mephosfolan	950-10-7	C8H16NO3PS2	269.0309	270.0382	268.0236	ESI+	6
472	Mepiquat	24307-26-4	C7H16N	114.1283	115.1356	113.1210	ESI+	6
473	Mepronil	55814-41-0	C17H19NO2	269.1416	270.1489	268.1343	ESI+	5
474	Meptyldinocap	6119-92-2	C18H24N2O6	364.1634	365.1707	363.1561	ESI-	6
475	Mesosulfuron-methyl	208465-21-8	C17H21N5O9S2	503.0781	504.0854	502.0708	ESI+	6
476	Mesotrione	104206-82-8	C14H13NO7S	339.0413	340.0486	338.0340	ESI+	6
477	Metaflumizone	139968-49-3	C24H16F6N4O2	506.1177	507.1250	505.1104	ESI+	6
478	Metalaxyl	57837-19-1	C15H21NO4	279.1471	280.1544	278.1398	ESI+	6
479	Metalaxyl-M	70630-17-0	C15H21NO4	279.1471	280.1544	278.1398	ESI+	6
480	Metamitron	41394-05-2	C10H10N4O	202.0855	203.0928	201.0782	ESI+	3
481	Metazachlor	67129-08-2	C14H16ClN3O	277.0982	278.1055	276.0909	ESI+	6
482	Metconazole	125116-23-6	C17H22ClN3O	319.1451	320.1524	318.1378	ESI+	4
483	Methabenzthiazuron	18691-97-9	C10H11N3OS	221.0623	222.0696	220.0550	ESI+	6
484	Methacrifos	62610-77-9	C7H13O5PS	240.0221	241.0294	239.0148	ESI+	12
485	Methamidophos	10265-92-6	C2H8NO2PS	141.0013	142.0086	139.9940	ESI+	6
486	Methfuroxam	28730-17-8	C14H15NO2	229.1103	230.1176	228.1030	ESI+	4
487	Methidathion	950-37-8	C6H11N2O4PS3	301.9619	302.9692	300.9546	ESI+	7
488	Methiocarb	2032-65-7	C11H15NO2S	225.0823	226.0896	224.0750	ESI+	6
489	Methiocarb-sulfone	2179-25-1	C11H15NO4S	257.0722	258.0795	256.0649	ESI+	9
490	Methiocarb-sulfoxide	2635-10-1	C11H15NO3S	241.0773	242.0846	240.0700	ESI+	6
491	Methomyl	16752-77-5	C5H10N2O2S	162.0463	163.0536	161.0390	ESI+	6
492	Methoprene	40596-69-8	C19H34O3	310.2508	311.2581	309.2435	ESI+	12
493	Methoprotryne	841-06-5	C11H21N5OS	271.1467	272.1540	270.1394	ESI+	6
494	Methoxyfenozide	161050-58-4	C22H28N2O3	368.2100	369.2173	367.2027	ESI+	6
495	Metobromuron	3060-89-7	C9H11BrN2O2	258.0004	259.0077	256.9931	ESI+	12
496	Metolachlor	51218-45-2	C15H22ClNO2	283.1339	284.1412	282.1266	ESI+	12
497	Metolcarb	1129-41-5	C9H11NO2	165.0790	166.0863	164.0717	ESI+	6
498	Metominostrobin	133408-50-1	C16H16N2O3	284.1161	285.1234	283.1088	ESI+	6
499	Metosulam	139528-85-1	C14H13Cl2N5O4S	417.0065	418.0138	415.9992	ESI+	24
500	Metoxuron	19937-59-8	C10H13ClN2O2	228.0666	229.0739	227.0593	ESI+	5
501	Metrafenone	220899-03-6	C19H21BrO5	408.0572	409.0645	407.0499	ESI+	12
502	Metribuzin	21087-64-9	C8H14N4OS	214.0888	215.0961	213.0815	ESI+	5
503	Metsulfuron-methyl	74223-64-6	C14H15N5O6S	381.0743	382.0816	380.0670	ESI+	6
504	Mevinphos	7786-34-7	C7H13O6P	224.0450	225.0523	223.0377	ESI+	5
505	Mexacarbate	315-18-4	C12H18N2O2	222.1368	223.1441	221.1295	ESI+	6
506	Molinate	2212-67-1	C9H17NOS	187.1031	188.1104	186.0958	ESI+	6
507	Monalide	7287-36-7	C13H18ClNO	239.1077	240.1150	238.1004	ESI+	20
508	Monocrotophos	6923-22-4	C7H14NO5P	223.0610	224.0683	222.0537	ESI+	12
509	Monolinuron	1746-81-2	C9H11ClN2O2	214.0509	215.0582	213.0436	ESI+	10
510	Monuron	150-68-5	C9H11ClN2O	198.0560	199.0633	197.0487	ESI+	10
511	Morpholine	110-91-8	C4H9NO	87.0684	88.0757	86.0611	ESI+	6
512	Moxidectin	113507-06-5	C37H53NO8	639.3771	640.3844	638.3698	ESI+	12
513	Myclobutanil	88671-89-0	C15H17ClN4	288.1142	289.1215	287.1069	ESI+	8
514	*N*-(2, 4-Dimethylphenyl) formamide	60397-77-5	C9H11NO	149.0841	150.0914	148.0768	ESI+	6
515	*N*-(2, 4-Dimethylphenyl) -*N*'-methylformamidine	33089-74-6	C10H14N2	162.1157	163.1230	161.1084	ESI+	6
516	*N*, *N*'-Diphenylurea	102-07-8	C13H12N2O	212.0950	213.1023	211.0877	ESI+	4
517	Naled	300-76-5	C4H7Br2Cl2O4P	377.7826	378.7899	376.7753	ESI+	6
518	Naproanilide	52570-16-8	C19H17NO2	291.1259	292.1332	290.1186	ESI+	2
519	Napropamide	15299-99-7	C17H21NO2	271.1572	272.1645	270.1499	ESI+	6
520	Naptalam	132-66-1	C18H13NO3	291.0895	292.0968	290.0822	ESI+	6
521	Neburon	555-37-3	C12H16Cl2N2O	274.0640	275.0713	273.0567	ESI+	9
522	Nicarbazin	330-95-0	C19H18N6O6	426.1288	427.1361	425.1215	ESI-	3
523	Nicosulfuron	111991-09-4	C15H18N6O6S	410.1009	411.1082	409.0936	ESI+	6
524	Nicotine	54-11-5	C10H14N2	162.1157	163.1230	161.1084	ESI+	6
525	Nitenpyram	150824-47-8	C11H15ClN4O2	270.0884	271.0957	269.0811	ESI+	6

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
526	Nitralin	4726-14-1	C13H19N3O6S	345.0995	346.1068	344.0922	ESI+	12
527	Nitrothal-isopropyl	10552-74-6	C14H17NO6	295.1056	296.1129	294.0983	ESI+	6
528	Norflurazon	27314-13-2	C12H9ClF3N3O	303.0386	304.0459	302.0313	ESI+	16
529	Norflurazon-desmethyl	23576-24-1	C11H7ClF3N3O	289.0230	290.0303	288.0157	ESI+	12
530	Novaluron	116714-46-6	C17H9ClF8N2O4	492.0123	493.0196	491.0050	ESI+	18
531	Noviflumuron	121451-02-3	C17H7Cl2F9N2O3	527.9690	528.9763	526.9617	ESI-	6
532	Nuarimol	63284-71-9	C17H12ClFN2O	314.0622	315.0695	313.0549	ESI+	11
533	Ofurace	58810-48-3	C14H16ClNO3	281.0819	282.0892	280.0746	ESI+	17
534	Omethoate	1113-02-6	C5H12NO4PS	213.0225	214.0298	212.0152	ESI+	4
535	Orbencarb	34622-58-7	C12H16ClNOS	257.0641	258.0714	256.0568	ESI+	12
536	Orthosulfamuron	213464-77-8	C16H20N6O6S	424.1165	425.1238	423.1092	ESI+	6
537	Oryzalin	19044-88-3	C12H18N4O6S	346.0947	347.1020	345.0874	ESI+	5
538	Oxabetrinil	94593-79-0	C12H12N2O3	232.0848	233.0921	231.0775	ESI+	2
539	Oxadiargyl	39807-15-3	C15H14Cl2N2O3	340.0381	341.0454	339.0308	ESI+	14
540	Oxadiazon	19666-30-9	C15H18Cl2N2O3	344.0694	345.0767	343.0621	ESI+	6
541	Oxadixyl	77732-09-3	C14H18N2O4	278.1267	279.1340	277.1194	ESI+	12
542	Oxamyl	23135-22-0	C7H13N3O3S	219.0678	220.0751	218.0605	ESI+	3
543	Oxasulfuron	144651-06-9	C17H18N4O6S	406.0947	407.1020	405.0874	ESI+	6
544	Oxaziclomefone	153197-14-9	C20H19Cl2NO2	375.0793	376.0866	374.0720	ESI+	2
545	Oxycarboxin	5259-88-1	C12H13NO4S	267.0565	268.0638	266.0492	ESI+	3
546	Oxydemeton-methyl	301-12-2	C6H15O4PS2	246.0149	247.0222	245.0076	ESI+	6
547	Paclobutrazol	76738-62-0	C15H20ClN3O	293.1295	294.1368	292.1222	ESI+	8
548	Paraoxon-ethyl	311-45-5	C10H14NO6P	275.0559	276.0632	274.0486	ESI+	6
549	Paraoxon-methyl	950-35-6	C8H10NO6P	247.0246	248.0319	246.0173	ESI+	3
550	Paraquat	1910-42-5	C12H14Cl2N2	256.0534	257.0607	255.0461	ESI+	5
551	Parathion	56-38-2	C10H14NO5PS	291.0330	292.0403	290.0257	ESI+	3
552	Pebulate	1114-71-2	C10H21NOS	203.1344	204.1417	202.1271	ESI+	6
553	Penconazole	66246-88-6	C13H15Cl2N3	283.0643	284.0716	282.0570	ESI+	12
554	Pencycuron	66063-05-6	C19H21ClN2O	328.1342	329.1415	327.1269	ESI+	10
555	Pendimethalin	40487-42-1	C13H19N3O4	281.1376	282.1449	280.1303	ESI+	6
556	Penoxsulam	219714-96-2	C16H14F5N5O5S	483.0636	484.0709	482.0563	ESI+	6
557	Pentachlorophenol	87-86-5	C6HCl5O	263.8470	264.8543	262.8397	ESI-	3
558	Pentoxazone	110956-75-7	C17H17ClFNO4	353.0830	354.0903	352.0757	ESI+	2
559	Permethrin	52645-53-1	C21H20Cl2O3	390.0790	391.0863	389.0717	ESI+	12
560	Pethoxamid	106700-29-2	C16H22ClNO2	295.1339	296.1412	294.1266	ESI+	7
561	Phenmedipham	13684-63-4	C16H16N2O4	300.1110	301.1183	299.1037	ESI+	6
562	Phenothrin	26002-80-2	C23H26O3	350.1882	351.1955	349.1809	ESI+	9
563	Phenthoate	2597-03-7	C12H17O4PS2	320.0306	321.0379	319.0233	ESI+	12
564	Phorate	298-02-2	C7H17O2PS3	260.0128	261.0201	259.0055	ESI+	6
565	Phorate-oxon	2600-69-3	C7H17O3PS2	244.0357	245.0430	243.0284	ESI+	6
566	Phorate-sulfone	2588-04-7	C7H17O4PS3	292.0027	293.0100	290.9954	ESI+	6
567	Phorate-sulfoxide	2588-03-6	C7H17O3PS3	276.0077	277.0150	275.0004	ESI+	6
568	Phosalone	2310-17-0	C12H15ClNO4PS2	366.9869	367.9942	365.9796	ESI+	12
569	Phosfolan	947-02-4	C7H14NO3PS2	255.0153	256.0226	254.0080	ESI+	6
570	Phosmet	732-11-6	C11H12NO4PS2	316.9945	318.0018	315.9872	ESI+	12
571	Phosphamidon	13171-21-6	C10H19ClNO5P	299.0689	300.0762	298.0616	ESI+	12
572	Phoxim	14816-18-3	C12H15N2O3PS	298.0541	299.0614	297.0468	ESI+	6
573	Picloram	1918-02-1	C6H3Cl3N2O2	239.9260	240.9333	238.9187	ESI+	9
574	Picolinafen	137641-05-5	C19H12F4N2O2	376.0835	377.0908	375.0762	ESI+	6
575	Picoxystrobin	117428-22-5	C18H16F3NO4	367.1031	368.1104	366.0958	ESI+	6
576	Pinoxaden	243973-20-8	C23H32N2O4	400.2362	401.2435	399.2289	ESI+	6
577	Piperonyl-butoxide	51-03-6	C19H30O5	338.2093	339.2166	337.2020	ESI+	12
578	Piperophos	24151-93-7	C14H28NO3PS2	353.1248	354.1321	352.1175	ESI+	6
579	Pirimicarb	23103-98-2	C11H18N4O2	238.1430	239.1503	237.1357	ESI+	3
580	Pirimicarb-desmethyl	30614-22-3	C10H16N4O2	224.1273	225.1346	223.1200	ESI+	6
581	Pirimicarb-desmethyl-formamido	27218-04-8	C11H16N4O3	252.1222	253.1295	251.1149	ESI+	2
582	Pirimiphos-ethyl	23505-41-1	C13H24N3O3PS	333.1276	334.1349	332.1203	ESI+	6
583	Pirimiphos-methyl	29232-93-7	C11H20N3O3PS	305.0963	306.1036	304.0890	ESI+	6
584	Prallethrin	23031-36-9	C19H24O3	300.1725	301.1798	299.1652	ESI+	6
585	Pretilachlor	51218-49-6	C17H26ClNO2	311.1652	312.1725	310.1579	ESI+	6
586	Primisulfuron-methyl	86209-51-0	C15H12F4N4O7S	468.0363	469.0436	467.0290	ESI+	9
587	Probenazole	27605-76-1	C10H9NO3S	223.0303	224.0376	222.0230	ESI+	4
588	Prochloraz	67747-09-5	C15H16Cl3N3O2	375.0308	376.0381	374.0235	ESI+	15
589	Profenofos	41198-08-7	C11H15BrClO3PS	371.9351	372.9424	370.9278	ESI+	12
590	Profoxydim	139001-49-3	C24H32ClNO4S	465.1741	466.1814	464.1668	ESI+	24
591	Promecarb	2631-37-0	C12H17NO2	207.1259	208.1332	206.1186	ESI+	6
592	Prometon	1610-18-0	C10H19N5O	225.1590	226.1663	224.1517	ESI+	6
593	Prometryn	7287-19-6	C10H19N5S	241.1361	242.1434	240.1288	ESI+	6
594	Propachlor	1918-16-7	C11H14ClNO	211.0764	212.0837	210.0691	ESI+	6
595	Propamocarb	24579-73-5	C9H20N2O2	188.1525	189.1598	187.1452	ESI+	6
596	Propanil	709-98-8	C9H9Cl2NO	217.0061	218.0134	215.9988	ESI+	9
597	Propaphos	7292-16-2	C13H21O4PS	304.0898	305.0971	303.0825	ESI+	10
598	Propaquizafop	111479-05-1	C22H22ClN3O5	443.1248	444.1321	442.1175	ESI+	12
599	Propargite	2312-35-8	C19H26O4S	350.1552	351.1625	349.1479	ESI+	6
600	Propazine	139-40-2	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	6

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
601	Propetamphos	31218-83-4	C10H20NO4PS	281.0851	282.0924	280.0778	ESI+	18
602	Propham	122-42-9	C10H13NO2	179.0946	180.1019	178.0873	ESI+	6
603	Propiconazole	60207-90-1	C15H17Cl2N3O2	341.0698	342.0771	340.0625	ESI+	12
604	Propisochlor	86763-47-5	C15H22ClNO2	283.1339	284.1412	282.1266	ESI+	12
605	Propoxur	114-26-1	C11H15NO3	209.1052	210.1125	208.0979	ESI+	6
606	Propoxycarbazone	181274-15-7	C15H18N4O7S	398.0896	399.0969	397.0823	ESI+	20
607	Propylene-thiourea	2122-19-2	C4H8N2S	116.0408	117.0481	115.0335	ESI+	6
608	Propyzamide	23950-58-5	C12H11Cl2NO	255.0218	256.0291	254.0145	ESI+	10
609	Proquinazid	189278-12-4	C14H17IN2O2	372.0335	373.0408	371.0262	ESI+	6
610	Prosulfocarb	52888-80-9	C14H21NOS	251.1344	252.1417	250.1271	ESI+	4
611	Prosulfuron	94125-34-5	C15H16F3N5O4S	419.0875	420.0948	418.0802	ESI+	7
612	Prothioconazole	178928-70-6	C14H15Cl2N3OS	343.0313	344.0386	342.0240	ESI+	10
613	Prothioconazole-desthio	120983-64-4	C14H15Cl2N3O	311.0592	312.0665	310.0519	ESI+	10
614	Prothiophos	34643-46-4	C11H15Cl2O2PS2	343.9628	344.9701	342.9555	ESI+	12
615	Prothoate	2275-18-5	C9H20NO3PS2	285.0622	286.0695	284.0549	ESI+	6
616	Pymetrozine	123312-89-0	C10H11N5O	217.0964	218.1037	216.0891	ESI+	4
617	Pyracarbolid	24691-76-7	C13H15NO2	217.1103	218.1176	216.1030	ESI+	3
618	Pyraclofos	89784-60-1	C14H18ClN2O3PS	360.0464	361.0537	359.0391	ESI+	12
619	Pyraclostrobin	175013-18-0	C19H18ClN3O4	387.0986	388.1059	386.0913	ESI+	11
620	Pyraflufen-ethyl	129630-19-9	C15H13Cl2F3N2O4	412.0204	413.0277	411.0131	ESI+	12
621	Pyrasulfotole	365400-11-9	C14H13F3N2O4S	362.0548	363.0621	361.0475	ESI+	9
622	Pyrazolynate	58011-68-0	C19H16Cl2N2O4S	438.0208	439.0281	437.0135	ESI+	2
623	Pyrazophos	13457-18-6	C14H20N3O5PS	373.0861	374.0934	372.0788	ESI+	12
624	Pyrazosulfuron-ethyl	93697-74-6	C14H18N6O7S	414.0958	415.1031	413.0885	ESI+	6
625	Pyrazoxyfen	71561-11-0	C20H16Cl2N2O3	402.0538	403.0611	401.0465	ESI+	12
626	Pyributicarb	88678-67-5	C18H22N2O2S	330.1402	331.1475	329.1329	ESI+	6
627	Pyridaben	96489-71-3	C19H25ClN2OS	364.1376	365.1449	363.1303	ESI+	12
628	Pyridalyl	179101-81-6	C18H14Cl4F3NO3	488.9680	489.9753	487.9607	ESI+	18
629	Pyridaphenthion	119-12-0	C14H17N2O4PS	340.0647	341.0720	339.0574	ESI+	6
630	Pyridate	55512-33-9	C19H23ClN2O2S	378.1169	379.1242	377.1096	ESI+	12
631	Pyrifenox	88283-41-4	C14H12Cl2N2O	294.0327	295.0400	293.0254	ESI+	8
632	Pyriftalid	135186-78-6	C15H14N2O4S	318.0674	319.0747	317.0601	ESI+	2
633	Pyrimethanil	53112-28-0	C12H13N3	199.1109	200.1182	198.1036	ESI+	6
634	Pyrimidifen	105779-78-0	C20H28ClN3O2	377.1870	378.1943	376.1797	ESI+	12
635	Pyriminobac-methyl (*E*)	136191-64-5	C17H19N3O6	361.1274	362.1347	360.1201	ESI+	6
636	Pyriproxyfen	95737-68-1	C20H19NO3	321.1365	322.1438	320.1292	ESI+	6
637	Pyroquilon	57369-32-1	C11H11NO	173.0841	174.0914	172.0768	ESI+	6
638	Pyroxsulam	422556-08-9	C14H13F3N6O5S	434.0620	435.0693	433.0547	ESI+	6
639	Quinalphos	13593-03-8	C12H15N2O3PS	298.0541	299.0614	297.0468	ESI+	6
640	Quinclorac	84087-01-4	C10H5Cl2NO2	240.9697	241.9770	239.9624	ESI+	11
641	Quinmerac	90717-03-6	C11H8ClNO2	221.0244	222.0317	220.0171	ESI+	12
642	Quinoclamine	2797-51-5	C10H6ClNO2	207.0087	208.0160	206.0014	ESI+	19
643	Quinoxyfen	124495-18-7	C15H8Cl2FNO	306.9967	308.0040	305.9894	ESI+	12
644	Quizalofop (free acid)	76578-12-6	C17H13ClN2O4	344.0564	345.0637	343.0491	ESI+	24
645	Quizalofop-ethyl	76578-14-8	C19H17ClN2O4	372.0877	373.0950	371.0804	ESI+	12
646	Quizalofop-methyl	76578-13-7	C18H15ClN2O4	358.0720	359.0793	357.0647	ESI+	12
647	Quizalofop-P	94051-08-8	C17H13ClN2O4	344.0564	345.0637	343.0491	ESI+	9
648	Quizalofop-P-ethyl	100646-51-3	C19H17ClN2O4	372.0877	373.0950	371.0804	ESI+	12
649	Rabenzazole	40341-04-6	C12H12N4	212.1062	213.1135	211.0989	ESI+	12
650	Resmethrin	10453-86-8	C22H26O3	338.1882	339.1955	337.1809	ESI+	6
651	Rimsulfuron	122931-48-0	C14H17N5O7S2	431.0569	432.0642	430.0496	ESI+	9
652	Rotenone	83-79-4	C23H22O6	394.1416	395.1489	393.1343	ESI+	6
653	Saflufenacil	372137-35-4	C17H17ClF4N4O5S	500.0544	501.0617	499.0471	ESI+	8
654	Sebuthylazine	7286-69-3	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	6
655	Sebuthylazine-desethyl	37019-18-4	C7H12ClN5	201.0781	202.0854	200.0708	ESI+	12
656	Secbumeton	26259-45-0	C10H19N5O	225.1590	226.1663	224.1517	ESI+	4
657	Sethoxydim	74051-80-2	C17H29NO3S	327.1868	328.1941	326.1795	ESI+	12
658	Siduron	1982-49-6	C14H20N2O	232.1576	233.1649	231.1503	ESI+	5
659	Silafluofen	105024-66-6	C25H29FO2Si	408.1921	409.1994	407.1848	ESI+	2
660	Silthiofam	175217-20-6	C13H21NOSSi	267.1113	268.1186	266.1040	ESI+	5
661	Simazine	122-34-9	C7H12ClN5	201.0781	202.0854	200.0708	ESI+	12
662	Simazine-2-hydroxy	2599-11-3	C7H13N5O	183.1120	184.1193	182.1047	ESI+	5
663	Simeconazole	149508-90-7	C14H20FN3OSi	293.1360	294.1433	292.1287	ESI+	6
664	Simetryn	1014-70-6	C8H15N5S	213.1048	214.1121	212.0975	ESI+	4
665	Spinetoram A	187166-40-1	C42H69NO10	747.4921	748.4994	746.4848	ESI+	2
666	Spinetoram B	187166-15-0	C43H69NO10	759.4921	760.4994	758.4848	ESI+	3
667	Spinosyn A	131929-60-7	C41H65NO10	731.4608	732.4681	730.4535	ESI+	4
668	Spinosyn D	131929-63-0	C42H67NO10	745.4765	746.4838	744.4692	ESI+	4
669	Spirodiclofen	148477-71-8	C21H24Cl2O4	410.1052	411.1125	409.0979	ESI+	11
670	Spiromesifen	283594-90-1	C23H30O4	370.2144	371.2217	369.2071	ESI+	4
671	Spirotetramat	203313-25-1	C21H27NO5	373.1889	374.1962	372.1816	ESI+	6
672	Spiroxamine	118134-30-8	C18H35NO2	297.2668	298.2741	296.2595	ESI+	6
673	Sulcotrione	99105-77-8	C14H13ClO5S	328.0172	329.0245	327.0099	ESI+	2
674	Sulfallate	95-06-7	C8H14ClNS2	223.0256	224.0329	222.0183	ESI+	9
675	Sulfaquinoxaline	59-40-5	C14H12N4O2S	300.0681	301.0754	299.0608	ESI+	6

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
676	Sulfometuron-methyl	74222-97-2	C15H16N4O5S	364.0841	365.0914	363.0768	ESI+	6
677	Sulfosulfuron	141776-32-1	C16H18N6O7S2	470.0678	471.0751	469.0605	ESI+	6
678	Sulfotep	3689-24-5	C8H20O5P2S2	322.0227	323.0300	321.0154	ESI+	6
679	Sulprofos	35400-43-2	C12H19O2PS3	322.0285	323.0358	321.0212	ESI+	6
680	Tau-Fluvalinate	102851-06-9	C26H22ClF3N2O3	502.1271	503.1344	501.1198	ESI+	15
681	Tebuconazole	107534-96-3	C16H22ClN3O	307.1451	308.1524	306.1378	ESI+	10
682	Tebufenozide	112410-23-8	C22H28N2O2	352.2151	353.2224	351.2078	ESI+	6
683	Tebufenpyrad	119168-77-3	C18H24ClN3O	333.1608	334.1681	332.1535	ESI+	12
684	Tebupirimfos	96182-53-5	C13H23N2O3PS	318.1167	319.1240	317.1094	ESI+	6
685	Tebutam	35256-85-0	C15H23NO	233.1780	234.1853	232.1707	ESI+	6
686	Tebuthiuron	34014-18-1	C9H16N4OS	228.1045	229.1118	227.0972	ESI+	6
687	Teflubenzuron	83121-18-0	C14H6Cl2F4N2O2	379.9742	380.9815	378.9669	ESI-	12
688	Tembotrione	335104-84-2	C17H16ClF3O6S	440.0308	441.0381	439.0235	ESI+	12
689	Temephos	3383-96-8	C16H20O6P2S3	465.9897	466.9970	464.9824	ESI+	6
690	Tepraloxydim	149979-41-9	C17H24ClNO4	341.1394	342.1467	340.1321	ESI+	8
691	Terbacil	5902-51-2	C9H13ClN2O2	216.0666	217.0739	215.0593	ESI-	10
692	Terbucarb	1918-11-2	C17H27NO2	277.2042	278.2115	276.1969	ESI+	12
693	Terbufos	13071-79-9	C9H21O2PS3	288.0441	289.0514	287.0368	ESI+	5
694	Terbufos-sulfone	56070-16-7	C9H21O4PS3	320.0340	321.0413	319.0267	ESI+	6
695	Terbufos-sulfoxide	10548-10-4	C9H21O3PS3	304.0390	305.0463	303.0317	ESI+	6
696	Terbumeton	33693-04-8	C10H19N5O	225.1590	226.1663	224.1517	ESI+	6
697	Terbumeton-desethyl	30125-64-5	C8H15N5O	197.1277	198.1350	196.1204	ESI+	3
698	Terbuthylazine	5915-41-3	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	10
699	Terbuthylazine-2-hydroxy	66753-07-9	C9H17N5O	211.1433	212.1506	210.1360	ESI+	6
700	Terbuthylazine-desethyl	30125-63-4	C7H12ClN5	201.0781	202.0854	200.0708	ESI+	12
701	Terbutryn	886-50-0	C10H19N5S	241.1361	242.1434	240.1288	ESI+	6
702	Tetrachlorvinphos (CVMP)	22248-79-9	C10H9Cl4O4P	363.8993	364.9066	362.8920	ESI+	16
703	Tetraconazole	112281-77-3	C13H11Cl2F4N3O	371.0215	372.0288	370.0142	ESI+	7
704	Tetraethylpyrophosphate	107-49-3	C8H20O7P2	290.0684	291.0757	289.0611	ESI+	6
705	Tetramethrin	7696-12-0	C19H25NO4	331.1784	332.1857	330.1711	ESI+	12
706	Thenylchlor	96491-05-3	C16H18ClNO2S	323.0747	324.0820	322.0674	ESI+	12
707	Thiabendazole	148-79-8	C10H7N3S	201.0361	202.0434	200.0288	ESI+	6
708	Thiacloprid	111988-49-9	C10H9ClN4S	252.0236	253.0309	251.0163	ESI+	6
709	Thiamethoxam	153719-23-4	C8H10ClN5O3S	291.0193	292.0266	290.0120	ESI+	12
710	Thiazafluron	25366-23-8	C6H7F3N4OS	240.0293	241.0366	239.0220	ESI+	6
711	Thiazopyr	117718-60-2	C16H17F5N2O2S	396.0931	397.1004	395.0858	ESI+	6
712	Thidiazuron	51707-55-2	C9H8N4OS	220.0419	221.0492	219.0346	ESI+	6
713	Thiencarbazone-methyl	317815-83-1	C12H14N4O7S2	390.0304	391.0377	389.0231	ESI+	3
714	Thifensulfuron-methyl	79277-27-3	C12H13N5O6S2	387.0307	388.0380	386.0234	ESI+	6
715	Thifluzamide	130000-40-7	C13H6Br2F6N2O2S	525.8421	526.8494	524.8348	ESI+	29
716	Thiobencarb	28249-77-6	C12H16ClNOS	257.0641	258.0714	256.0568	ESI+	11
717	Thiodicarb	59669-26-0	C10H18N4O4S3	354.0490	355.0563	353.0417	ESI+	6
718	Thiofanox	39196-18-4	C9H18N2O2S	218.1089	219.1162	217.1016	ESI+	2
719	Thiofanox-sulfone	39184-59-3	C9H18N2O4S	250.0987	251.1060	249.0914	ESI+	9
720	Thiofanox-sulfoxide	39184-27-5	C9H18N2O3S	234.1038	235.1111	233.0965	ESI+	12
721	Thiometon	640-15-3	C6H15O2PS3	245.9972	247.0045	244.9899	ESI+	2
722	Thionazin	297-97-2	C8H13N2O3PS	248.0384	249.0457	247.0311	ESI+	6
723	Thiophanate-ethyl	23564-06-9	C14H18N4O4S2	370.0769	371.0842	369.0696	ESI+	6
724	Thiophanate-methyl	23564-05-8	C12H14N4O4S2	342.0456	343.0529	341.0383	ESI+	6
725	Thiram	137-26-8	C6H12N2S4	239.9883	240.9956	238.9810	ESI+	6
726	Tolclofos-methyl	57018-04-9	C9H11Cl2O3PS	299.9544	300.9617	298.9471	ESI+	12
727	Tolylfluanid	731-27-1	C10H13Cl2FN2O2S2	345.9780	346.9853	344.9707	ESI+	22
728	Topramezone	210631-68-8	C16H17N3O5S	363.0889	364.0962	362.0816	ESI+	12
729	Tralkoxydim	87820-88-0	C20H27NO3	329.1991	330.2064	328.1918	ESI+	6
730	Tralomethrin	66841-25-6	C22H19Br4NO3	660.8098	661.8171	659.8025	ESI+	7
731	Triadimefon	43121-43-3	C14H16ClN3O2	293.0931	294.1004	292.0858	ESI+	12
732	Triadimenol	55219-65-3	C14H18ClN3O2	295.1088	296.1161	294.1015	ESI+	7
733	Tri-allate	2303-17-5	C10H16Cl3NOS	303.0018	304.0091	301.9945	ESI+	16
734	Triapenthenol	76608-88-3	C15H25N3O	263.1998	264.2071	262.1925	ESI+	12
735	Triasulfuron	82097-50-5	C14H16ClN5O5S	401.0561	402.0634	400.0488	ESI+	12
736	Triazamate	112143-82-5	C13H22N4O3S	314.1413	315.1486	313.1340	ESI+	4
737	Triazophos	24017-47-8	C12H16N3O3PS	313.0650	314.0723	312.0577	ESI+	6
738	Triazoxide	72459-58-6	C10H6ClN5O	247.0261	248.0334	246.0188	ESI+	11
739	Tribenuron-methyl	101200-48-0	C15H17N5O6S	395.0900	396.0973	394.0827	ESI+	5
740	Trichlorfon	52-68-6	C4H8Cl3O4P	255.9226	256.9299	254.9153	ESI+	10
741	Triclopyr	55335-06-3	C7H4Cl3NO3	254.9257	255.9330	253.9184	ESI-	2
742	Tricyclazole	41814-78-2	C9H7N3S	189.0361	190.0434	188.0288	ESI+	6
743	Tridemorph	81412-43-3	C19H39NO	297.3032	298.3105	296.2959	ESI+	6
744	Trietazine	1912-26-1	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	6
745	Triethanolamine	102-71-6	C6H15NO3	149.1052	150.1125	148.0979	ESI+	6
746	Trifloxystrobin	141517-21-7	C20H19F3N2O4	408.1297	409.1370	407.1224	ESI+	6
747	Trifloxysulfuron	145099-21-4	C14H14F3N5O6S	437.0617	438.0690	436.0544	ESI+	9
748	Triflumizole	68694-11-1	C15H15ClF3N3O	345.0856	346.0929	344.0783	ESI+	9
749	Triflumizole Metabolite	131549-75-2	C12H14ClF3N2O	294.0747	295.0820	293.0674	ESI+	2
750	Triflumuron	64628-44-0	C15H10ClF3N2O3	358.0332	359.0405	357.0259	ESI+	8

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	Compound	CAS	Formula	M	[M+H]+	[M-H]-	Ionisation Mode	MRM Transitions
751	Triflusulfuron-methyl	126535-15-7	C17H19F3N6O6S	492.1039	493.1112	491.0966	ESI+	8
752	Triforine	26644-46-2	C10H14Cl6N4O2	431.9248	432.9321	430.9175	ESI+	7
753	Trinexapac-ethyl	95266-40-3	C13H16O5	252.0998	253.1071	251.0925	ESI+	6
754	Triphenyl phosphate	115-86-6	C18H15O4P	326.0708	327.0781	325.0635	ESI+	6
755	Tris (2-chloro-1-(chloromethyl)ethyl) phosphate	13674-87-8	C9H15Cl6O4P	427.8839	428.8912	426.8766	ESI+	26
756	Triticonazole	131983-72-7	C17H20ClN3O	317.1295	318.1368	316.1222	ESI+	9
757	Tritosulfuron	142469-14-5	C13H9F6N5O4S	445.0279	446.0352	444.0206	ESI+	4
758	Valifenalate	283159-90-0	C19H27ClN2O5	398.1608	399.1681	397.1535	ESI+	16
759	Vamidothion	2275-23-2	C8H18NO4PS2	287.0415	288.0488	286.0342	ESI+	6
760	Vamidothion-sulfone	70898-34-9	C8H18NO6PS2	319.0313	320.0386	318.0240	ESI+	6
761	Vamidothion-sulfoxide	20300-00-9	C8H18NO5PS2	303.0364	304.0437	302.0291	ESI+	6
762	Vernolate	1929-77-7	C10H21NOS	203.1344	204.1417	202.1271	ESI+	5
763	Warfarin	81-81-2	C19H16O4	308.1049	309.1122	307.0976	ESI+	6
764	XMC (3, 5-xylyl methylcarbamate)	2655-14-3	C10H13NO2	179.0946	180.1019	178.0873	ESI+	12
765	Ziram	137-30-4	C6H12N2S4Zn	303.9175	304.9248	302.9102	ESI+	2
766	Zoxamide	156052-68-5	C14H16Cl3NO2	335.0247	336.0320	334.0174	ESI+	18

Further Information

Application News No.C136 describes the analysis of 646 pesticides in a single multi-residue method built using the Shimadzu Pesticide Library.

Scope and Legal Disclaimers

Whilst every effort has been made to ensure the accuracy of the Library, the method will need to be verified in a laboratory as conditions may differ marginally. The influence of sample matrices, extraction protocols, LC behaviour and technical experience may affect the performance of the LC/MS/MS analysis.

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Application News

No.C136

Liquid Chromatography Mass Spectrometry

Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060

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Abstract

With an increasing global population, food security is increasingly under threat and there is a growing challenge for agriculture to produce more food, safely and more sustainably. The use of herbicides, insecticides, and fungicides reduce crop losses both before and after harvest, and increase crop yields. However, pesticide residues resulting from the use of plant protection products on crops may pose a risk to human health and require a legislative framework to monitor pesticide residues in food.

National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRL's) or tolerance information (EPA) for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL enforcement, which therefore requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This application note describes the expanded capability of the LCMS-8060 to help accelerate method development workflows and support increased pesticide monitoring programs. Using the Shimadzu Pesticide MRM Library (the Library includes information on 766 certified reference materials) a single multi-residue LC/MS/MS method was developed for 646 pesticides (3 MRM transitions for over 99 % targeted pesticides resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

Keywords: Pesticides; food safety; LCMS-8060; Pesticide MRM Library, 776 compound library

Introduction

There are more than 1,000 pesticides used globally on soil and crops. With the ever increasing international trade of the food industry, regulatory bodies around the world have increased the number of regulated pesticides and the maximum residue levels (MRLs) allowed in food commodities. In the EU, regulation 396/2005/EC and its annexes set MRLs for over 500 pesticides in 370 food products.[1)] In the US, tolerances for more than 450 pesticides and other ingredients are established by the US EPA[2)] and Japan's positive list system for agricultural chemical residues in foods contains MRLs for over 400 pesticides in various commodities.[3)]

National pesticide monitoring programs create new challenges for food safety laboratories as the number of pesticides required for analysis is increasing together with an expanded range of food products.

In this application paper we present the development of a LC-MS/MS method for screening and quantifying over 646 pesticides in a single method. The method was quickly and efficiently set up using the Shimadzu Pesticide MRM Library. For each target pesticide analysis, up to 3 MRMs (Multiple Reaction Monitoring) transitions were imported from the library. 3 MRMs transitions provided additional data confidence in reporting results in comparison to the conventional 2 transitions used in most methods. As the LCMS-8060 has a high data acquisition speed 1,919 transitions were acquired using a polarity switching speed of 5 msec over a 10.5 minutes gradient elution.

To evaluate the method QuEChERS extracts of mint, tomato and apple were provided by a commercial laboratory as raw acetonitrile extracts and spiked with 646 pesticides (data is presented on the mint extract as it is the more complex sample matrix). The method was evaluated in matrix to ensure that the reporting limits were in agreement with recognised MRL's.

Experiment

Food extracts of mint, tomato and apple were supplied by Phytocontrol, France, following established QuEChERS protocols. Final extracts were prepared in acetonitrile without any dilution. Certified reference materials for the Shimadzu Pesticide MRM Library were obtained from ACSD, France as stock solutions. All solvents were of LCMS quality purchased from Sigma-Aldrich.

A six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/µL) were generated using internal standard method. Two internal standards (Atrazine-d5 and Diuron-d6) were spiked in during the auto-sampler sequence for quantitation.

The robustness of the LCMS-8060 was assessed by peak area response for 646 pesticides spiked into mint, tomato and apple matrix extracts at 0.05 mg/kg.

LC/MS/MS method development

The Shimadzu Pesticide MRM Library has 766 pesticides in its database (Application News No. C135). For each pesticide several MRM's are included in the database and in this analysis the default value used was 3 MRM's. For this method, 1,919 transitions were selected in both positive and negative ionisation mode using a switching time of 5 msec (1,819 MRM transitions were in positive mode and 100 MRM transitions in negative mode).

To optimize ion source conditions (for example, DL temperature, interface temperature, heating block temperature, heating gas flow, drying gas flow and nebulizer gas flow) the interface setting software was used. This tool provides an optimized response for all compounds.

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Table 1 LC and MS/MS Acquisition Parameters

Liquid chromatography	
UHPLC	Nexera LC system
Analytical column	Restek Raptor Biphenyl (2.1 mm I.D. × 100 mm L., 2.7 µm)
Column temperature	35 °C
Flow rate	0.4 mL/min
Solvent A	2 mmol/L ammonium formate + 0.002 % formic acid - Water
Solvent B	2 mmol/L ammonium formate + 0.002 % formic acid - Methanol
Binary Gradient B.Conc.	3 % (0 min) - 10 % (1.00 min) - 55 % (3.00 min) - 100 % (10.50 - 12.00 min) - 3 % (12.01 - 15.00 min)
Injection volume	2 µL sample (plus 40 µL water)

Mass spectrometry	
LC/MS/MS	LCMS-8060
Ionisation mode	Heated electrospray
Polarity switching time	5 msec
Pause time	1 msec
Total MRM transitions	1,919 (1,819 positive; 100 negative)
MRM Dwell	4 msec (target ion); 1 msec (reference ion)
Interface temperature	350 °C
Heating block	300 °C
Desolvation line	150 °C
Heating gas	10 L/min
Drying gas	10 L/min
Nebulizer gas	3 L/min

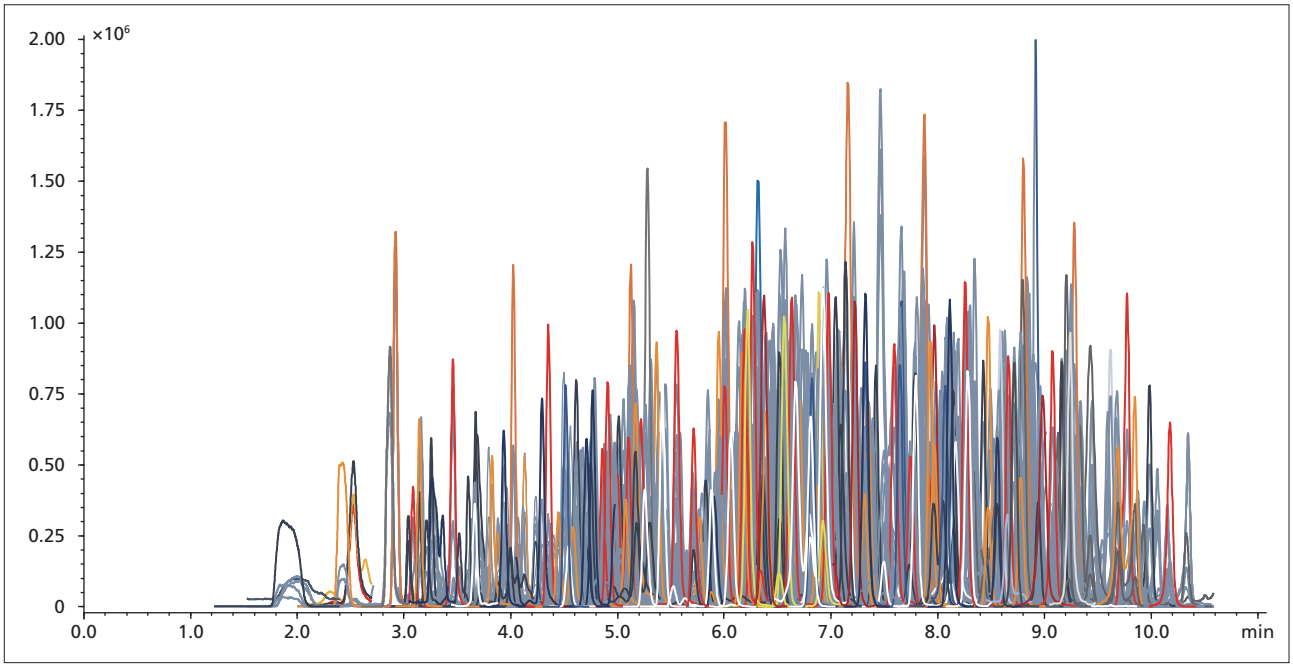

Fig. 1 MRM chromatograms of 646 pesticides spiked into a mint extract at 0.01 mg/kg (Up to 3 MRMs per compound and 5 msec polarity switching time).

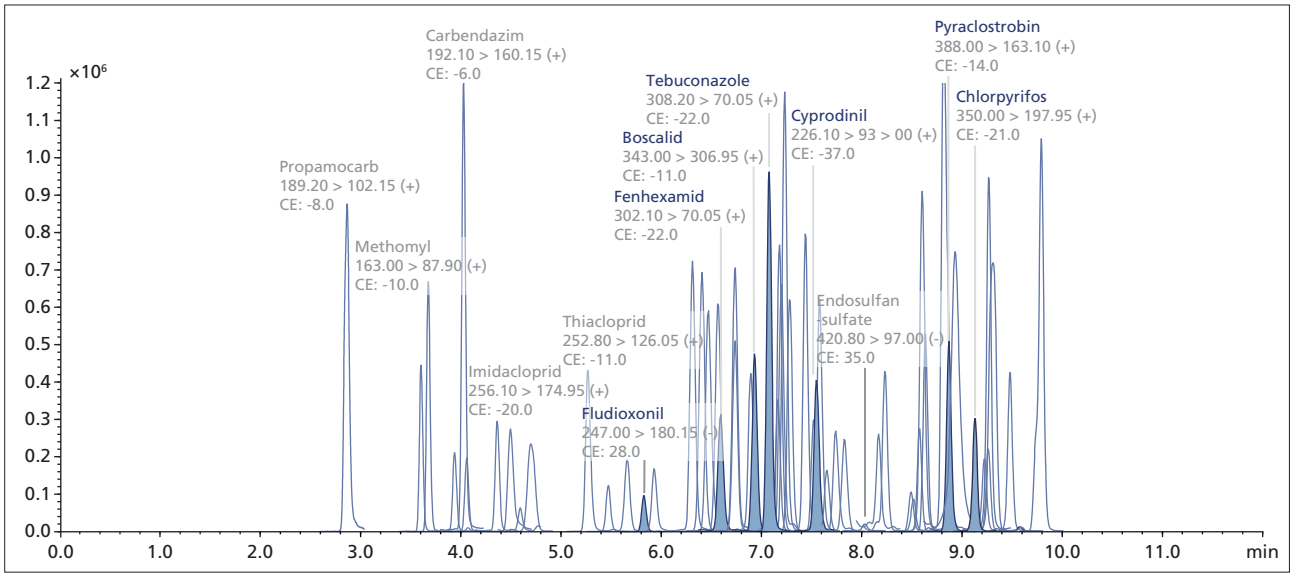

Fig. 2 MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyriphos, cyprodinil, fenhexamid, fludioxonil, pyraclostrobin and tebuconazole (highlighted in the MRM chromatogram) are some of the most frequently detected compounds present in more than 4 % of the samples analyzed.

The MRM chromatograms show the response to each pesticide spiked into a food matrix at the default MRL of 0.01 mg/kg.

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Results and Discussion

Shimadzu Pesticide MRM Library

(Application News No. C135)

A flexible tool for expanding capabilities in pesticide monitoring programs

The Pesticide MRM Library has been created using 766 certified reference materials and is designed to help accelerate method development and compound management.

The library contains an average of 8 optimized MRM transitions for each compound (including positive and negative ion modes). In total, more than 6,000 MRM transitions are held within the 766 compound library. The library itself documents CAS#, formula, activity, mono-isotopic mass and adduct masses, rank of MRM transitions, synonyms, InChI, InChIKey, compound names translation (Japanese and Chinese) and links to websites offering further information (for example; alanwood.net, PAN pesticide database, Chemical Book, ChemSpider).

The library also serves as a powerful data repository for reporting and checking pesticide data sources.

Creating flexible pesticide monitoring methods

Building a new LC/MS/MS method

To create new pesticide LC/MS/MS methods the user simply needs to select the target compounds from the library, identify the required number of MRMs for each compound and confirm the analytical column for the analysis. (The new method can be used to expand current capabilities or to create focused methods with a limited number of pesticides). The new method is simply imported into LabSolutions.

As the LCMS-8060 has a high data acquisition speed of 30,000 u/sec, high sensitivity and a polarity switching speed of 5 msec, the capabilities of the library can be expanded to meet the future needs of any laboratory.

Expanded capability of the LCMS-8060

The LCMS-8060 has a data acquisition speed of 30,000 u/sec which creates new opportunities for expanding compound lists.

As one example, between 6.45 and 6.60 minutes 25 pesticide compounds elute (Fig. 3). Even with high data density acquisitions the average variation in peak area response was less than 3 %RSD (varying between 1.1 - 5.9 %RSD).

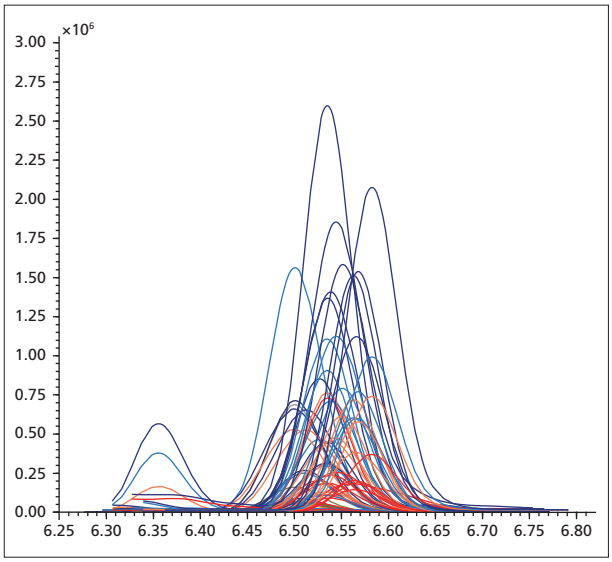

Fig. 3 The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density. Between 6.45 and 6.60 minutes 25 compounds were monitored (Table 2).

Table 2 Peak area variation (%RSD; n=6) for 25 pesticides eluting over a nine-second time window (6.45 - 6.60 minutes) spiked into a mint matrix extract at the reporting limit of 0.01 mg/kg.

Compound Name	CAS number	Formula	M	Polarity	MRM Quantitation Ion	RT	Average Peak Area	%RSD (n=6)
Trinexapac-ethyl	95266-40-3	C13H16O5	252.0998	+	252.90 > 69.05	6.45	1,780,015	3.1
Iprovalicarb	140923-17-7	C18H28N2O3	320.2100	+	321.20 > 119.15	6.46	1,442,486	2.8
Dodemorph	1593-77-7	C18H35NO	281.2719	+	282.30 > 116.15	6.47	658,920	4.2
Fluopyram	658066-35-4	C16H11ClF6N2O	396.0464	+	397.00 > 145.00	6.47	2,439,146	1.9
Flutolanil	66332-96-5	C17H16F3NO2	323.1133	+	324.10 > 242.00	6.48	3,372,285	2.7
Trifloxysulfuron	145099-21-4	C14H14F3N5O6S	437.0617	+	438.00 > 182.15	6.48	1,822,340	2.5
Azaconazole	60207-31-0	C12H11Cl2N3O2	299.0228	+	300.00 > 159.00	6.50	1,580,445	2.0
Terbutryn	886-50-0	C10H19N5S	241.1361	+	242.10 > 157.95	6.50	755,446	3.4
Prometryn	7287-19-6	C10H19N5S	241.1361	+	242.10 > 158.00	6.50	1,300,193	2.6
Azimsulfuron	120162-55-2	C13H16N10O5S	424.1026	+	425.10 > 182.10	6.50	2,498,050	1.8
Metominostrobin	133408-50-1	C16H16N2O3	284.1161	+	285.10 > 193.95	6.51	2,929,500	1.7
Thifluzamide	130000-40-7	C13H6Br2F6N2O2S	525.8421	+	528.60 > 148.05	6.51	193,982	5.9
Nicarbazin	330-95-0	C13H10N4O5	302.0651	-	301.10 > 137.15	6.52	973,101	2.6
Bromobutide	74712-19-9	C15H22BrNO	311.0885	+	312.10 > 194.10	6.53	1,829,781	2.1
Saflufenacil	372137-35-4	C17H17ClF4N4O5S	500.0544	+	501.00 > 198.00	6.53	465,224	2.3
Cyproconazole	94361-06-5	C15H18ClN3O	291.1138	+	292.10 > 70.05	6.54	1,174,967	1.7
Clomazone	81777-89-1	C12H14ClNO2	239.0713	+	239.90 > 125.00	6.54	3,409,656	1.7
Fensulfothion	115-90-2	C11H17O4PS2	308.0306	+	309.00 > 281.00	6.54	4,267,514	1.4
Oxasulfuron	144651-06-9	C17H18N4O6S	406.0947	+	407.10 > 150.15	6.54	2,911,533	1.1
Rimsulfuron	122931-48-0	C14H17N5O7S2	431.0569	+	432.00 > 182.00	6.55	4,722,065	1.8
Fenthion-oxon	6552-12-1	C10H15O4PS	262.0429	+	263.10 > 231.00	6.55	3,075,195	1.4
Nitrothal-isopropyl	10552-74-6	C14H16NO6Na	317.0875	+	295.10 > 230.95	6.56	2,199,581	3.0
Chlorantraniliprole	500008-45-7	C18H14BrCl2N5O2	480.9708	+	483.90 > 452.90	6.57	2,407,025	2.7
Fipronil-sulfone	120068-36-2	C12H4Cl2F6N4O2S	451.9336	-	451.00 > 414.90	6.57	2,843,708	2.0
Valifenalate	283159-90-0	C19H27ClN2O5	398.1608	+	399.20 > 155.00	6.59	3,845,335	1.9

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Final method performance for 646 pesticides

In order to test the performance of the developed method, linearity, repeatability and longer term robustness were assessed for all 646 pesticides.

Linearity

Linearity was assessed over a six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/µL). All 646 pesticides achieved excellent R^2 values greater than 0.99 in both tomato and mint spiked extracts with typical values greater than 0.996. Calibration curves were generated using a linear curve fit type and 1/C weighting. Typical calibration curve data is presented below in Fig. 4.

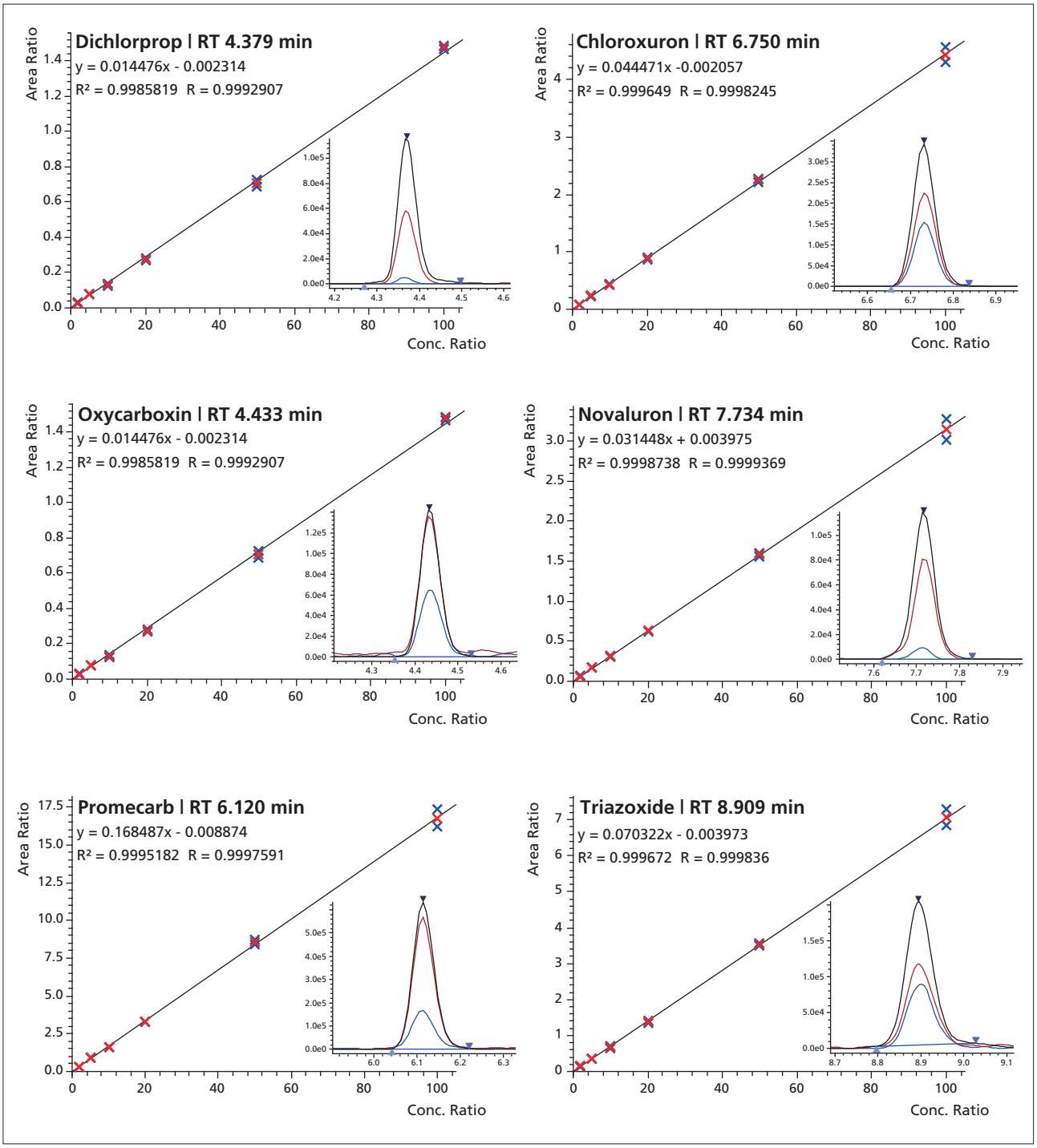

Fig. 4 Calibration curves for selected pesticides spiked into a mint matrix extract in the range 0.002 - 0.1 mg/kg. The quantitation MRM chromatogram is shown in black (qualifier ion MRM chromatograms are shown in red and blue).

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Repeatability

To assess the robustness of the system and the developed method during routine analysis, repeat injections of a mint matrix sample spiked with 646 pesticides at 0.05 mg/kg, were analyzed over a 24 hour period.

The results for selected compounds are displayed below in Fig. 5.

Compounds were selected throughout the run at equi-distant points (closest elution points to 3, 4, 5, 6, 7, 8, 9 and 10 minutes), including positive and negative ion detection, (Table 3).

The peak area variance was less than 5.7 % for all pesticides measured.

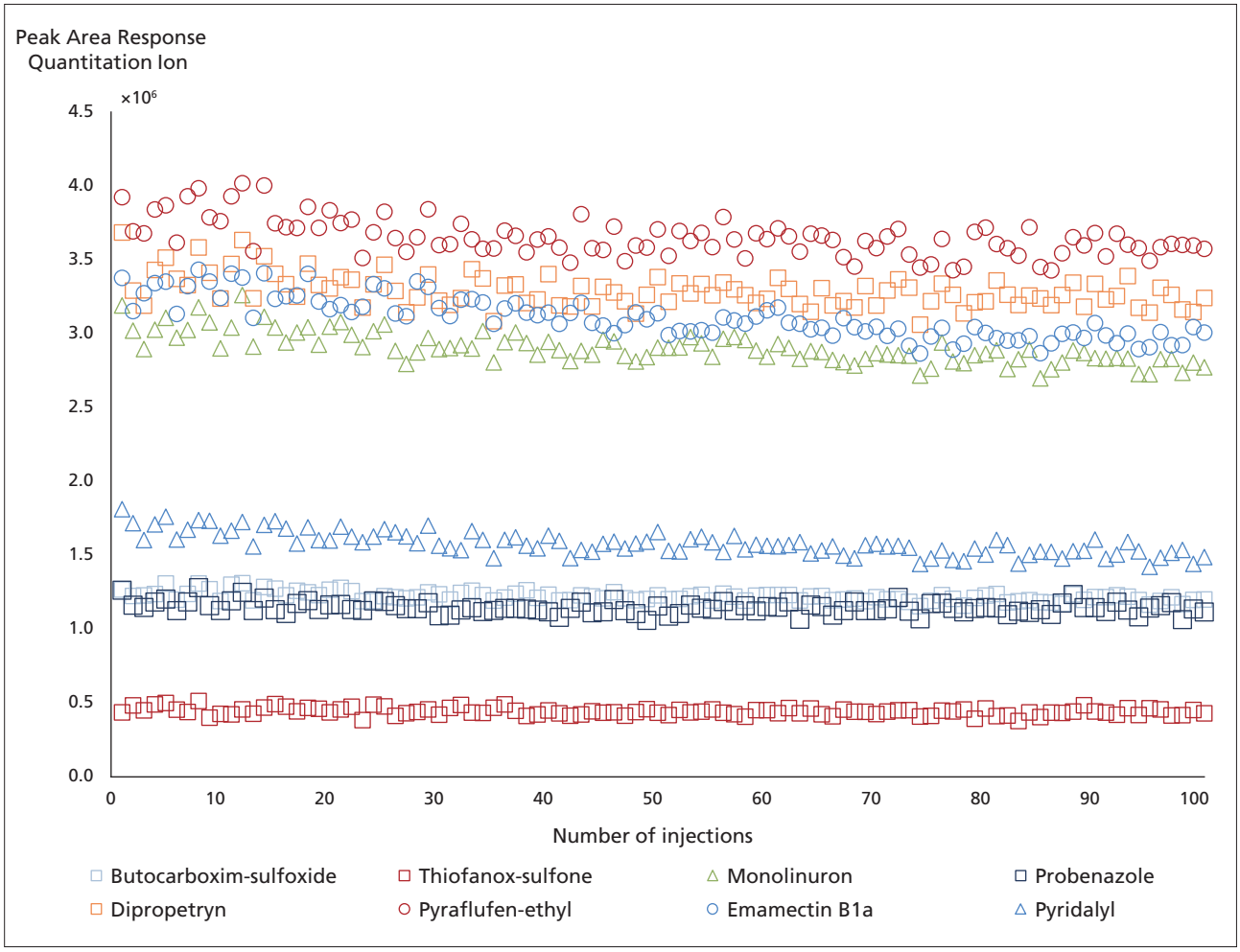

Fig. 5 Peak area response for several pesticides following 100 repeat injections of a 0.05 mg/kg spiked into mint matrix extract.

Table 3 Peak area variance for selected following the repeated injection of a 0.05 mg/kg spiked into mint matrix extract (number of sample replicates was 100; the analysis sequence was 24 hours).

Compound Name	CAS Number	Formula	M	Polarity	MRM Quantitation Ion	RT (mins)	Average Peak Area	%RSD (n=100)
Butocarboxim-sulfoxide	34681-24-8	C7H14N2O3S	206.0725	+	207.10 > 75.10	3.042	1,220,391	2.6
Thiofanox-sulfone	39184-59-3	C9H18N2O4S	250.0987	+	268.10 > 57.00	4.001	442,724	5.7
Monolinuron	1746-81-2	C9H11ClN2O2	214.0509	+	215.10 > 99.10	4.985	2,904,116	3.7
Probenazole	27605-76-1	C10H9NO3S	223.0303	+	224.00 > 41.05	5.995	1,145,189	3.5
Dipropetryn	4147-51-7	C11H21N5S	255.1518	+	256.20 > 144.05	6.999	3,289,597	3.4
Pyraflufen-ethyl	129630-19-9	C15H13Cl2F3N2O4	412.0204	+	413.00 > 339.00	8.004	3,653,333	3.5
Emamectin B1a	138511-97-4	C56H81NO15	1007.5606	+	886.40 > 158.20	9.008	3,109,562	4.5
Pyridalyl	179101-81-6	C18H14Cl4F3NO3	488.9680	-	491.90 > 109.05	10.171	1,579,422	5.0

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Response to differing matrices

One of the major challenges in the quantitative LC/MS/MS analysis for pesticides in food is that compound and matrix-dependent response suppression or enhancement may occur. Although matrix effects can affect the peak area response between different food types following a QuEChERS extraction protocol, the peak area variance should be minimized within a single matrix.

Food extracts of apple, mint and tomato following QuEChERS extraction were spiked with 646 pesticides at 0.05 mg/kg and were repeatedly injected on the LCMS-8060 (n=100 repeat injections for each matrix; 300 injections in the same batch sequence). Fig. 6 shows the response for 3 selected pesticides analyzed in a single batch sequence corresponding to a 72 hour analysis sequence. Within a matrix, variance was less than 5.9 %RSD for all compounds.

Although the absolute peak area changes with different food matrices, the response between injection 1 and injection 100 for 2 pesticides (probenazole and dipropetryn) within a single matrix has a variance less than 5.7 %RSD.

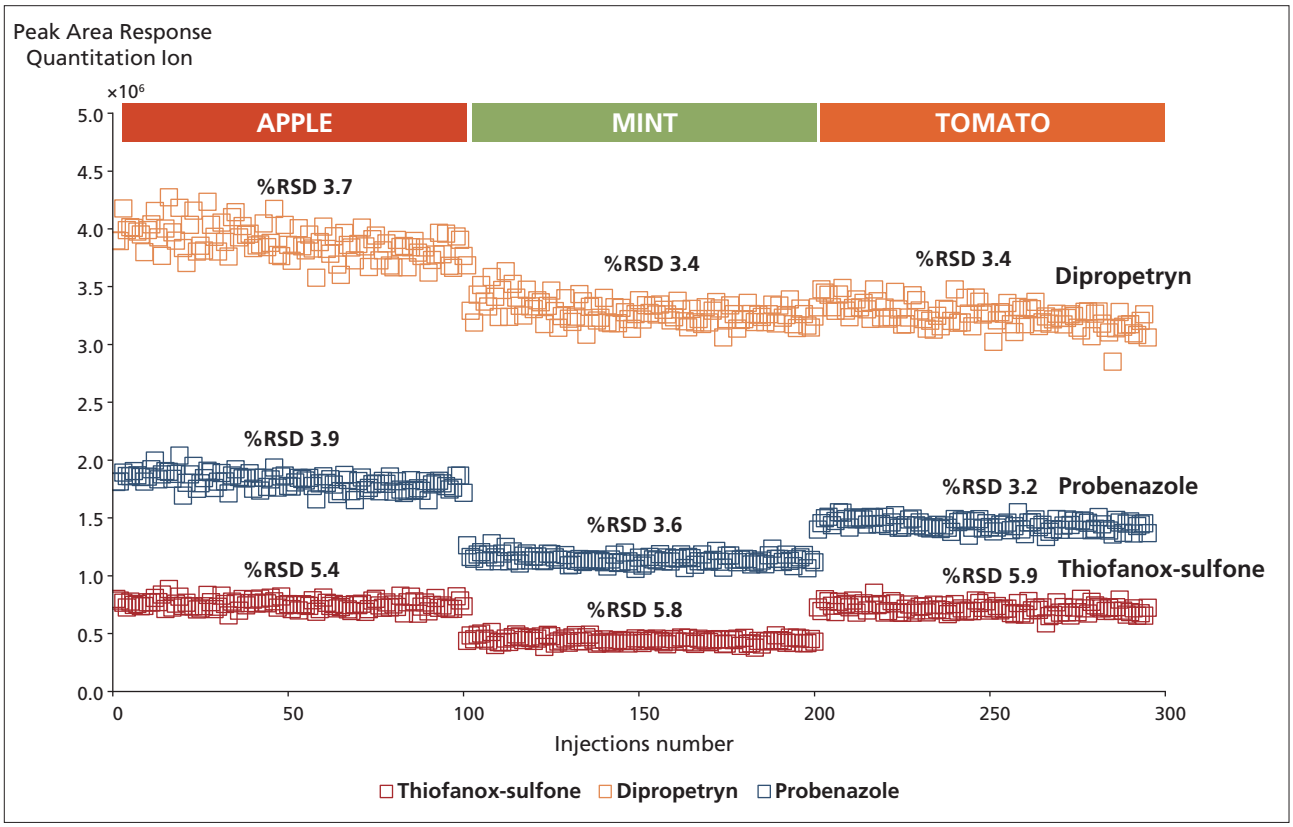

Fig. 6 Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Fig. 5, compounds were selected to reflect peak area response throughout the chromatographic run (Table 3).

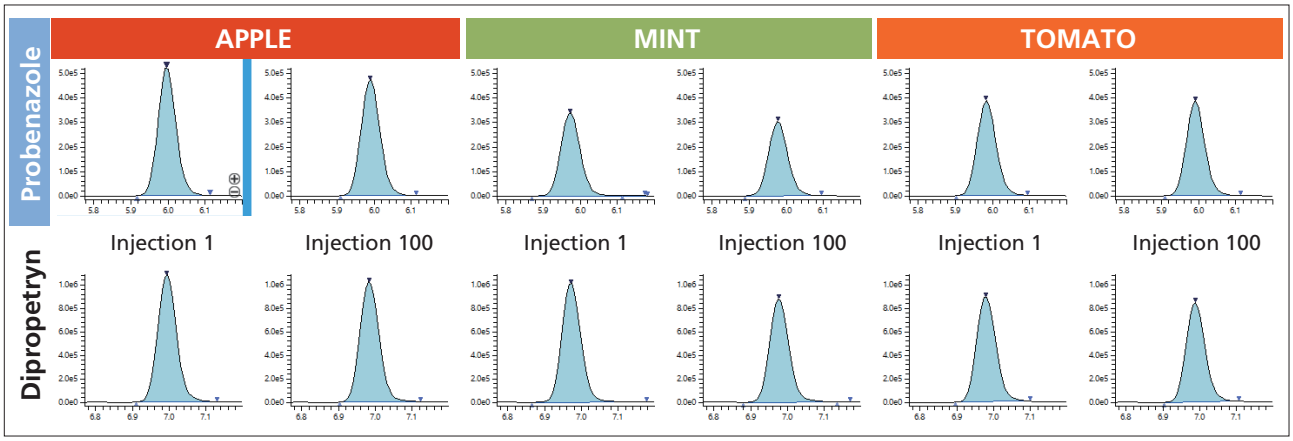

Fig. 7 MRM chromatograms for probenazole (RT 5.995 minutes) and dipropetryn (RT 6.999 minutes) for injection 1 and injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.

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Reducing matrix effects by extensively diluting the sample

The need to test for more pesticides in a wider range of samples at high sensitivity is very challenging as matrix effects from the sample extraction will influence both ion suppression and enhancement. Ion suppression can lead to errors in the detection capability, accuracy and precision of the method.

To reduce the effect of interfering compounds in the quantitation of complex samples extensive sample dilution is now widely used in routine analysis. It is an approach which is simple to build into multi-residue extraction methods and is cost effective.

This approach leads to greater robustness as a consequence of a reduced sample injection in the LC/MS/MS, higher data quality and increased instrument uptime.

Fig. 8 shows the results of diluting a matrix sample spiked at 0.005 mg/kg with dilution factors of 1:5, 1:10, 1:20, 1:50 and 1:100.

As matrix effects can be both significant and variable for different compounds Table 4 shows recovery data for a series of pesticides diluted from 0 to a dilution factor of 1:100.

Matrix suppression was reduced for most compounds when the sample was diluted 1:10 with recoveries in the range of 70 - 120 % with an associated repeatability RSDr ≤ 20 %. Relative standard deviations in relation to the mean values were typically less than 10 %.

Diluting the sample by a factor of 20 or 50 resulted in acceptable signal suppression from the matrix.

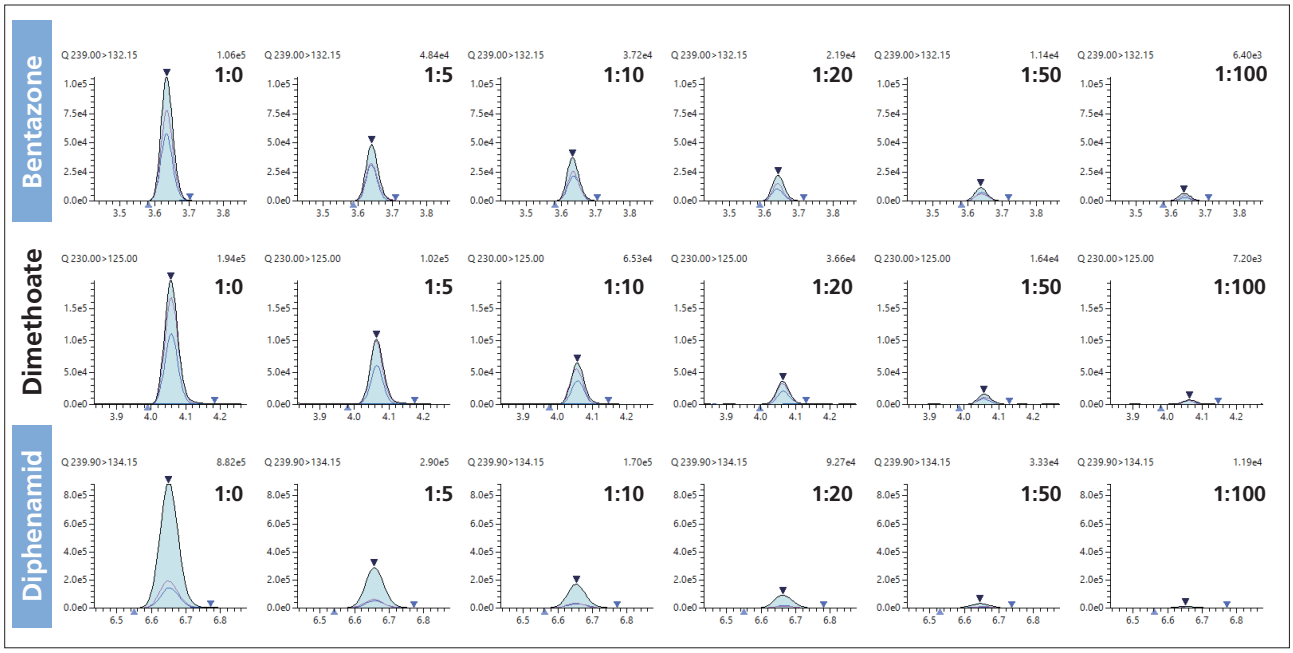

Fig. 8 MRM chromatograms for 3 selected compounds spiked into a mint extract at 0.005 mg/kg and diluted 1:5, 1:10, 1:20, 1:50 and 1:100 with water.

Table 4 Diluting a sample matrix extract spiked with 0.005 mg/kg with water reduced matrix ion suppression.

				Dilution series					
Compound	CAS	Formula	M	0	1:5	1:10	1:20	1:50	1:100
				Recovery					
Bentazone	25057-89-0	C10H12N2O3S	240.0569	32.1	44.6	65.5	72.7	91.7	98.1
Demeton-S-methyl-sulfone	17040-19-6	C6H15O5PS2	262.0099	51.1	78.5	89.6	91.1	114.2	116.8
Dimethoate	60-51-5	C5H12NO3PS2	228.9996	36.2	65.3	88.5	92.2	92.4	94.2
Isocarbamid	30979-48-7	C8H15N3O2	185.1164	28.8	57.1	81.8	98.7	102.5	96.4
Vamidothion	2275-23-2	C8H18NO4PS2	287.0415	53.6	76.3	98.2	98.5	101.5	114.1
Thiazafluron	25366-23-8	C6H7F3N4OS	240.0293	32.8	62.9	80.5	84.2	87.1	97.4
Demeton-S-methyl	919-86-8	C6H15O3PS2	230.0200	57.8	82.1	93.1	87.6	108.5	102.4
Sebuthylazine	7286-69-3	C9H16ClN5	229.1094	28.7	53.3	69.8	79.8	88.5	95.8
Flutriafol	76674-21-0	C16H13F2N3O	301.1027	27.3	46.1	71.4	76.1	81.8	87.3
Furametpyr	123572-88-3	C17H20ClN3O2	333.1244	48.3	69.8	86.9	86.2	97.6	101.9
Fenobucarb	3766-81-2	C12H17NO2	207.1259	60.9	79.2	100.7	96.1	102.8	103.9
Benodanil	15310-01-7	C13H10INO	322.9807	50.9	69.8	86.3	96.5	102.4	94.8
Terbuthylazine	5915-41-3	C9H16ClN5	229.1094	50.4	66.6	83.2	87.2	89.8	91.0
Dimethachlor	50563-36-5	C13H18ClNO2	255.1026	75.1	86.1	106.0	107.1	106.2	108.0
Dimethenamid	87674-68-8	C12H18ClNO2S	275.0747	72.6	84.9	102.9	100.0	103.6	97.3
Furalaxyl	57646-30-7	C17H19NO4	301.1314	82.2	89.1	106.6	108.6	106.2	102.4
Bixafen	581809-46-3	C18H12Cl2F3N3O	413.0310	66.8	79.3	99.0	95.6	103.7	97.1
Triflumuron	64628-44-0	C15H10ClF3N2O3	358.0332	54.2	71.8	95.5	84.9	95.3	101.7
Epoxiconazole	133855-98-8	C17H13ClFN3O	329.0731	61.6	77.2	98.8	95.3	90.0	101.2
Teflubenzuron	83121-18-0	C14H6Cl2F4N2O2	379.9742	41.8	50.9	80.1	86.8	100.0	97.7

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Conclusion

A fast, selective and highly sensitive method has been developed for the quantitation of 646 pesticides using a single method with 1,919 transitions (corresponding to up to 3 MRM transitions per compound) and a LC gradient time of only 10.5 minutes.

As the LCMS-8060 has a rapid polarity switching time of 5 msec, the single multi-residue LC/MS/MS method supported the analysis of 34 pesticides in negative ion mode and 612 compounds in positive ion mode.

The enhanced performance and higher sensitivity of the LCMS-8060 has created new opportunities in sample dilution to reduce ion signal suppression and matrix effects. For most compounds a dilution factor of 1:20 or 1:50 was sufficient to provide recoveries in the range 70 - 120 %.

References

1. Commission Regulation (EC). 2005. No 396/2005 of the European Parliament and of the Council, maximum residue levels of pesticides in or on food and feed of plant and animal origin. Official Journal of the European Union, L 70: 1-16

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3. Japanese Ministry of Health, Labour and Welfare, Department of Food Safety. 2006. Director Notice about Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Syoku-An No. 0124001 January 24, 2005; amendments May 26, 2006).

First Edition: Jun. 2016

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Application News

No.C140

Liquid Chromatography Mass Spectrometry

Ultra-Sensitive and Rapid Assay of Neonicotinoids, Fipronil and Some Metabolites in Honey by UHPLC-MS/MS [LCMS-8060]

Neonicotinoids are a class of insecticides widely used to protect fields as well as fruits and vegetables.
Recently the use of these compounds became very controversial as they were pointed as one cause of the honeybees colony collapse disorder. Since pollination is essential for agriculture, extensive studies have been conducted to evaluate the impact of neonicotinoids on bee health. Following this the European Food Security Authoritiy (EFSA) limited the use of thiamethoxam, clothianidin and imidacloprid. Fipronil, a pesticide from a different chemical class, has been also banned by EFSA for maize seed treatment due to its high risk for honeybee health.
In order to better understand the effect of these compounds on bees and their contamination in pollen and honey, a highly sensitive assay method was necessary. A method was set up using Nexera X2 with LCMS-8060.

Sample Preparation

Thiamethoxam-d3, imidacloprid-d4 and chlothianidin-d3 were used as internal standards.
Compound extraction was performed using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with an additional dispersive Solid Phase Extraction (dSPE) step.
5 g of honey (±1 %) were weighted in a 50 mL polypropylene tube. 5 µL of internal standard solution at 5 µg/mL of each compound in acetonitrile was added on honey and let dry for 10 minutes. 10 mL of ultra pure water were added and the samples were homogenized by vortex mixing for 1 minute. 10 mL of acetonitrile were then added followed by vortex mixing for 1 minute.
After incubation at room temperature for one hour with gentle shaking, a commercially available salt mix from Biotage (4 g MgSO4, 1 g Sodium Citrate, 0.5 g Sodium Citrate sesquihydrate, 1g NaCl) was added. After manual shaking, samples were centrifuged at 3000 g for 5 minutes at 10 °C.
Supernatant (6 mL) was transferred into a 15 mL tube containing 1200 mg of MgSO4, 400 mg PSA and 400 mg C18 from Biotage. After centrifugation at 3000 g and 10 °C for 5 minutes the supernatant was transferred into a LCMS certified inert glass vial for analysis (Shimadzu LabTotal 227-34001-01).

Recovery

An "all-flowers" honey from the local supermarket was extracted with or without spike at 50 ppt. A blank extract (no honey) was prepared to evaluate losses or non specific interactions. Results are presented in Table 1.
Calculated recoveries are within acceptance values 70-120 % from EU SANTE/11945/2015.

Table 1 Measured Recoveries in Honey

Compound	Recovery	Compound	Recovery
Acetamiprid	78.8 %	Fipronil sulfone	74.2 %
Acetamiprid-N-desmethyl	93.4 %	Imidaclorpid	83.2 %
Chlothianidin	70.6 %	Nitenpyram	87.0 %
Dinotefuran	76.5 %	Thiacloprid	82.2 %
Fipronil	78.1 %	Thiamethoxam	75.6 %

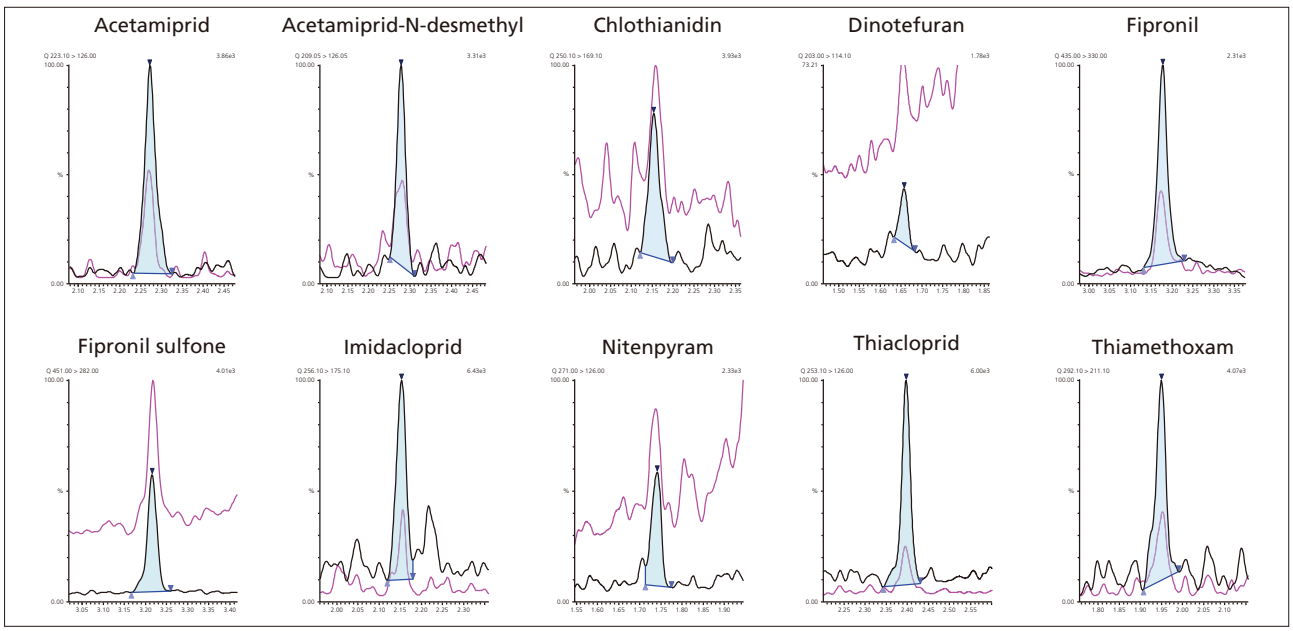

Fig. 1 Chromatogram of the Target Compounds at Their Lower Limit of Quantification

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Table 2 Analytical Conditions

System	: Nexera X2	System	: LCMS-8060
Column	: ACE SuperC18 (100 mm L. × 2.1 mm I.D., 2 µm)	Ionization	: Heated ESI
Column Temperature	: 30 °C	Probe Voltage	: +1 kV (positive ionization) / -1.5 kV (negative ionization)
Mobile Phases	: A: Water = 0.05 % ammonia B: Methanol + 0.05 % ammonia	Temperature	: Interface: 400 °C Desolvation Line: 200 °C Heater Block: 400 °C
Flowrate	: 600 µL/min		
Gradient	: 5 %B to 100 %B in 3 min 100 %B to 5 %B in 0.1 min	Gas Flow	: Nebulizing Gas: 3 L/min Heating Gas: 10 L/min Drying Gas: 5 L/min
Total Run Time	: 4 min		
Injection Volume	: 2 µL (POISe mode with 10 µL of water)		

Table 3 MS/MS Acquisition Parameters

MRM Transitions	Name	Polarity	MRM Quan	MRM Qual	ISTD
	Acetamiprid	+	223.1 > 126.0	223.1 > 56.1	2
	Acetamiprid-N-desmethyl	+	209.1 > 126.0	211.1 > 128.0	2
	Clothianidin	+	250.1 > 169.1	250.1 > 132.0	3
	Dinotefuran	+	203.0 > 114.0	203.0 > 87.0	1
	Fipronil	-	435.0 > 330.0	435.0 > 250.0	3
	Fipronil sulfone	-	451.0 > 415.0	451.0 > 282.0	3
	Imidacloprid	+	256.1 > 175.1	258.1 > 211.1	2
	Nitenpyram	+	271.0 > 126.0	271.0 > 225.0	3
	Thiacloprid	+	253.1 > 126	253.1 > 90.1	1
	Thiamethoxam	+	292.1 > 211.1	292.1 > 181.1	1
	Thiamethoxam-D3	+	295.1 > 214.05	---	1
	Imidacloprid-D4	+	260.1 > 179.1	---	2
	Clothianidin-D3	+	253.1 > 132.05	---	3

Dwell Time	: 3 to 34 msec depending upon the number of concomitant transitions to ensure to have at least 30 points per peak (max total loop time 140 msec).
Pause Time	: 1 msec
Quadrupole Resolution	: Q1: Unit Q3: Unit

Calibration

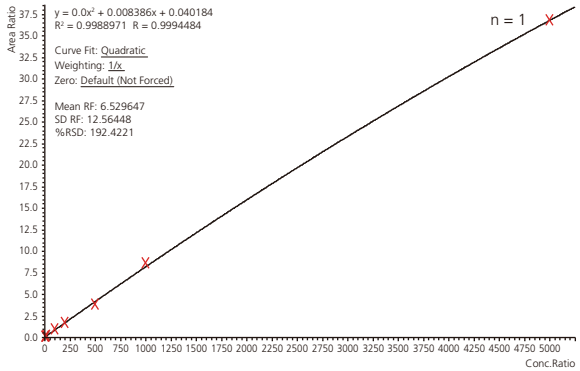
Calibration curves were prepared in acetonitrile to obtain final concentrations ranging from 0.5 pg/mL (1 fg on column) to 5 ng/mL. These concentrations corresponds to 1 ng/kg and 10 µg/kg in honey, respectively.

For each compound, the lower limit of quantification was selected to give an accuracy between 80-120 % (see table 4).

A typical calibration curve is shown in Fig. 2.

Table 4 Limits of Quantification in Honey

Compound	LOQ (µg/kg)	Compound	LOQ (µg/kg)
Acetamiprid	0.005	Fipronil sulfone	0.001
Acetamiprid-N-desmethyl	0.005	Imidacloprid	0.020
Chlothianidin	0.020	Nitenpyram	0.020
Dinotefuran	0.010	Thiacloprid	0.005
Fipronil	0.001	Thiamethoxam	0.005



Standard (µg/kg)	Accuracy (%)
0.005	106
0.010	97.2
0.020	95.6
0.100	107
0.200	98.4
0.500	91.5
1.000	104
5.000	99.9
10.000	100

Fig. 2 Calibration Curve of Acetamiprid

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Real Samples Analysis

Nine honey samples purchased at the local supermarket or used as raw materials in cosmetics (orange tree honey) were assayed as unknowns.

All tested honeys showed concentrations far below the authorized maximum residue limit. But thanks to the very high sensitivity reached, even low concentrations of neonicotinoids were quantified. Results are presented in table 5.

A representative chromatogram of a sample honey is shown in Fig. 3.

Table 5 Honey Samples Results (concentrations in µg/kg)

Honey	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
1. Provence creamy	---	---	0.20	---	0.010
2. Italy creamy	0.15	---	0.17	---	---
3. Pyrenees liquid	0.38	---	0.043	0.020	---
4. French-Spanish creamy	0.27	---	0.047	0.020	---
5. Thyme liquid	---	---	---	---	---
6. Lemon tree creamy	1.7	---	0.15	0.033	---
7. Orange tree liquid	1.2	---	0.62	---	---
8. Flowers creamy	0.14	---	0.055	0.39	---
9. Flowers liquid	0.34	---	0.11	0.010	---

Honey	Dinotefuran	Nitenpyram	Acetamiprid-N-desmethyl	Fipronil	Fipronil sulfone
1. Provence creamy	---	0.052	0.005	---	---
2. Italy creamy	---	0.040	---	---	---
3. Pyrenees liquid	---	---	0.015	0.004	---
4. French-Spanish creamy	---	0.032	---	---	---
5. Thyme liquid	---	---	---	---	---
6. Lemon tree creamy	---	---	0.020	---	---
7. Orange tree liquid	---	0.024	0.018	---	---
8. Flowers creamy	---	---	0.016	---	---
9. Flowers liquid	---	---	0.006	---	---

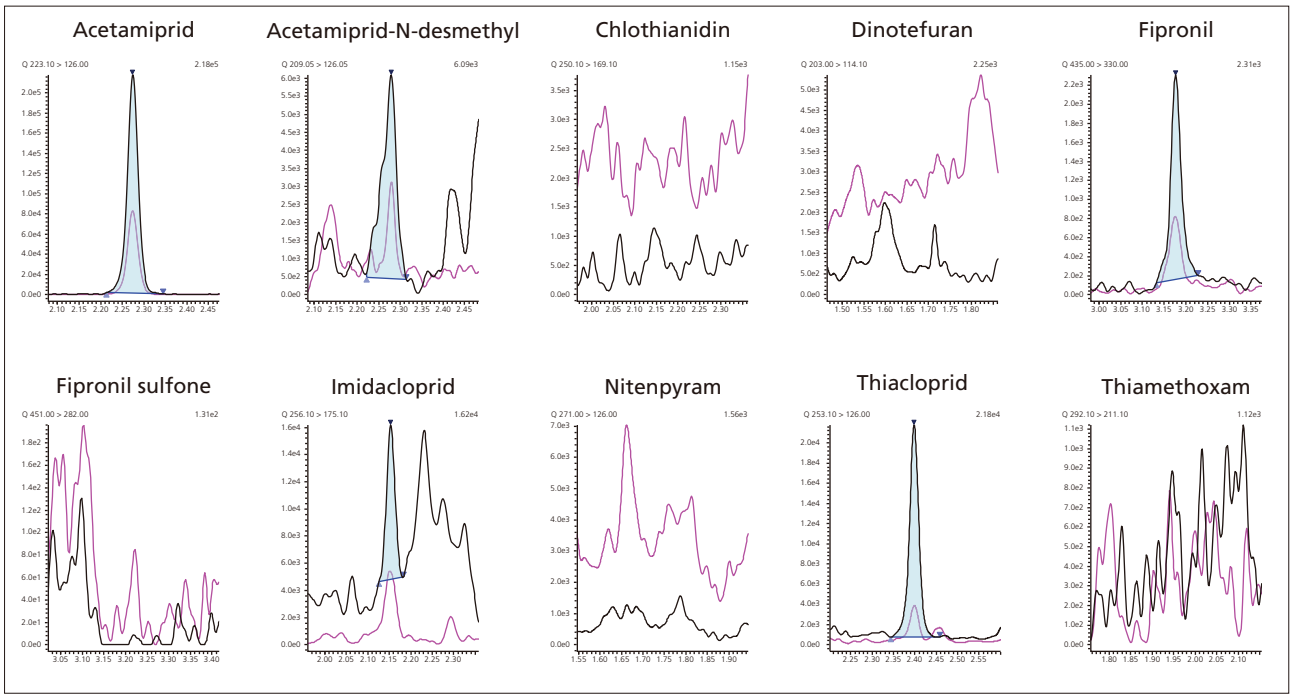

Fig. 3 Chromatogram of a Sample Honey (Pyrenees)

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Stability

The thyme honey sample with no detectable target compound was spiked at 50 ng/kg with all compounds prior to extraction. The extract obtained was then consecutively injected 150 times in the system.
The results presented in Fig. 4 show excellent stability of the signal even at these low concentrations. This demonstrates that the excellent sensitivity can be maintained over long series of real sample analysis thanks to the ion source ruggedness.

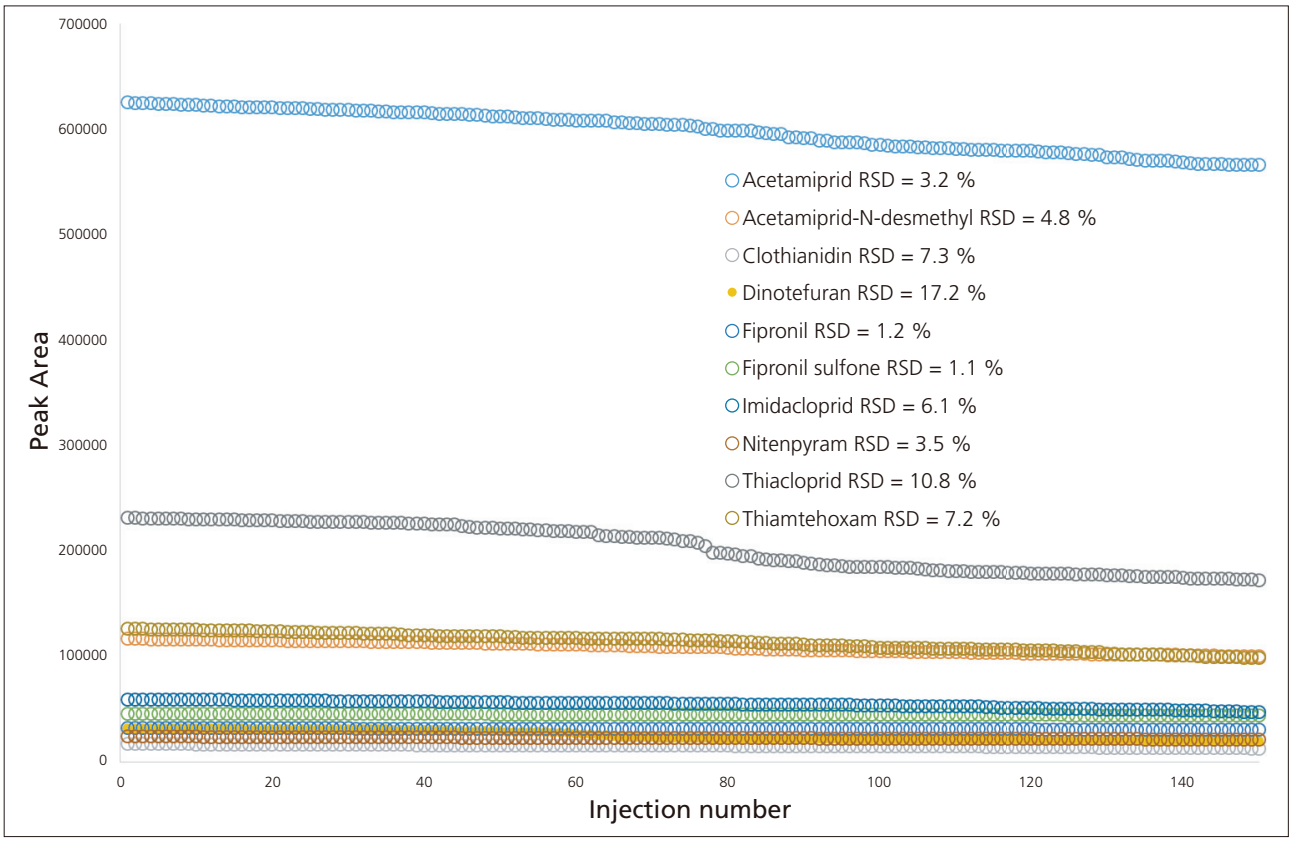

Fig. 4 Stability of Peak Areas in Real Honey Samples

Conclusion

A method for ultra sensitive assay of neonicotinoids in honey was set up. The sample preparation was simple but provided excellent recoveries. The injection mode used prevented the use of tedious evaporation/reconstitution or dilution steps.
Thanks to the high sensitivity obtained enabled assay in real samples at very low levels far under the regulated residue levels. Furthermore, even at low measured concentrations, the system demonstrated its stability after long analytical series of real samples.
This method can be a very efficient support tool to better understand the impact of neonicotinoids on honey bee colonies and could be easily transposed to pollen or bee samples.

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Application News

No. C154

Liquid Chromatography Mass Spectrometry

Applying 'MRM Spectrum Mode' and Library Searching for Enhanced Reporting Confidence in Routine Pesticide Residue Analysis

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Abstract

To help reduce the incidence of false positive and false negative reporting in pesticide residue monitoring routine multiple-reaction monitoring (MRM) methods have been enhanced to monitor a higher number of fragment ion transitions to increase specificity and reporting confidence. In this workflow, typically 6-10 fragment ion transitions were monitored for each target pesticide as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target pesticide had a corresponding fragmentation spectra which could be used in routine library searching and compound verification using reference library match scores. This 'MRM Spectrum Mode' was applied to quantify and identify 193 pesticides using 1,291 MRM transitions without compromising limits of detection, linearity or repeatability.

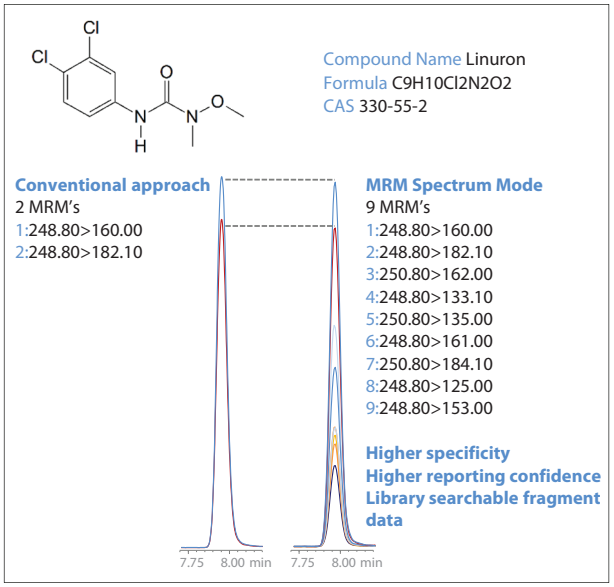

Fig. 1 Using a higher number of fragment ions in MRM data acquisition increases the specificity of detection and reduces false negative and false positive reporting. In the case of linuron, 9 precursor-fragment ion transitions were used to increase confidence in assay specificity. There is no compromise in data quality between methods despite a higher number of fragment ions monitored. Signal intensity, linearity, reproducibility are in good agreement between both methods.

Introduction

Multiple Reaction Monitoring (MRM) based LC-MS/MS techniques are widely used on triple quadrupole platforms for targeted quantitation as a result of high selectivity, sensitivity and robustness. In a regulated environment such as food safety there is a growing need to enhance the capability in routine monitoring programs by increasing the number of pesticides measured in a single analysis and at the same time delivering the highest confidence in compound identification to reduce false detect reporting. For pesticide analysis in the EU, identification criteria in SANTE/11945/2015 requires the retention time and the ion ratio from at least 2 MRM transitions to be within acceptable tolerance limits.*[1] However, even applying this criteria it is well reported that false positives can occur in certain pesticide/commodity combinations.*[2-*4]

To reduce false negative and false positive reporting a higher number of MRM transitions were used for each target pesticide to increase the level of confidence in assay specificity. The number of fragment ion transitions monitored for each target pesticide was dependent upon the chemical structure with typically between 6-10 fragment ions for each compound. MRM Spectrum mode combines conventional MRM quantitation with the generation of a high quality MRM product ion spectrum which can be used in routine library searching and compound verification and identification.

In this application paper we present the development of a method for 193 pesticides, with 1,291 MRM transitions, and a 15 minute cycle time. In order to acquire this number of MRM transitions using a short run time a 3 msec dwell time was applied to each MRM transition and a 5 msec polarity switch was used. On average 7 MRM transitions were applied to each compound. The method was quickly set up using the Shimadzu Pesticide Method Package, a data base with more than 750 pesticides and over 6,000 MRM transitions designed to accelerate method set-up and help compound verification. MRM Spectrum mode was also compared to a conventional pesticide monitoring method with 2 MRMs per compound (386 MRMs in total) in order to assess the effect on data quality when adding additional MRM transitions to the method. Several different food commodities were analysed with varying complexity (turmeric, plum, peppermint, parsnip, cherry, lime, pumpkin, tomato, potato). Data was processed using LabSolutions Insight software which provides automated library searching of target MRM spectrum.

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Experimental

Pesticide spiked samples, extracted using established QuEChERS based methods, were provided by Scientific Analysis Laboratories, UK. In order to test the performance of the MRM Spectrum Mode database and library searching a number of matrices were tested including turmeric, plum, peppermint, parsnip, cherry, lime, pumpkin, tomato and potato. Final extracts were prepared in acetonitrile without any dilution and directly injected into the LC-MS/MS. A water co-injection method, performed automatically in the auto-sampler, was used to improve early eluting peak shapes in addition to a sub 2 micron particle size column to improve peak capacity (Table 1) .

Calibration curves were prepared in the range 0.01 to 0.2 mg/kg. Repeatability of the method was tested using avocado matrix at 0.1 mg/kg. In the final method samples were analysed in ESI +/- using a polarity switching time of 5 msec.

On average 7 MRM transitions were applied to each compound, with more than 10 MRM transitions applied to 34 compounds. All MRM transitions were acquired throughout the MRM window without the need for triggering thresholds. The method includes a total of 1,291 MRM transitions for 193 pesticides in a run time of only 15 minutes. A dwell time of 3 msecs was applied to every MRM transition. In order to evaluate the data quality from the MRM Spectrum Mode method, the same method was set up with 2 MRMs applied to each compound (386 MRMs in total) using the same acquisition method (Table 2).

LabSolutions software was used to automatically optimize the fragmentation for all pesticides and generate a MRM Spectrum mode method. The MRM Spectrum Mode method for library searching and compound verification could be simply and quickly set up using the Shimadzu pesticide database. This database contains more than 6,000 MRM transitions for over 750 pesticides.

LabSolutions Insight v3.0 software was used to review quantitative data and MRM Spectrum mode library searching with advanced filtering tools to review by exception and to reduce false detect reporting.

Table 1 LC acquisition parameters

Liquid chromatography		
UHPLC	Nexera LC system	
Analytical column	HSS T3 (100 × 2.1, 1.7 µm)	
Column temperature	40 °C	
Flow rate	0.4 mL/minute	
Solvent A	5 mmol/L ammonium formate and 0.004 % formic acid	
Solvent B	5 mmol/L ammonium formate and 0.004 % formic acid in methanol	
Binary Gradient	**Time (mins)**	**%B**
	1.50	35
	11.50	100
	13.00	100
	13.01	3
	15.00	Stop
Injection volume	0.1 µL (plus 30 µL water)	

Table 2 MS/MS methods used to acquire data in MRM Spectrum Mode and a conventional MRM method with 2 MRM transitions per compound. As part of the comparative study, the same LC conditions were used for both methods.

LC-MS/MS Mass spectrometry	MRM Spectrum Mode: generating library searchable spectra	2 MRM method
Target number of compounds	193	193
Total number of MRM transitions	1,291 transitions (1,229 in ESI+ and 62 in ESI-)	386 (374 in ESI+ and 12 in ESI-)
Pause time/dwell time	1 msec./3 msec.	1 msec./3 msec.
Ionisation mode	ESI +/-	ESI +/-
Polarity switching time	5 msec	5 msec
Interface temperature	350 °C	350 °C
Heat bl°Ck temperature	300 °C	300 °C
Desolvation line temperature	150 °C	150 °C
Nebulising gas	3 L/min	3 L/min
Heating gas	10 L/min	10 L/min
Drying gas	10 L/min	10 L/min

Results and Discussion

In developing monitoring programs for chemical contamination methods are designed to determine a list of known analytes with a focus on delivering a rapid, cost-effective analysis that generates no false-negative or false-positive results. Guidelines for compound identification have been published by the EU in directive SANTE/11945/2015 . This identification criteria requires at least two MRM transitions with an ion ratio and retention time within defined tolerance limits.

To help reduce false detect reporting in pesticide monitoring programs, a MRM method was developed with a higher number of MRM transitions for each target pesticide to increase the level of confidence in assay specificity. By combining multiple MRM transitions for a compound into a product ion spectrum, pesticide identification can be verified and confirmed against a MS/MS reference spectral library. Using MRM Spectrum mode can help markedly reduce false detect reporting without affecting the data quality for optimized quantitation or identification.

Fig. 2, shows the MRM chromatogram for all 193 pesticides spiked at 0.010 mg/kg measured with MRM Spectrum mode. Using this mode 1,291 MRM transitions were measured for 193 pesticides. Despite the high data density acquired with MRM Spectrum Mode (for example, 151 MRM transitions were registered in the same time window during the analysis, see Fig. 3) sensitivity was not affected by the high data acquisition rate.

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Method performance

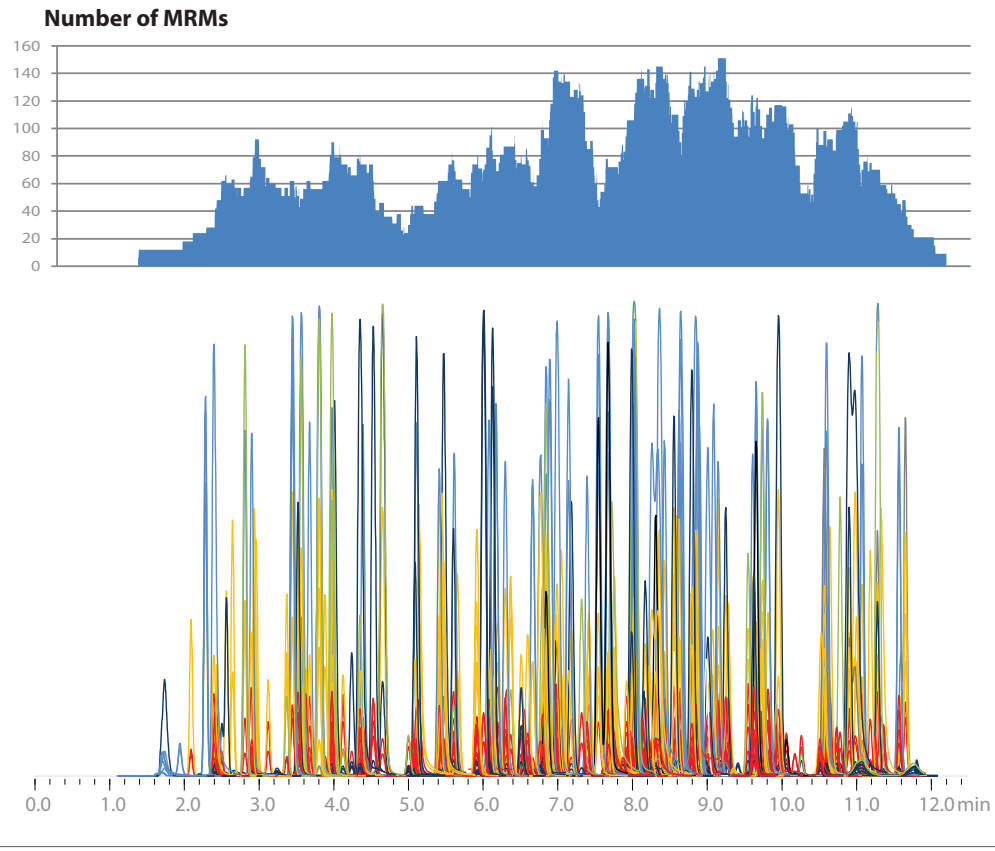

Fig. 2 Histogram showing the number of MRM transitions monitored at each time point and chromatogram showing all 193 target compounds. The highest number of overlapping MRM's acquired was 151. Even at such a high data sampling rate the response was in agreement with a conventional 2 MRM method with peak area variation less than 5.2% (n=5). This data is displayed below in more detail, Fig. 3.

Table 3 Between 8.80 mins and 9.30 mins151 MRM transitions in both positive and negative ion were monitored. Peak area repeatability for the 22 compounds eluting in this time period is shown below.

	Ret. Time	# MRMs	Polarity	Peak Area %RSD (n=5)
Dichlofluanid	8.80	6	ESI+	2.2
Dichlofluanid 2	8.80	6	ESI+	3.4
Dichlofluanid 1	8.80	5	ESI+	2.6
Fluoxastrobin	8.82	12	ESI+	2.0
Fenhexamid	8.83	11	ESI+	2.2
Iprovalicarb	8.88	6	ESI+	2.3
Spirotetramat	8.89	6	ESI+	2.6
Azinphos-ethyl	8.90	5	ESI+	3.1
Chromafenozide	8.91	5	ESI+	3.2
Triticonazole	8.93	5	ESI+	2.1
Cyazofamid	9.01	5	ESI+	2.1
Prothioconazole desthio	9.07	10	ESI+	1.9
Diflubenzuron	9.09	4	ESI+	2.0
Pyrifenox	9.11	8	ESI+	2.0
Dodemorph	9.17	6	ESI+	2.1
Fenoxycarb	9.17	6	ESI+	2.0
Rotenone	9.17	6	ESI+	2.4
Fipronil	9.20	10	ESI-	5.2
Bixafen	9.25	8	ESI-	2.8
Tebufenozide	9.27	6	ESI+	3.9
Bensulide	9.27	6	ESI+	2.6
Neburon	9.30	9	ESI+	1.7
		Total MRM's 151		Average 2.6 %RSD

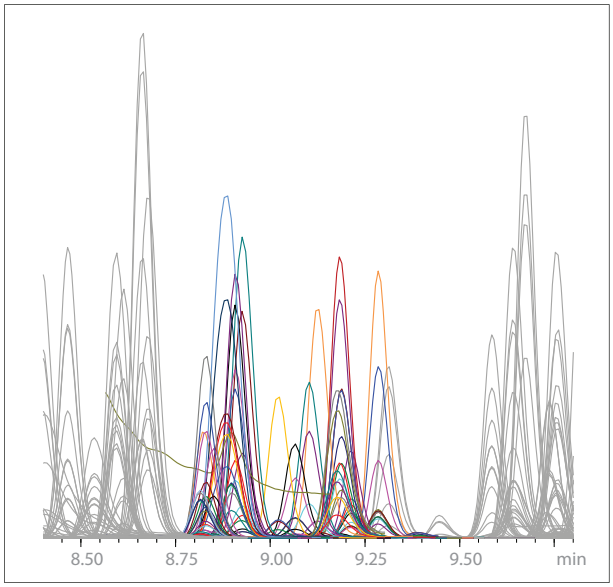

Fig. 3 Between 8.80 mins and 9.30 mins151 MRM transitions in both positive and negative ion were monitored. During this time period 22 target pesticides eluted with a peak area variation less than 5.2 % RSD. Data was acquired in an avocado sample matrix at a concentration of 0.1 mg/kg.

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Method performance

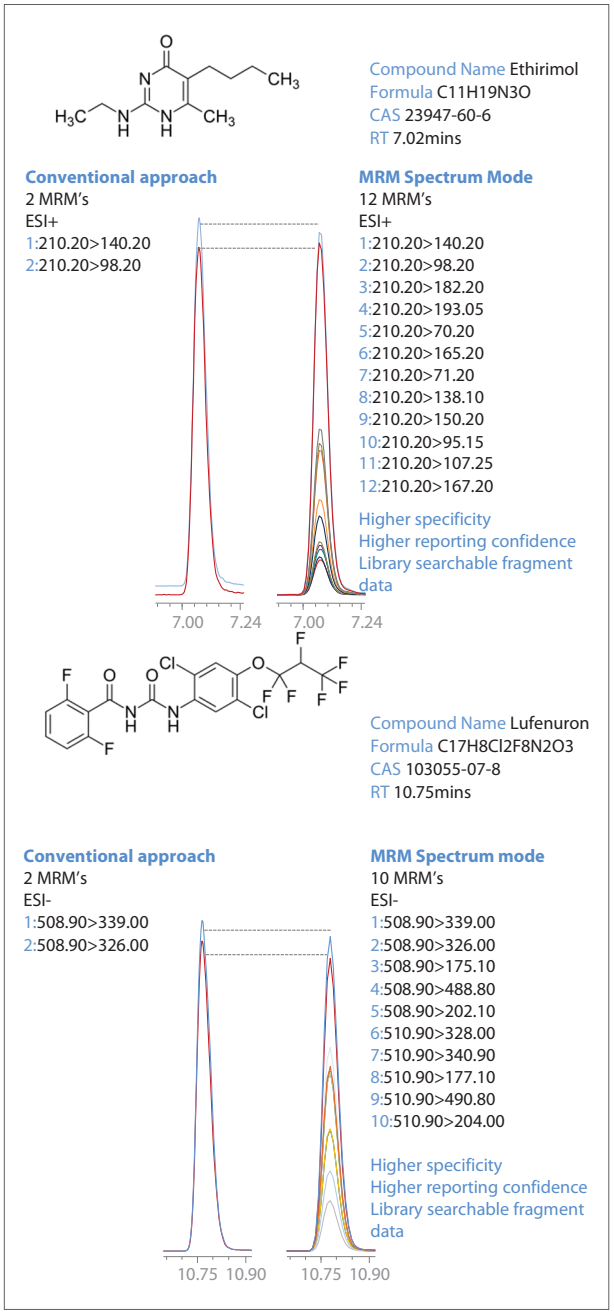

Fig. 4 MRM chromatograms for ethirimol (positive ion) and lufenuron (negative ion) acquired using a conventional 2 fragment ion MRM method and compared to a method with a higher number of precursor-fragment ions to increase confidence in assay specificity and reporting.

Despite acquiring a higher number of MRM transitions the library searchable MRM approach (acquiring 1,291 transitions in a single method) results in the same signal intensity compared to a conventional 2 fragment ion MRM method (acquiring 386 MRM transitions in a single method). The repeatability for each MRM method was evaluated by repeatedly injecting (n=5) an avocado extract corresponding to a concentration of 0.1 mg/kg. In each MRM method the %RSD was less than 3.5% for both compounds.

To minimize the possibility of false positive and false negative reporting LC-MS/MS methods were developed with a high number of MRM transitions for each pesticide. The performance of this approach was compared with a conventional MRM method monitoring 2 transitions for each pesticide.

In Fig. 4, the MRM chromatograms for 2 compounds, ethirimol and lufenuron, are shown for the same sample extract acquired using different MRM methods (the sample is avocado spiked at 0.1 mg/kg). The MRM chromatograms show un-smoothed data and are scaled to the same signal intensity for each compound. Ethirimol and lufenuron elute at 7.02 and 10.75 mins corresponding to time windows of high data density with more than one hundred MRM transations monitored in the same time segment. However, regardless of the high number of fragment ions monitored, the absolute signal intensity for both approach's is near identical in positive and negative ion mode.

Fig. 5 shows the correlation between the peak areas for all pesticides measured using 2 different MRM methods. The linear regression curve shows a good agreement between the peak areas measured for all pesticides spiked into sample matrix with a slope value near unity and an intercept near zero.

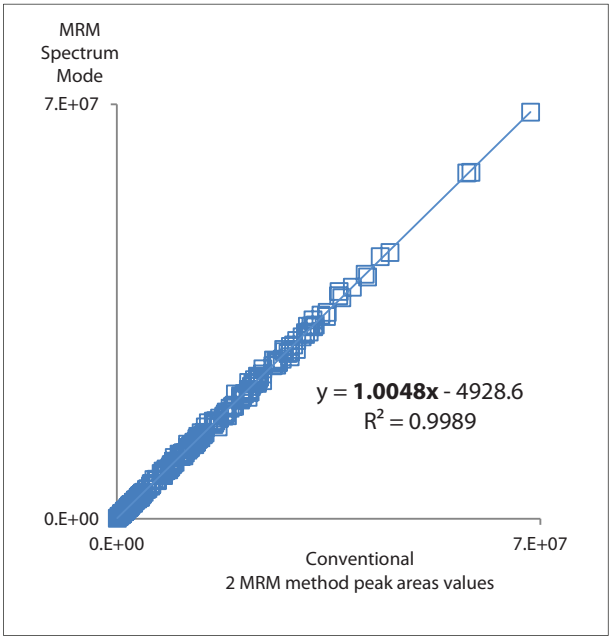

Fig. 5 Absolute peak area response for all 193 pesticides acquired using a conventional MRM method with 386 transitions compared to a MRM method with 1,291 transitions designed for library searchable verification. Both approaches result in near identical peak areas regardless of the number of fragment ions used to verify and identify each pesticide.

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Spectrum based identification

In this study, the number of qualifier fragment ion transitions was increased for each pesticide and the combined transitions were used to create a MRM product ion spectrum. This product ion spectrum derived from MRM acquisitions was used in conventional library matching routines comparing against a reference spectrum to generate a similarity score.

In Fig. 6, demeton-S-methyl sulphone was to highlight library matching in different matrices including cumin, potato, mucuna pruriens powder, tomato, black pepper, peppermint tea and turmeric. Even in the presence of complex spice matrices the library matching approach identified demeton-S-methyl sulphone with a high similarity score and a high degree of confidence for data reporting.

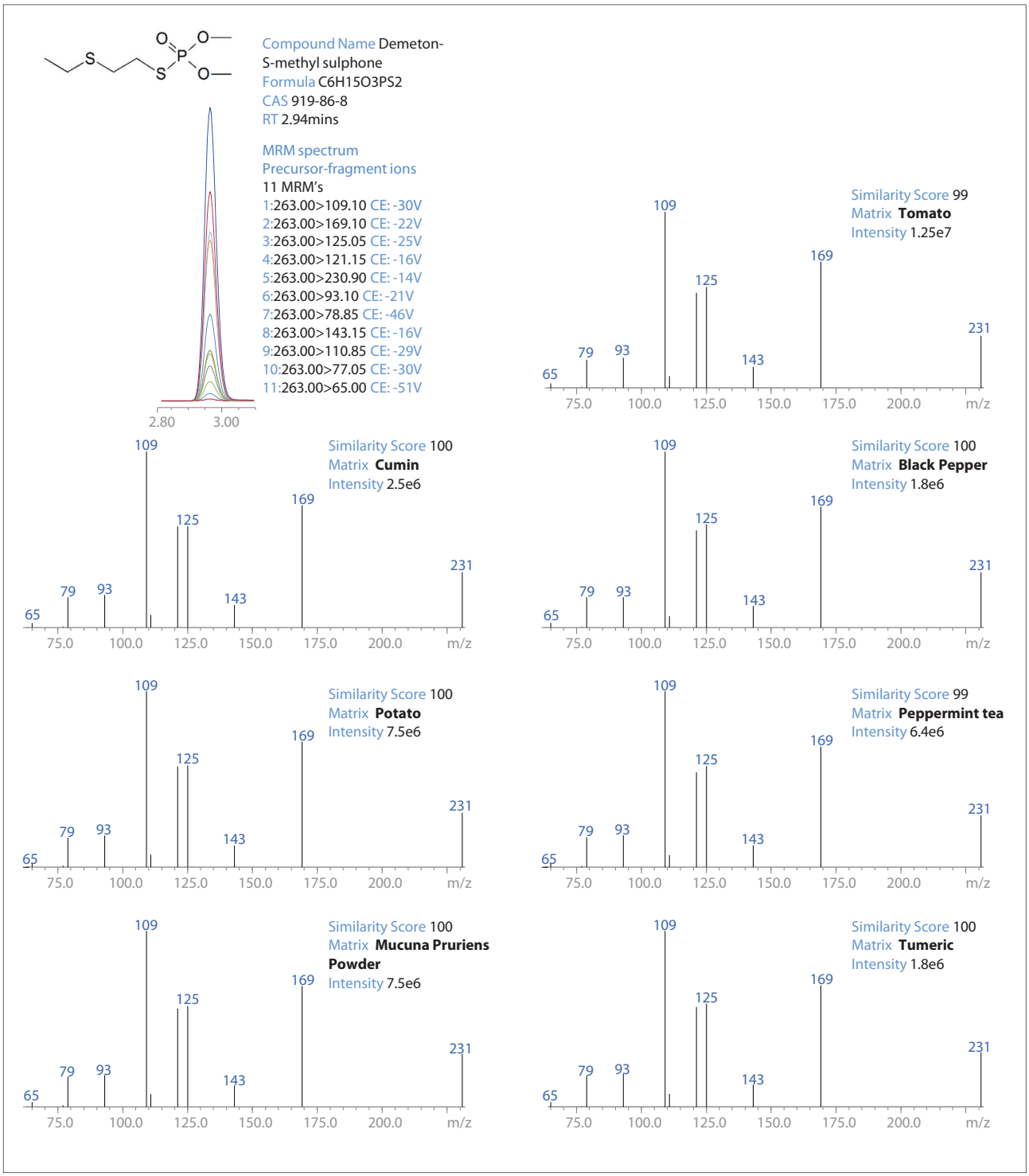

Fig. 6 MRM spectrum identification in different matrices for demeton-S-methyl sulphone

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Spectrum based identification

To increase the confidence in reporting results the number of qualifier transitions was increased for each pesticide and the combined MRM transitions were used to create a product ion spectrum. This MRM product ion spectrum can then be automatically compared against a reference spectrum to generate a product ion spectrum match score using conventional library matching.

Fig. 7 highlights the advantage of using a library searchable fragment ion spectrum in identifying and quantifying desmedipham and phenmedipham. Both desmedipham and phenmedipham share several common fragment ions and have similar retention times. Using MRM Spectrum Mode and comparing to a library searchable spectra, both desmedipham and phenmedipham are positively identified (fragment ions at *m/z* 154 and 182 are absent in product ion spectrum for phenmedipham).

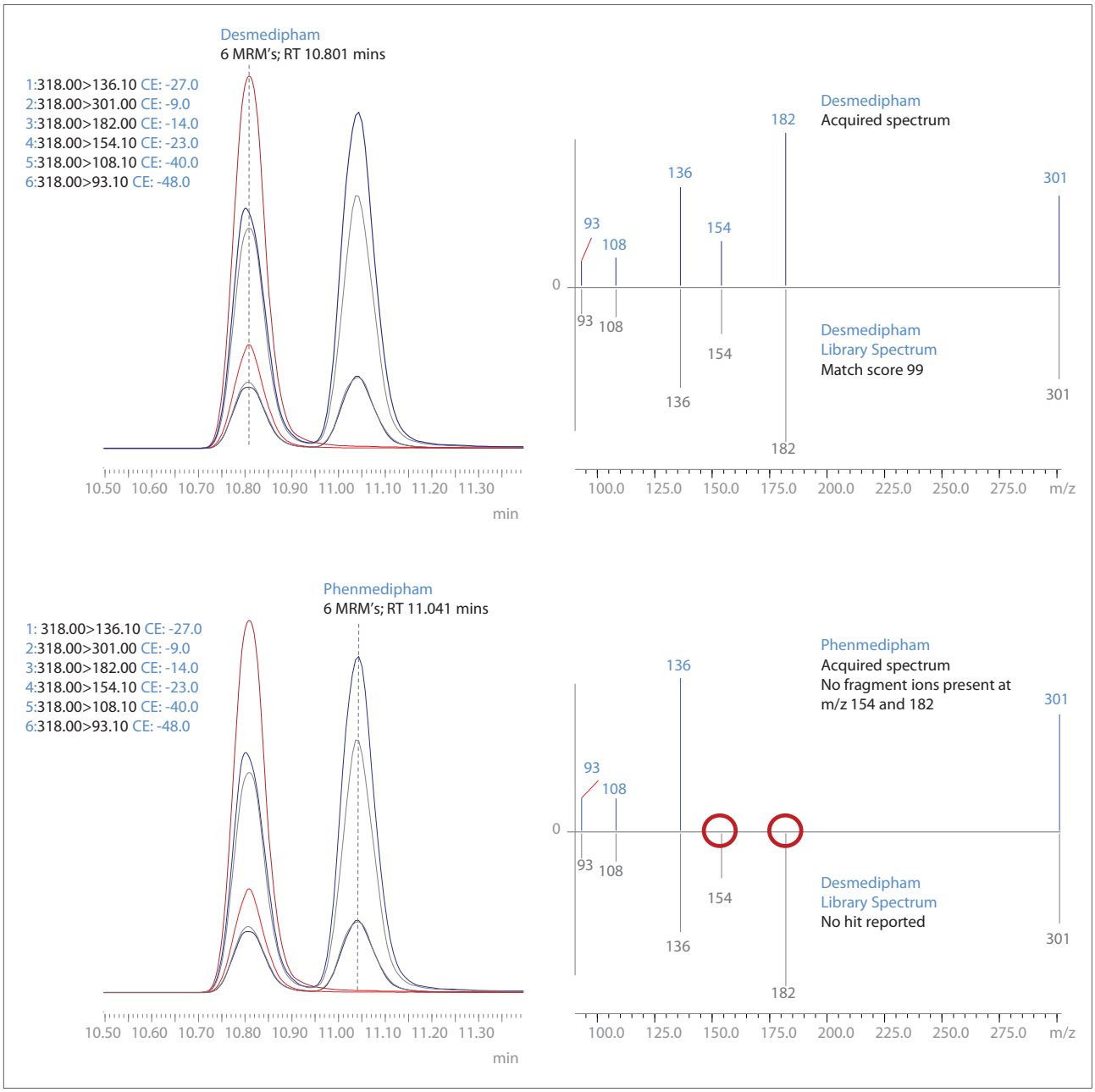

Fig. 7 MRM chromatogram for desmedipham and phenmedipham spiked into a cumin extract at 0.1 mg/kg. As phenmedipham shares common transitions and elutes at a similar retention time as desmedipham the MRM spectrum can be used to distinguish between both pesticides to avoid false positive reporting.

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Quantitation

As one example, carbendazim was spiked into a matrix at three different concentration levels. In Fig. 8, all MRM transitions were detected even at the reporting level of 0.010mg/kg with a signal to noise for all fragment ion transitions greater than 9. The response was linear for all transitions throughout the calibration range (0.010-0.200mg/kg) as shown Fig. 9.

The limit on the number of MRM transations used to generate a product ion spectrum is dependent on the chemical structure of the pesticide molecule. In the case of carbendazim, several bonds could be broken using collision energies between 10-60V resulting in a product ion spectrum of 12 fragment ions. The product ion spectrum can then be used for library search and analyte confirmation as shown in Fig. 10. For each calibration level ranging from 0.010-0.200mg/kg the library similarity score was greater than 99 confidently confirming the target analyte. The advantage of this technique is that library searchable product ion spectrum data is used in target compound identification without compromising sensitivity, accuracy and robustness in quantitative data reporting.

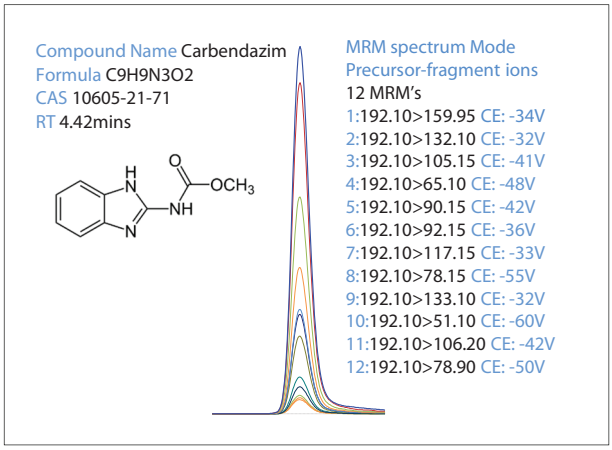

Fig. 8 By applying a range of collision energies to carbendazim 12 precursor-fragment ions are generated. MRM 192.10>159.95 was used in generating sensitive and robust quantitation whilst the product ion spectrum using all 12 fragment ions was used in confirming peak identification.

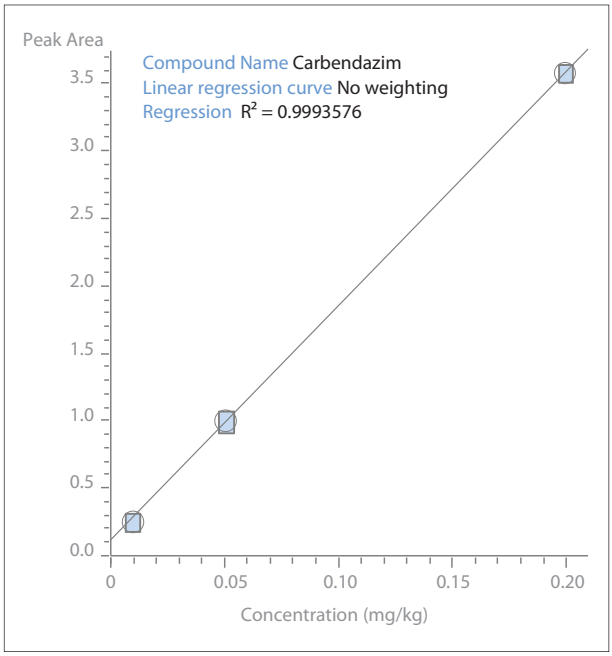

Fig. 9 Calibration curve for carbendazim using the optimized quantitation ion transition (MRM 192.10>159.95). The response was linear for all calibration and QC samples. All 12 fragment ions were above a signal to noise ratio of 10 even at the reporting level of 0.010mg/kg.

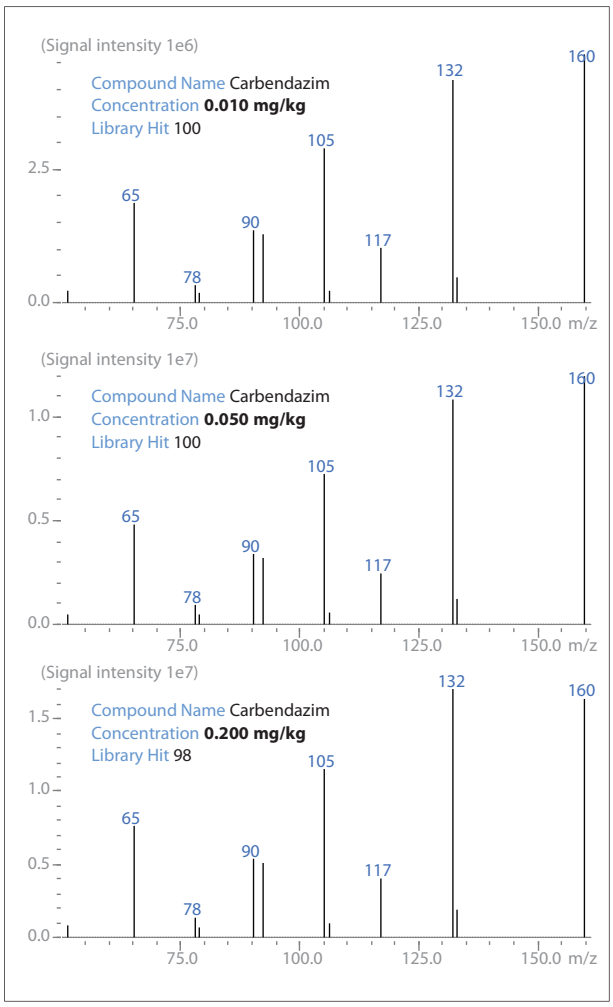

Fig. 10 MRM Product ion spectrum data for carbendazim in 3 calibration levels (0.010-0.200mg/kg) spiked into a food matrix was compared with an authentic library spectrum of carbendazim. In all library searches the similarity score was greater than 99 indicating a very high confidence in compound verification and reporting.

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Data Reporting

Automated reference library matching and quantitation results can be simply viewed using LabSolutions Insight software (Fig 11).

LabSolutions Insight software helps to review by exception and to reduce false positive reporting by verifying compound identification using library matching scores and retention time variation from a calibration standard.

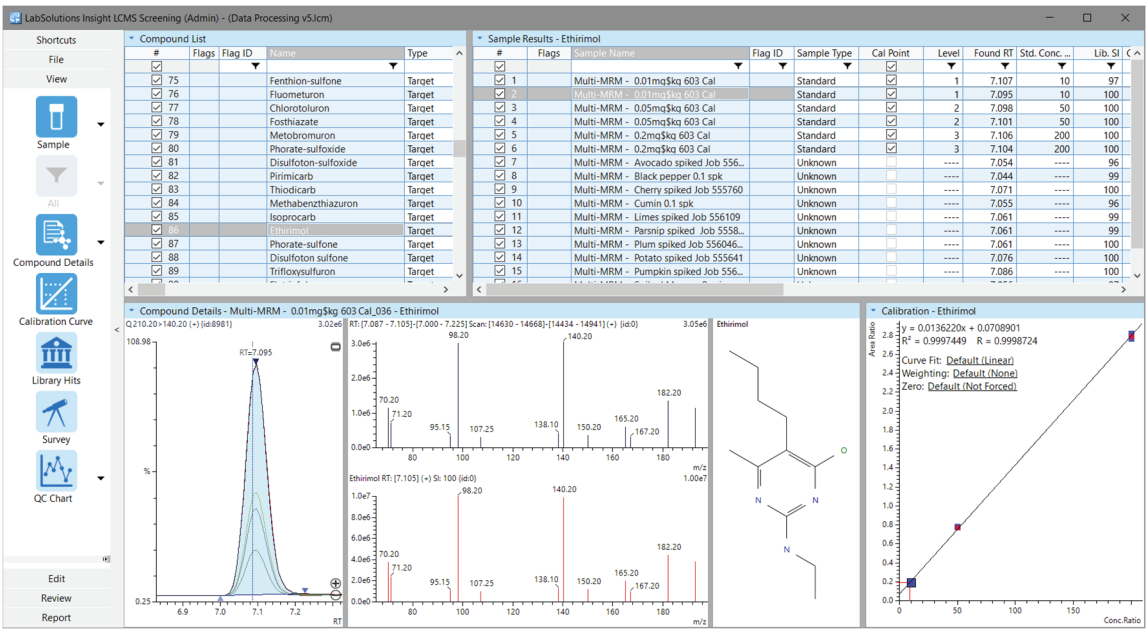

Fig. 11 LabSolutions Insight software helps to review quantitative and reference library matching results quickly and easily. Flexible filtering and sorting tools can be used to help reduce reporting false detects, especially in high throughput laboratories by filtering results based upon a similarity score with a reference library product ion spectrum.

Conclusions

False positive results are a major issue for all pesticide residue monitoring laboratories. EU regulations require that retention time and the ion ratio between 2 MRM transitions are within a set threshold. However, even applying this criteria false positives may occur for certain pesticide/commodity combinations.

In this application paper, we have applied MRM Spectrum Mode to identify and quantify 193 target pesticides in a number of different sample matrices. The library score is used as an additional identification criterion in order to improve identification confidence.

Acquisition of the MRM Spectrum mode method (1,291 MRM transitions) did not compromise data quality when compared to a conventional 2 MRM per compound method (386 MRM transitions) with consistent signal response and repeatability in both methods. The MRM product ion spectrums were demonstrated to be consistent across the linear range and between different matrices. The method acquired data in both positive and negative ion modes with a polarity switching time of 5 msec enabling fast cycle times and a high data collection rate.

All 1,291 MRM transitions were acquired throughout the MRM window. No 'triggering' of MRM transitions was necessary due to the short dwell times that were applied using the LCMS-8060. Therefore, MRM transitions can be swapped between qualifier and qualifier if needed and the peak shape of the additional MRM transitions can be assessed.

References

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Application News

No. C162

Liquid Chromatograph Mass Spectrometry

Quantitative Analysis of Highly Polar Pesticides in Food Using SFC/MS

Since achieving sufficient retention and favorable separation in normal batch analysis of highly polar pesticides has proved difficult due to their chemical characteristics, a number of individual analysis methods are employed for LC/MS/MS analysis. To rectify this situation, EURL-SRM (Stuttgart, Germany), an EU Reference Laboratories member in charge of individual analysis method development, is developing a batch analysis method called "QuPPe (Quick Polar Pesticides)" for highly polar pesticides that are difficult to analyze using pretreatment with the QuEChERS method as well as normal batch analysis methods. This method proposes multiple methods to suit each sample and target chemical compound (M. Anastassiades et al; QuPPe of EURL-SRM (Version 9.1; 2016)).

Until now, analysis of highly polar pesticides using LC/MS/MS has used a variety of separation methods including HILIC mode, mixed mode, normal phase, and reversed phase. However, all of these methods have restrictions on the chemical compounds that can be analyzed together and this remains a problem. On the contrary, supercritical fluid chromatography (SFC) has the advantage of being able to separate a wide array of chemical compounds at once due to the characteristics of the mobile phase that is used. In addition, since the separation behavior with SFC differs from that with LC even when using a column of the same separation mode, SFC may be effective for the analyses of chemical compounds for which retention and separation are difficult in LC. This article introduces an example of batch analysis of highly polar pesticides using SFC.

Y.Fujito, D. Baker, A. Barnes, C. Titman, J. Horner, N. Loftus

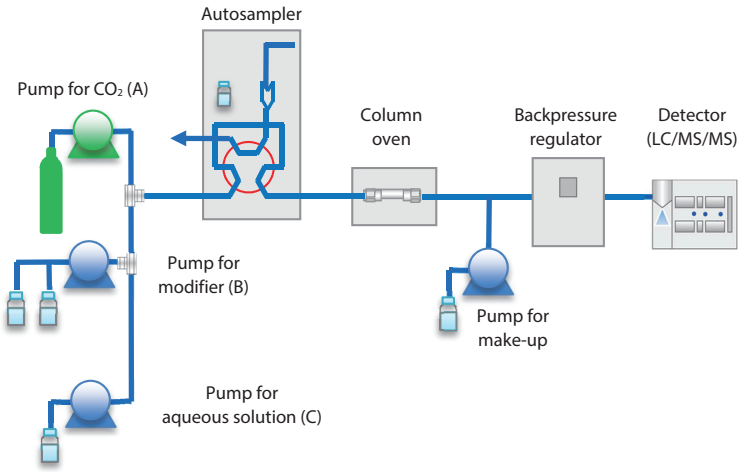

Fig. 1 SFC/MS System Configuration Diagram

In this experiment, an examination of adding a small amount of water to a modifier was performed for the purpose of eluting and separating highly polar pesticides.
In order to simplify this examination, a low-pressure gradient pump (LPGE) was used as pump B and the modifier was automatically prepared by mobile phase blending.

Table 1 SFC/MS Analysis Conditions

Supercritical fluid chromatography		Mass spectrometry	
SFC	Nexera UC system	**LC-MS/MS**	LCMS-8060
Analytical column	Restek Ultra Silica (150 × 2.1 mm 3 µm)	**Ionisation mode**	Heated ESI
Column temperature	50 °C	**Scan speed**	15,000 u/sec
Flow rate	0.8 mL/min (0.6 mL/min 13-22 min)	**MRM Dwell time**	3 msec
Pump A	CO_2	**Pause time**	1 msec
Pump B (modifier solvent)	Acetonitrile + 0.5 % formic acid + 10 mM ammonium formate	**Interface temp.**	300 °C
Pump C (modifier solvent)	Water + 0.5 % formic acid + 10 mM ammonium formate	**Heating block**	350 °C
Pump D (make up solvent)	Methanol	**Desolvation line**	250 °C
Makeup solvent flow rate	0.2 mL/min		

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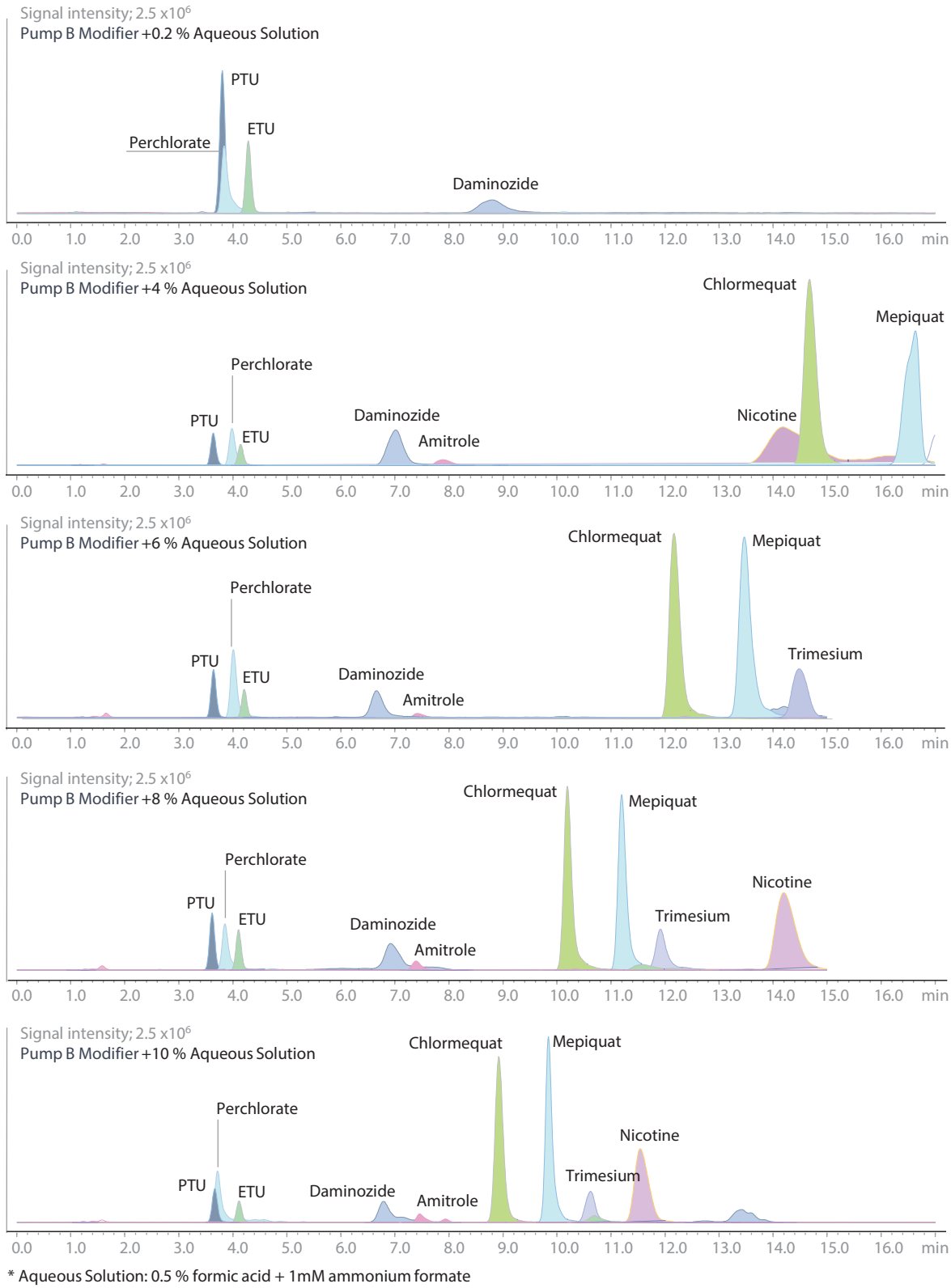
Examination of SFC Separation Conditions

Normally, SFC performs gradient separation using supercritical carbon dioxide and an organic solvent (such as methanol and acetonitrile), which is referred to as a modifier. However, some highly polar chemical compounds exhibit strong retention in columns resulting in cases where separation and elution is insufficient even with 100 % organic solvent. In this experiment, since a number of highly polar pesticides could not be eluted with 100 % organic solvent, separation was examined by adding a small amount of water to the modifier.

Supercritical carbon dioxide has low polarity and low miscibility with water. This means that only a limited amount of water can be added to the modifier (normally about 0.1 to 10 %). We therefore examined separation behavior by adding water by the amount equivalent to 0.2, 4, 6, 8, and 10 % to the modifier. Through examination based on the peak profiles and separation patterns of the eluted components, we adopted a water content of 6 %. However, there were chemical compounds that could not be eluted even with this condition.



* Aqueous Solution: 0.5 % formic acid + 1mM ammonium formate

Fig. 2 Effect of Water on Separation Behavior of Highly Polar Pesticides in SFC/MS

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Optimization of SFC Separation Conditions

When we examined addition of water to the modifier, we were able to confirm elution of most chemical compounds with the 6 % aqueous solution. However, nicotine and kasugamycine, which both exhibit strong retention, could not be eluted. Any further addition of aqueous solution in the presence of carbon dioxide adversely affects gradient accuracy and may impair the stability of the analysis method. For this reason, aqueous solution was added using a separate pump (pump C) after the modifier reached 100 % (Fig. 4).

This allowed elution of the remaining highly polar pesticides and enabled batch separation of the highly polar pesticides from logP-3.47 to 1.96.

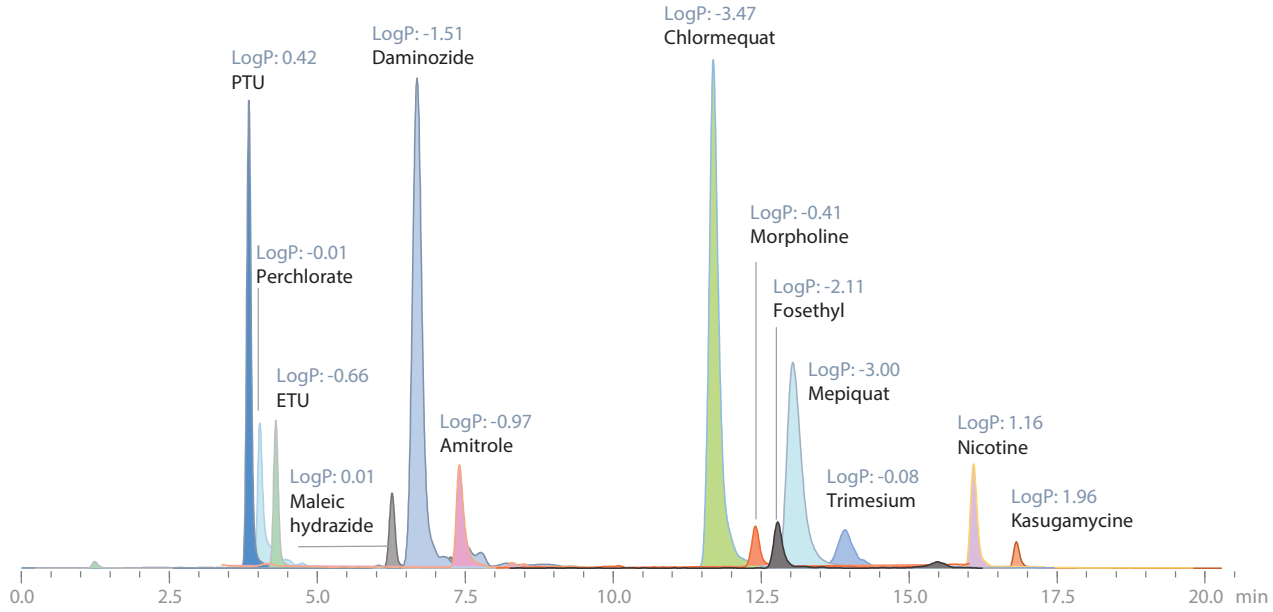
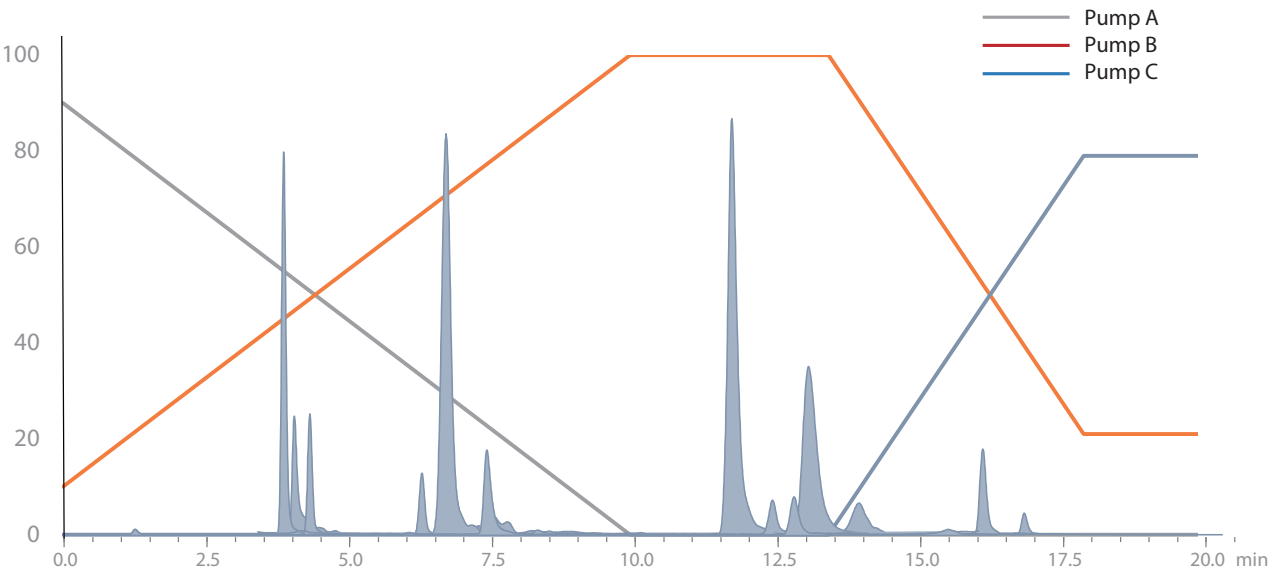


Fig. 3 MRM Chromatogram of Highly Polar Pesticides Using SFC-MS
(Addition of 200 ppb Pesticide Standard Solution into Flaxseed Extract Using QuPPe)



The initial SFC/MS conditions;
Pump A 90 % : Carbon Dioxide
Pump B 10 % : 6 % Water in Acetonitrile containing 0.5 % formic acid and 10 mM ammonium formate
Pump C 0 % : Aqueous solution containing 0.5 % formic acid + 10 mM ammonium formate

Fig. 4 Ternary Gradient Program

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Sample Preparation and Analysis

Flaxseed and lemon were used as food samples and extraction was performed using a method compliant with QuPPe. (The extracts were provided by Concept Life Sciences, a contract analytical laboratory located in the U.K.) Standard solution of highly polar pesticides was added to these matrix solutions, which were then directly injected into the SFC-MS/MS.

Quantitative Analysis of Highly Polar Pesticides

In order to verify the quantitative performance of the developed SFC/MS analysis method, matrix calibration curves were created using each food extract to which standard solution of the highly polar pesticides was added. The calibration curve range was 10 to 200 ppb and accuracy was verified using the internal standard method regarding components for which an internal standard substance labeled with a stable isotope was obtained.

The calibration curve created for each sample showed favorable linearity for all chemical compounds regardless of the sample matrix.

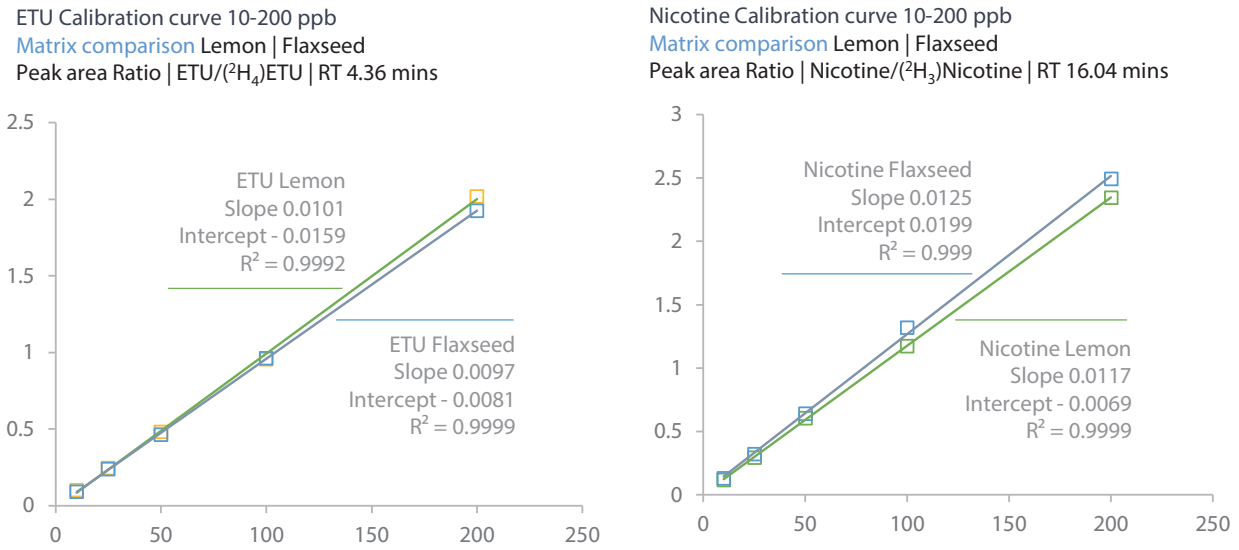

Fig. 5 Matrix Calibration Curves of Representative Highly Polar Pesticides
(ETU: fast eluting compound, Nicotine: slow eluting compound, Samples: lemon, flaxseed)

Table 2 Calibration Curve Linearity and Repeatability at 100 ppb of Eight Highly Polar Pesticide Components

Compound	RT (min)	Internal Standard	IS RT (min)	Quan MRM	%RSD 100ppb	R^2
Perchlorate	3.95	$^{18}O_4$ Perchlorate	3.91	99.00 > 82.90	4.98	0.968
ETU	4.36	$^{2}H_4$ ETU	4.26	103.10 > 44.05	4.84	0.999
Maleic hydrazide	6.28	$^{2}H_2$ Maleic hydrazide	6.28	113.00 > 67.10	6.81	0.997
Chlormequat	11.58	$^{2}H_4$ Chlormequat	11.54	121.90 > 58.10	1.75	1.000
Fosethyl	12.50	$^{2}H_{15}$ Fosethyl	12.50	109.00 > 80.95	6.78	0.999
Morpholine	12.19	$^{2}H_8$ Morpholine	12.23	87.90 > 70.05	10.74	0.996
Mepiquat	12.72	$^{2}H_3$ Mepiquat	12.69	114.30 > 98.10	7.66	0.998
Nicotine	16.06	$^{2}H_3$ Nicotine	16.03	163.00 > 130.00	2.31	0.999

First Edition: Nov. 2017

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Application News

No. C207A

Liquid Chromatograph Mass Spectrometry

Analysis of Residual Pesticides (No. 1: in Soybeans) Using Triple Quadrupole LC/MS/MS <LCMS™-8060>

With a recent increase in the number of regulated pesticides, more effective methods for simultaneous analysis of residual pesticides in food are required.

QuEChERS, which was introduced by the United States Department of Agriculture (USDA) in 2003, is known as a quick and simple pretreatment method and approved as an official method by AOAC and CEN. This method requires no special instruments for extraction of pesticides, but the contaminants that cannot be completely removed by means of purification procedures may affect accurate quantitative analysis. In such cases, sample dilution or review of the purification process is also required.

This article introduces an example of the analysis of 158 pesticides among those specified in the Multi-residue Method I and II for Agricultural Chemicals by LC-MS (Agricultural Products)[1)] by measuring these pesticides in the sample solutions pretreated using the QuEChERS method, resulting in good recovery.

M. Kawashima, N. Kato

Sample Pretreatment

The soybean sample was pretreated using the QuEChERS method. The workflow of sample pretreatment is shown in Fig. 1. The concentration of samples extracted was 0.5 g/mL.

PL2005MIX-4, 5, 6, 7, 8, 9 and 10, mixtures of pesticides manufactured by Hayashi Pure Chemical Ind.,Ltd., were used as the standard samples. The matrix effect was identified using the matrix standard solution (10 ng/mL pesticide in the solution) made by adding each pesticide to the sample solution pretreated with the QuEChERS method to reach a concentration of 0.02 mg/kg in the soybean extract.

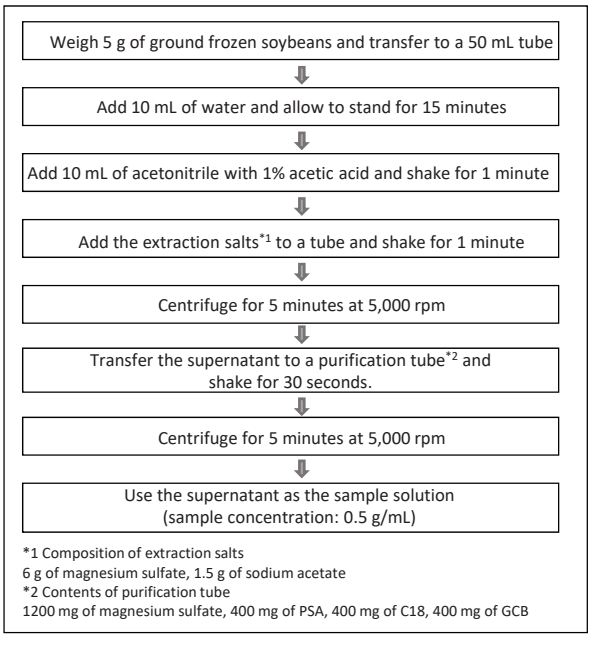

Fig. 1 Pretreatment Workflow

Analytical conditions

The analytical conditions for HPLC and MS are shown in Table 1.

Table 1. Analytical Conditions

[HPLC conditions] (Nexera™ X2)		
Column	:	Shim-pack Scepter™ C18-120 (100 mm x 2.1 mm I.D., 3 µm)
Mobile phases	:	A) 5 mM ammonium formate, 0.02% acetic acid in H_2O B) 5 mM ammonium formate, 0.02% acetic acid in MeOH
Gradient Program	:	B 5% (0-2 min) – B 50% (5 min) – B 97% (13-16 min) – B 5% (16.1-20 min)
Flow rate	:	0.3 mL/min
Column Temp.	:	40°C
Injection volume	:	1 µL
[MS conditions] (LCMS-8060)		
Ionization	:	ESI (Positive and negative mode)
Probe Voltage	:	+2.0 kV / -1.5 kV
Mode	:	MRM
Nebulizing gas flow	:	3.0 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
DL Temp.	:	200°C
Heat Block Temp.	:	300°C
Interface Temp.	:	200°C
Probe position	:	+2.0 mm

MRM Measurement of Matrix Standard Solution

Fig. 2 shows the MRM chromatogram of the matrix standard solution made by adding pesticide standard solution to the soybean extract.

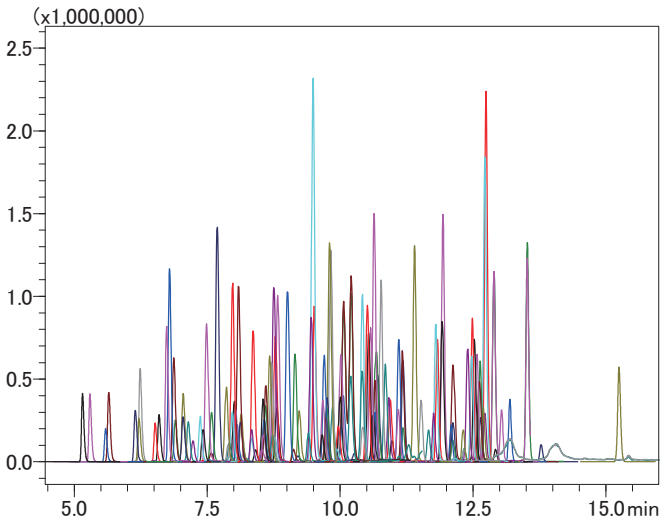

Fig. 2 Example of Peak Detections of 158 Pesticides (Soybean Extract Added to 10 ng/mL Standard Solution)

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Recovery

The recovery and peak area repeatabilities (n=6) of the matrix standard solutions for 158 pesticides were determined. The results of determination are shown in Table 2. Details of the recovery are shown in Fig. 3.

The recovery for 156 of 158 pesticides were in the range of 70 to 120%. Even in the test solution containing a high concentration of sample, 98.7% of these pesticides were not significantly affected by the matrix, resulting in good recovery and repeatabilities.

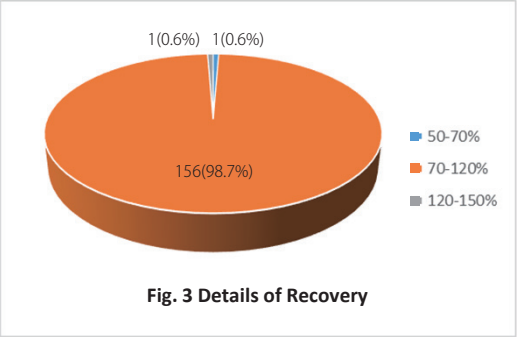

Fig. 3 Details of Recovery

Table 2. Recovery and Peak Area Repeatability of Sample Solutions

Compound name	Recovery(%)	%RSD
1-Naphthaleneacetic Acid	76.3	8.32
2,4-D	97.3	4.66
4-Chlorophenoxyacetic acid	76.5	5.34
Abamectin B1a	93.5	1.37
Acibenzolar-S-methyl	90.0	8.84
Acifluorfen	88.7	7.50
Aldicarb	92.8	4.24
Aldoxycarb	96.5	1.15
Anilofos	93.3	3.08
Aramite	95.8	3.88
Azamethiphos	93.5	4.68
Azimsulfuron	84.9	8.46
Azinphos-methyl	95.2	3.91
Azoxystrobin	93.6	6.70
Bendiocarb	99.2	2.14
Bensulfuron-methyl	97.9	5.57
Benzofenap	97.4	1.42
Boscalid	98.0	2.53
Bromoxynil	92.7	8.96
Butafenacil	99.0	2.63
Carbaryl(NAC)	98.5	5.77
Carbofuran	93.5	5.35
Carpropamid	94.4	3.72
Chloridazon	92.1	2.82
Chlorimuron-ethyl	101.8	7.76
Chloroxuron	95.5	5.95
Chlorsulfuron	96.9	5.36
Chromafenozide	95.1	1.65
Cinosulfuron	98.4	9.43
Clodinafop acid	91.9	8.00
Clofentezine	84.7	4.06
Clomeprop	87.6	3.77
Cloprop	97.8	9.36
Cloquintocet-mexyl	97.8	3.64
Cloransulam-methyl	101.9	6.16
Clothianidin	85.9	5.42
Cumyluron	98.5	2.16
Cyazofamid	95.7	1.39
Cyclanilide	96.8	4.10
Cycloate	94.9	3.31
Cycloprothrin	72.6	5.13
Cyclosulfamuron	96.8	5.74
Cyflufenamid	91.9	1.72
Cyprodinil	94.6	3.10
Diallate	94.1	4.05
Dichlorprop	97.5	9.08
Diclomezine	100.7	8.89
Diclosulam	95.9	2.23
Diflubenzuron	87.4	3.15
Dimethirimol	94.7	3.20
Dimethomorph(E)	98.1	2.86
Dimethomorph(Z)	98.1	2.86
Diuron	96.6	2.34
Dymuron	91.8	5.36
Epoxiconazole	90.0	1.87
Ethametsulfuron-methyl	95.3	6.52
Ethoxysulfuron	101.2	4.22
Fenamidone	92.8	3.74
Fenhexamid	92.0	6.06
Fenobucarb	96.5	4.83
Fenoxaprop-ethyl	88.0	2.68
Fenoxycarb	92.9	3.94
Fenpyroximate E	93.4	3.11
Fenpyroximate Z	93.9	2.90
Ferimzone(E)	95.2	2.66
Ferimzone(Z)	96.9	2.08
Flazasulfuron	97.3	4.79
Florasulam	97.6	7.50
Fluazifop	94.1	6.73
Flufenacet	95.3	4.66
Flufenoxuron	93.1	7.23
Flumetsulam	101.8	6.45
Fluridone	93.6	2.22
Fluroxypyr	91.2	8.65
Fomesafen	103.4	4.83
Foramsulfuron	115.1	8.65
Forchlorfenuron	92.0	5.85
Furametpyr	97.5	3.02
Furathiocarb	93.5	1.76
Gibberellic acid	63.5	10.61
Halosulfuron-methyl	80.2	7.45
Haloxyfop	82.7	7.70
Haloxyfop	85.0	8.78
Hexaflumuron	96.4	7.41
Hexythiazox	93.8	3.31
Imazalil	106.8	3.52
Imazaquin	95.5	4.25
Imazosulfuron	94.0	5.67
Imidacloprid	89.9	1.12
Indanofan	94.3	3.07
Indoxacarb	99.9	4.16
Iodosulfuron-methyl	93.0	7.59
Ioxynil	98.8	7.08
Iprovalicarb	95.6	3.46
Isoxaflutole	92.8	6.43
Lactofen	90.5	2.10
Linuron	95.4	3.54
Lufenuron	93.2	4.48
MCPA	96.1	4.16
MCPB	86.6	2.15
Mecoprop+Mecoprop-P	85.2	2.79
Mepanipyrim	94.5	3.97
Mesosulfuron-methyl	95.1	3.50
Methabenzthiazuron	96.6	2.11
Methiocarb	95.0	4.16
Methomyl	97.8	1.44
Methoxyfenozide	92.0	6.35
Metosulam	102.7	7.44
Metsulfuron-methyl	96.5	5.54
Monolinuron	96.0	2.52
Naproanilide	91.3	5.53
Naptalam	95.9	9.97
Novaluron	90.9	6.44
Oryzalin	90.5	9.95
Oxamyl	93.6	2.71
Oxaziclomefone	89.5	4.16
Oxycarboxin	96.3	3.21
Pencycuron	95.8	3.89
Penoxsulam	99.9	2.92
Pentoxazone	79.9	9.20
Phenmedipham	95.5	1.35
Pirimicarb	94.6	5.96
Primisulfuron-methyl	95.0	3.81
Propaquizafop	93.6	3.13
Propoxycarbazone	142.5	8.39
Prosulfuron	99.6	6.03
Pyraclostrobin	96.5	4.62
Pyrazolynate	93.7	2.96
Pyrazosulfuron-ethyl	96.8	8.99
Pyriftalid	95.7	4.52
Quizalofop-ethyl	81.4	1.31
Silafluofen	84.9	6.25
Simeconazole	95.1	2.63
SpinosynA	100.9	6.04
SpinosynD	105.9	4.00
Sulfentrazone	86.4	7.29
Sulfosulfuron	97.4	7.18
Tebufenozide	95.3	5.07
Tebuthiuron	91.6	4.51
Teflubenzuron	87.9	7.57
Tetrachlorvinphos	94.2	3.80
Thiabendazole	94.0	3.67
Thiacloprid	94.3	1.85
Thiamethoxam	96.0	1.72
Thidiazuron	82.8	7.17
Thifensulfuron-methyl	96.6	6.43
Thiodicarb	95.8	2.97
Tralkoxydim 1	104.0	5.25
Tralkoxydim 2	93.9	4.25
Triasulfuron	96.8	3.77
Tribenuron-methyl	94.1	7.77
Triclopyr	94.5	7.21
Tridemorph 1	97.5	4.28
Tridemorph 2	96.3	2.18
Trifloxysulfuron	96.3	7.75
Triflumuron	92.9	3.70
Triflusulfuron-methyl	99.5	5.49
Triticonazole	94.2	2.68

1) Ministry of Health, Labour and Welfare: Testing Method of Agricultural Chemical Residues in Food, Feed Additives or Components of Animal Pharmaceuticals (PFSB/DFS Notification No. 1129002)P

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Simultaneous Determination of Pesticide Residues in Vegetable Extract by LC/MS/MS [LCMS™-8050]

To protect food safety, it is important to establish detection criteria for pesticide residues and methods to improve accuracy when measuring the concentration of the target substances. Generally, the standard addition method and matrix-matched calibration curve are more useful techniques for reducing the matrix effect than the absolute calibration method. However, these techniques are not necessarily simple, since an independent calibration curve is required for each sample of a wide variety of samples. In this report, we introduce an LC/MS/MS analysis technique which is capable of obtaining high recovery accuracy with the absolute calibration method.

N. Maeshima

Methods and Materials

The test matrix solution (carrot extract) was prepared by a solid-phase extraction technique with QuEChERS (STQ method). The range of the calibration curve for the standard concentrations was set from 0.1 to 50 ng/mL, and was determined by the absolute calibration method. Tables 1 and 2 below show the LC/MS analysis conditions.

Table 1 LC Conditions

[LC] Nexera™X2 system		
Column	:	Shim-pack Scepter™ C18-120 (100 mm × 2.0 mm, 1.9 µm)
Column temp.	:	40 °C
Solvent A	:	5 mmol/L ammonium acetate/water
Solvent B	:	5 mmol/L ammonium acetate/methanol
Gradient	:	B conc. 3% (0 min) → 10% (2 min) → 55% (6 min) → 100% (21-26 min) → 3% (26.01-32 min)
Flow rate	:	0.4 mL/min (0-21 min) → 0.6 mL/min (21.01-27 min) → 0.4 mL/min (27.01-32 min)
Injection vol.	:	5 µL

Table 2 MS Conditions

[MS] LCMS-8050		
Ionization	:	ESI positive and negative
DL temp.	:	150 °C
Interface temp.	:	200 °C
Block heater temp.	:	500 °C
Nebulizer gas flow	:	2 L/min
Drying gas flow	:	10 L/min
Heating gas flow	:	10 L/min
Probe position	:	3 mm
Dwell time	:	1-200 ms
Pause time	:	1 ms

Spike and Recovery Test

For analysis of the carrot extract spiked with 1 ng/mL as the final concentration of the target pesticides, the number of targets with recovery rates within 70% to 120% was 82 of a total of 89 pesticides (Fig. 1). Moreover, reproducibility under 3% (n = 10, Fig. 2) was achieved with 70 pesticides. Table 3 shows the details of the MRM transition, recovery rate, and reproducibility. Fig. 4 shows the MS chromatogram of some compounds and the calibration curves of them.

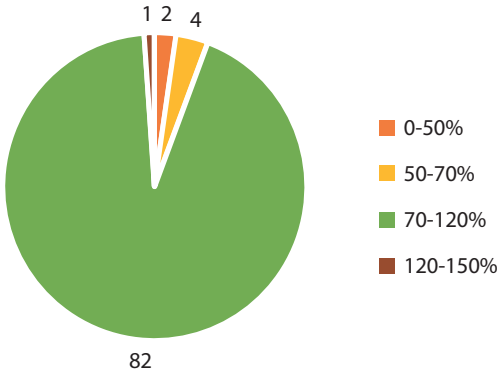

Fig. 1 Recovery Rate of Target Pesticides

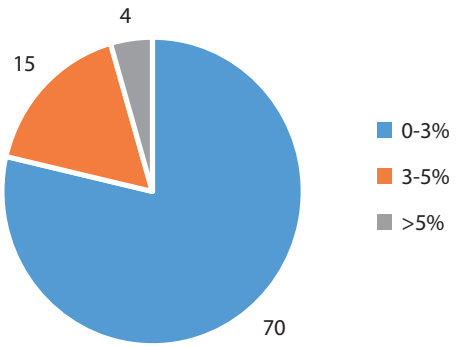

Fig. 2 Reproducibility of Target Pesticides

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Table 3-1 MRM Transition, Recovery Rate, and Reproducibility of Target Pesticides (1 ng/mL)

No.	Name	Retention time (min)	+/-	MRM transition	Recovery rate (%)	Repro-ducibility (%)	Determination range (ng/mL)
1	Abamectin B1a	17.70	+	890.30 > 305.30	52.3	3.7	0.1-50
2	Acibenzolar-S-methyl	10.30	+	210.90 > 136.05	97.9	5.2	0.1-50
3	Aldicarb	6.65	+	208.20 > 115.85	95.8	2.5	0.1-50
4	Aldicarb-sulfone (Aldoxycarb)	4.31	+	240.10 > 86.20	98.3	0.5	0.1-50
5	Anilofos	12.60	+	368.00 > 125.00	97.6	3.1	0.1-50
6	Azamethiphos	7.17	+	325.00 > 182.90	98.1	1.1	0.1-20
7	Azinphos-methyl	9.60	+	318.00 > 132.05	100.0	3.1	0.1-50
8	Azoxystrobin	10.08	+	404.00 > 371.95	103.1	2.8	0.1-50
9	Bendiocarb	7.39	+	224.20 > 109.10	86.0	2.1	0.1-50
10	Benzofenap	14.57	+	431.15 > 105.25	95.2	1.2	0.1-50
11	Boscalid	10.29	+	343.00 > 306.95	80.1	1.1	0.1-50
12	Butafenacil	11.26	+	492.10 > 330.85	103.0	2.2	0.1-50
13	Carbaryl (NAC)	7.84	+	202.10 > 145.10	79.2	4.1	0.1-50
14	Carbofuran	7.41	+	222.10 > 123.15	87.2	1.3	0.1-50
15	Carpropamid	12.68	+	334.10 > 139.10	129.1	4.7	0.1-50
16	Chloridazon	6.10	+	222.10 > 104.10	94.2	1.1	0.1-50
17	Chloroxuron	11.02	+	291.10 > 72.15	101.8	1.5	0.1-50
18	Chromafenozide	11.41	+	395.20 > 175.15	95.0	1.2	0.1-50
19	Clofentezine	13.82	+	303.00 > 138.15	77.8	1.9	0.1-50
20	Cloquintocet-mexyl	15.24	+	336.10 > 237.90	102.6	2.7	0.1-50
21	Clothianidin	5.66	+	250.00 > 132.05	96.5	1.4	0.1-20
22	Cumyluron	10.91	+	303.20 > 185.10	106.1	2.7	0.1-50
23	Cyazofamid	11.69	+	325.00 > 108.10	95.4	3.1	0.1-50
24	Cycloate	13.57	+	216.10 > 154.00	104.9	8.6	0.5-50
25	Cycloprothrin	16.87	+	499.00 > 181.10	90.4	2.0	0.1-20
26	Cyflufenamid	13.52	+	413.10 > 295.05	96.8	2.0	0.1-50
27	Cyprodinil	12.83	+	226.10 > 108.00	98.6	4.7	0.1-50
28	Daimuron (Dymron)	10.69	+	269.25 > 151.15	104.8	2.1	0.1-50
29	Diflubenzuron	11.96	+	311.00 > 158.10	46.2	2.4	0.1-50
30	Dimethirimol	8.22	+	210.20 > 71.00	97.6	1.1	0.1-50
31	Dimethomorph (*E,Z*)	10.11	+	388.10 > 301.00	93.4	2.9	0.1-20
32		10.58			97.7	3.3	0.1-50
33	Diuron (DCMU)	8.92	+	233.00 > 72.10	99.5	1.2	0.1-50
34	Epoxiconazole	11.57	+	330.00 > 121.10	98.2	2.6	0.1-50
35	Fenamidone	10.13	+	312.10 > 236.00	99.2	1.4	0.1-50
36	Fenoxaprop-ethyl	14.65	+	362.10 > 287.90	99.1	1.2	0.1-50
37	Fenoxycarb	12.20	+	302.10 > 88.00	94.1	1.5	0.1-50
38	Fenpyroximate (*E,Z*)	15.66	+	422.30 > 366.20	96.9	1.2	0.1-50
39		16.90			98.2	1.4	0.1-50
40	Ferimzone (*E,Z*)	10.27	+	255.20 > 91.05	98.7	3.6	0.1-50
41		10.43			103.7	1.8	0.1-50
42	Flufenacet	11.29	+	364.10 > 152.05	90.0	2.0	0.1-50
43	Flufenoxuron	16.44	+	489.00 > 158.10	91.9	1.2	0.1-50
44	Fluridone	9.85	+	330.10 > 309.00	106.9	1.9	0.1-50
45	Furametpyr	8.55	+	334.10 > 157.10	94.8	1.5	0.1-50
46	Furathiocarb	14.84	+	383.20 > 195.00	97.9	1.9	0.1-50
47	Hexaflumuron	14.68	-	458.80 > 439.00	115.5	1.3	0.1-50
48	Hexythiazox	15.71	+	353.10 > 228.00	85.1	1.1	0.1-50
49	Imazalil	12.46	+	297.10 > 159.05	94.6	3.3	0.1-50
50	Imidacloprid	5.62	+	256.10 > 174.95	98.3	1.2	0.1-20

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Table 3-2 MRM Transition, Recovery Rate and Reproducibility of Target Pesticides (1 ng/mL)

No.	Name	Retention time (min)	+/-	MRM transition	Recovery rate (%)	Repro-ducibility (%)	Determination range (ng/mL)
51	Indanofan	11.67	+	341.10 > 175.15	97.8	2.6	0.1-50
52	Indoxacarb	14.19	+	528.10 > 203.00	96.1	1.5	0.1-50
53	Iprovalicarb	11.16	+	321.20 > 119.15	51.9	4.0	0.1-50
54	Isoxaflutole	8.78	+	360.10 > 251.00	81.9	5.7	0.1-50
55	Linuron	9.96	+	248.80 > 182.05	88.6	2.0	0.1-50
56	Lufenuron	15.97	-	508.90 > 339.00	115.1	2.3	0.1-50
57	Mepanipyrim	11.53	+	224.10 > 77.00	87.7	4.4	0.1-50
58	Methabenzthiazuron	8.67	+	222.10 > 150.10	95.7	2.0	0.1-50
59	Methiocarb	10.02	+	226.10 > 121.10	93.1	6.6	0.2-50
60	Methomyl	4.82	+	163.00 > 87.90	95.4	1.7	0.1-50
61	Methoxyfenozide	10.86	+	369.20 > 149.15	103.4	1.3	0.1-50
62	Monolinuron	8.08	+	215.10 > 99.10	88.7	2.1	0.1-50
63	Naproanilide	12.13	+	292.25 > 171.25	96.1	1.7	0.1-20
64	Novaluron	14.81	+	493.00 > 158.00	68.0	2.0	0.1-20
65	Oryzalin	11.36	+	347.10 > 288.00	36.1	3.5	0.1-50
66	Oxamyl	4.53	+	237.10 > 72.10	98.1	1.4	0.1-50
67	Oxaziclomefone	14.70	+	376.20 > 190.15	99.2	3.7	0.1-50
68	Oxycarboxin	6.24	+	268.10 > 175.00	90.8	2.6	0.1-50
69	Pencycuron	13.61	+	329.10 > 125.00	100.0	2.0	0.1-50
70	Pentoxazone	14.82	+	371.10 > 286.00	85.2	1.9	0.1-20
71	Pirimicarb	8.37	+	239.20 > 72.00	99.9	1.7	0.1-50
72	Propaquizafop	15.09	+	444.10 > 100.15	83.1	2.4	0.1-50
73	Pyrazolynate	13.69	+	439.10 > 91.15	102.6	3.0	0.1-50
74	Pyriftalid	9.77	+	319.10 > 139.10	100.2	1.9	0.1-50
75	Quizalofop-ethyl	14.67	+	373.10 > 298.90	79.8	2.5	0.1-50
76	Silafluofen	19.93	+	426.30 > 287.15	101.8	1.1	0.1-50
77	Simeconazole	11.11	+	294.10 > 69.95	63.0	1.9	0.1-50
78	Spinosyn A	18.07	+	732.60 > 142.20	102.9	1.5	0.1-50
79	Spinosyn D	18.65	+	746.60 > 142.10	105.0	1.7	0.1-50
80	Tebufenozide	12.11	+	353.20 > 133.10	97.1	1.1	0.1-50
81	Tebuthiuron	7.59	+	229.10 > 172.00	97.7	1.4	0.1-50
82	Teflubenzuron	15.32	-	378.80 > 339.00	99.0	2.7	0.1-50
83	Tetrachlorvinphos (CVMP)	12.13	+	366.90 > 127.15	98.1	3.2	0.1-50
84	Thiabendazole	7.21	+	202.00 > 175.00	104.3	2.8	0.1-50
85	Thiacloprid	6.44	+	253.00 > 126.05	95.5	1.3	0.1-50
86	Thiamethoxam	4.95	+	292.00 > 211.10	93.9	1.5	0.1-50
87	Thiodicarb	8.40	+	355.00 > 88.00	99.6	2.5	0.1-50
88	Triflumuron	13.35	+	359.00 > 156.05	91.6	0.9	0.1-50
89	Triticonazole	11.18	+	318.10 > 70.15	87.1	2.1	0.1-50

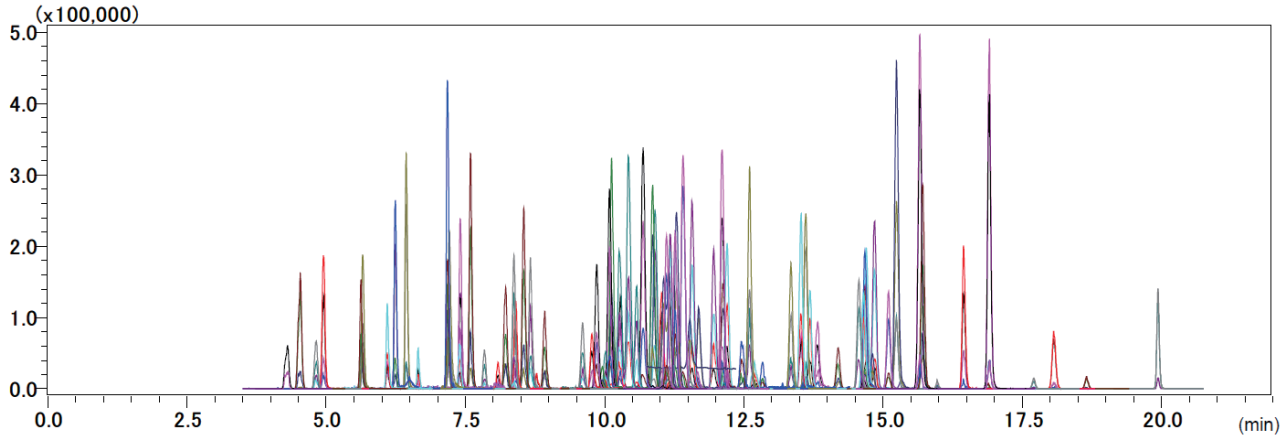

Fig. 3 MS Chromatogram of Pesticides (1 ng/mL)

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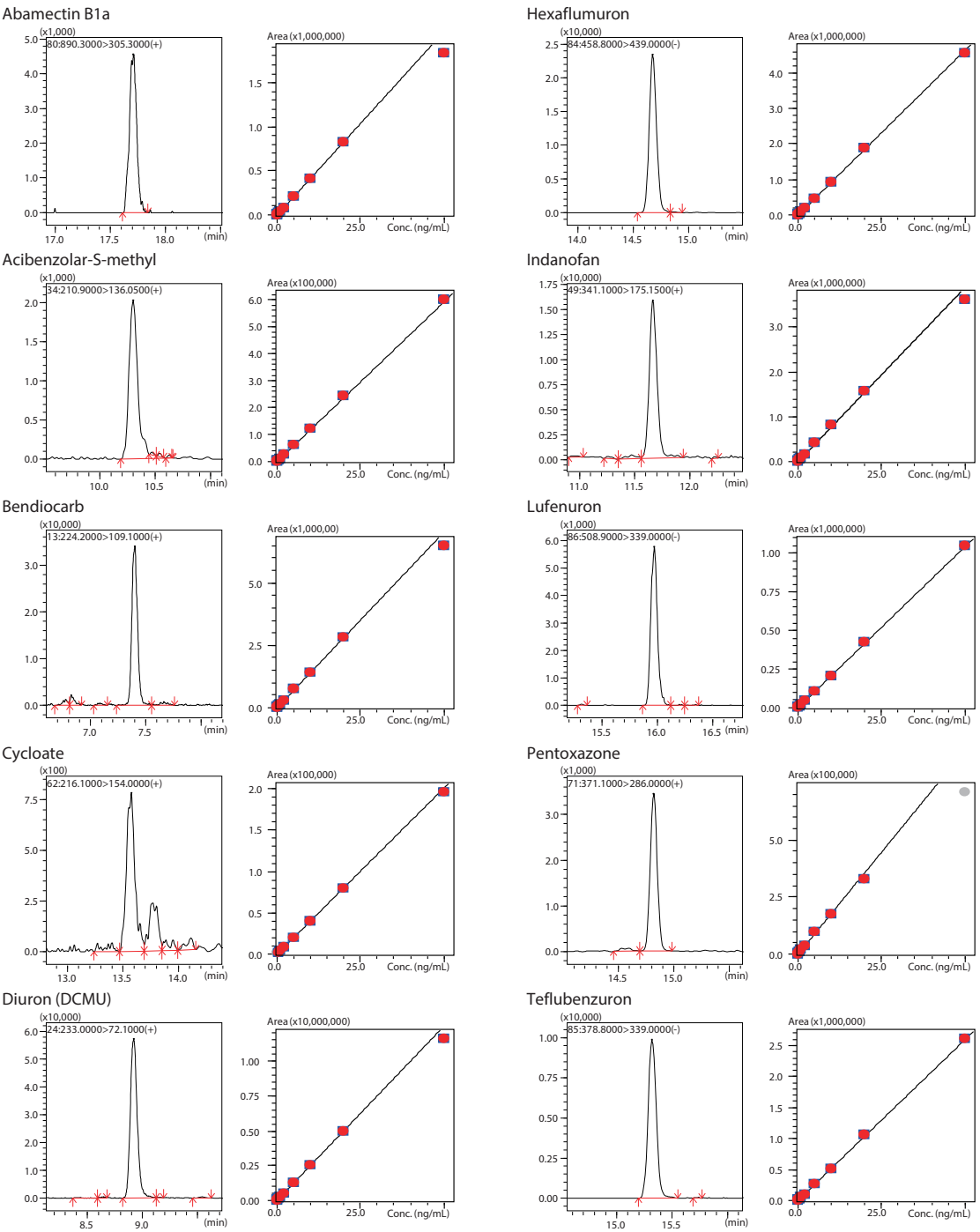

Fig. 4 MS Chromatograms of Spiked Samples (Final Concentration: 1 ng/mL) and Calibration Curves of Pesticides

Conclusion

Using an LCMS-8050 triple quadrupole mass spectrometer with Nexera X2 UHPLC, it was possible to obtain a high recovery rate and high reproducibility with the absolute calibration method.

<Acknowledgements>
We would like to thank the Institute of Public Health in Sagamihara for their cooperation.

First Edition: Feb. 2020

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Application News

No.C99

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Foods in which chemical residues, like pesticides, feed additives, and veterinary drugs found in excess of maximum residue levels have been banned from sale in many countries around the world. Compounds that are subject to residue standards vary widely and the list is expected to grow. Because of this, there is a need for a highly sensitive and rapid analytical technique to analyze as many of these compounds as possible in a single run. This Application News introduces an example of the high-sensitivity analysis of 89 veterinary drugs in a crude extract of livestock and fishery products.

Sample Preparation

The typical samples used in the analysis of veterinary drugs contain large amounts of lipids because they are commonly meat and fish samples. Sample preparation is extremely important to ensure excellent sensitivity and repeatability. To avoid the typical time-consuming and laborious solid phase extraction sample preparation procedure, the QuEChERS method, which is typically used for the preparation of vegetables, was selected to simplify sample preparation.
The QuEChERS method normally consists of two steps, the first is an acetonitrile extraction and the second a cleanup step, but this time only the acetonitrile extraction step was used.

* QuEChERS Extraction Salts kit: Restek Q-sep™ AOAC2007.01

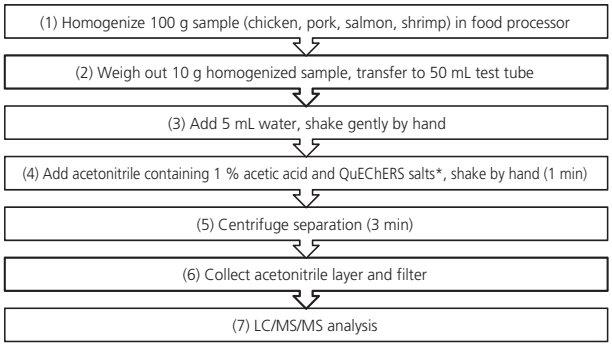
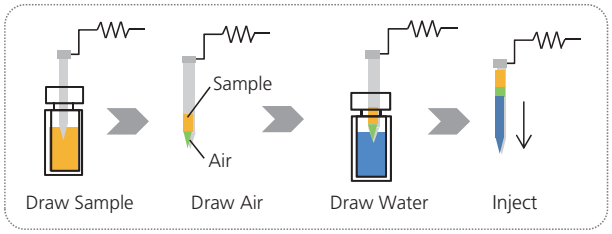


Fig. 1 Sample Preparation Procedure

Improved Peak Shape Using Sample / Water Co-Injection

When conducting reversed phase chromatography, the peaks of polar compounds may split or collapse depending on the relationship between the sample solvent and mobile phase. In cases where the sample solvent is rich in organic solvent, the elution strength must be lowered (by substitution or dilution) with the addition of water. As the pretreated sample solvent in this analysis consists of 100 % acetonitrile, injection in that state into the LC/MS will result in split peaks for some of the substances (Fig. 2 left).
To eliminate as much of the time and effort typically associated with sample preparation, the pretreatment features of the autosampler (SIL-30A) were utilized to conduct co-injection of sample and water, which resulted in improved peak shapes.



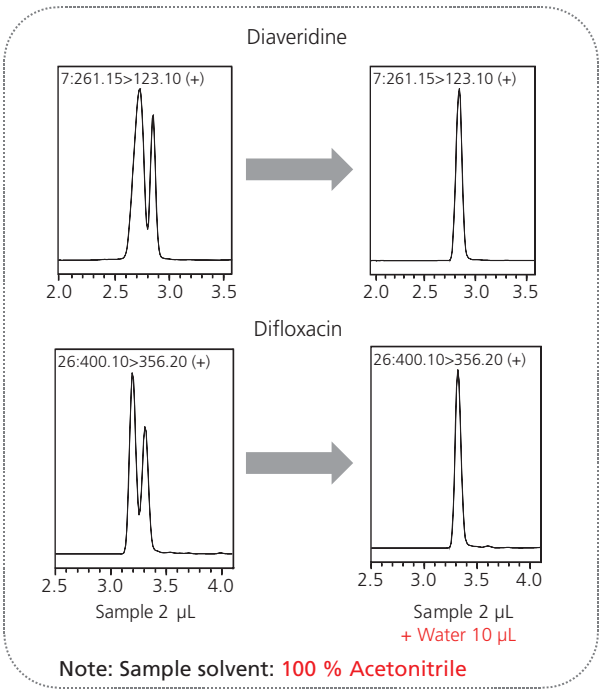


Fig. 2 Comparison of Peak Shape

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MRM Analysis of Matrix Standards

Fig. 3 shows the MRM chromatogram of the matrix standard solution consisting of the sample solution with added standard solution (data obtained using pork extract solution). Table 1 shows the lower limits of quantitation for the standard solution without added matrix and with added matrix, respectively. In a crude extract obtained by acetonitrile extraction alone, sensitivity was comparable to that obtained for most of the compounds using only standard solution. Although there were several compounds for which the lower limit of quantitation was different in the standard solution than the matrix-added solution, rather than attributing this to matrix effects, it is thought to be caused by elevated background due to ions derived from contaminating components (Refer to Fig. 5).

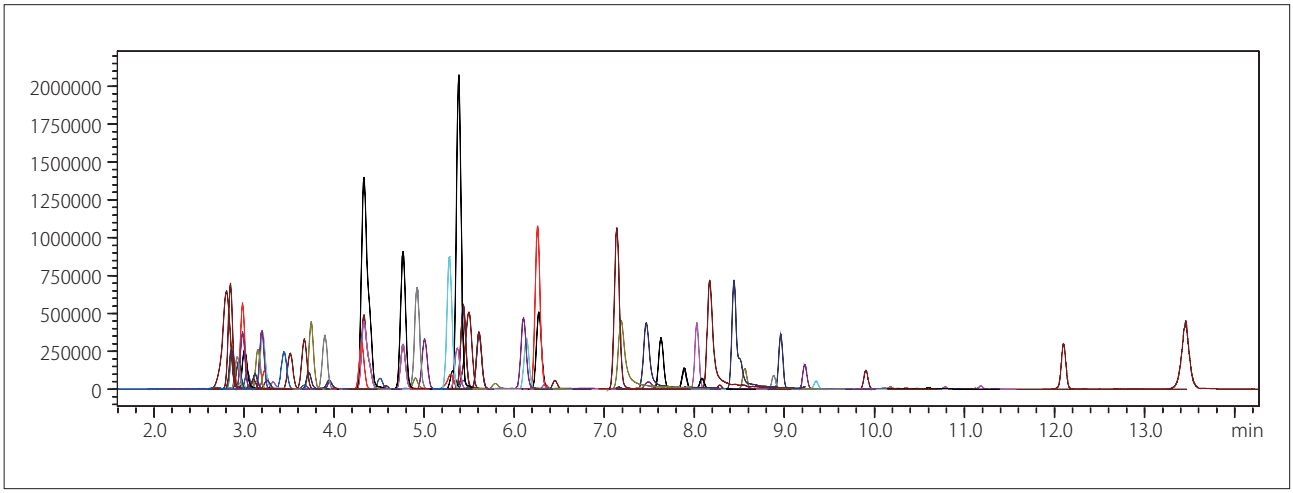

Fig. 3 MRM Chromatograms of 89 Veterinary Drugs (10 µg/L pork extract solution with added standard solution)

Table 1 LOQs of Veterinary Drugs in Neat Standards and Matrix Standards and Calibration Range of Veterinary Drugs in Matrix Standards

	Std. Solution	Matrix-Added Std. Solution	
	Min. Conc.	Min. Conc.	Max. Conc.
Gentamicin	0.5	1	50
Sulfanilamide	1	1	50
Levamisole	0.05	0.05	50
Lincomycin	0.01	0.01	10
5-Propylsulfonyl-1-benzimidazole-2-amine	0.05	0.05	10
Diaveridine	0.01	0.01	10
Trimethoprim	0.02	0.02	20
Marbofloxacin	0.01	0.01	50
Sulfisomidine	0.02	0.02	20
Norfloxacin	0.5	0.5	50
Ormetoprim	0.02	0.02	10
Thiabendazole	0.01	0.01	10
Ciprofloxacin	0.05	0.5	10
Neospiramycin I	0.01	0.05	10
Danofloxacin	0.1	0.1	10
Enrofloxacin	0.05	0.1	50
Oxytetracycline	0.01	0.1	50
Xylazine	0.01	0.01	10
Orbifloxacin	0.05	0.05	50
Sulfacetamide	1	1	50
Clenbuterol	0.01	0.01	10
Tetracycline	0.05	0.01	50
Spiramycin I	0.01	0.01	50
Sarafloxacin	0.5	0.5	50
Difloxacin	0.05	0.1	50
Sulfadiazine	0.02	0.1	20
Sulfathiazole	0.02	0.1	20
Sulfapyridine	0.02	0.1	20
Carbadox	0.05	0.05	10
Pyrimethamine	0.02	0.02	20
Sulfamerazine	0.02	0.02	20
Chlortetracycline	0.1	0.1	50
Tilmicosin	0.1	0.1	50
Thiamphenicol	1	1	50
Sulfadimidine	0.02	0.02	20
Sulfametoxydiazine	0.01	0.02	10
Sulfamethoxypyridazine	0.02	0.02	20
Sulfisozole	0.01	0.01	50
Trichlorfon (DEP)	0.05	0.05	50
Sulfamonomethoxine	0.02	0.02	20
Furazolidone	1	1	50
Difurazone	0.05	0.05	50
Erythromycin A	0.01	0.01	50
Cefazolin	0.5	0.5	50

	Std. Solution	Matrix-Added Std. Solution	
	Min. Conc.	Min. Conc.	Max. Conc.
Sulfachloropyridazine	0.02	0.02	20
Sulfadimethoxine	0.02	0.02	10
Tylosin	0.05	0.05	50
Sulfamethoxazole	0.02	0.1	10
Sulfaethoxypyridazine	0.02	0.02	10
Tiamulin	0.01	0.01	50
Florfenicol	0.5	10	50
2Acetylamino 5nitrothiazole	0.05	0.05	50
Sulfatroxazole	0.01	0.01	5
Leucomycin	0.01	0.01	50
Sulfisoxazole	0.01	0.05	50
Oxolinic acid	0.01	0.1	50
Chloramphenicol	0.5	1	50
Clorsulon	0.5	1	50
Sulfabenzamide	0.01	0.01	10
Ethopabate	0.01	0.01	10
Sulfadoxine	0.02	0.02	20
Sulfaquinoxaline	0.02	0.02	10
Prednisolone	0.1	0.05	20
Ofloxacin	0.5	0.5	50
Flubendazole	0.01	0.01	50
Methylprednisolone	0.5	0.5	50
Nalidixic acid	0.01	0.01	50
Dexamethasone	0.5	0.5	50
Flumequine	0.01	0.01	50
Benzylpenicillin	0.5	0.5	50
Sulfanitran	0.2	0.2	50
Sulfabromomethazine	0.01	0.01	50
betaTrenbolone	0.02	0.1	50
Emamectin B1a	0.01	0.01	50
alphaTrenbolone	0.02	0.1	50
Piromidic acid	0.01	0.05	50
Zeranol	1	0.1	50
Ketoprofen	0.01	0.05	50
Testosterone	0.01	0.05	10
Famphur	0.05	0.05	50
Fenobucarb (BPMC)	0.01	0.01	50
Clostebol	0.05	0.05	50
Dichlofenac	0.01	0.01	50
Melengestrol Acetate	0.05	0.05	50
Temephos (Abate)	0.01	0.5	50
Allethrin	0.1	1	50
Closantel	0.01	0.01	10
Monensin	0.01	0.01	10

(Unit: µg/L)

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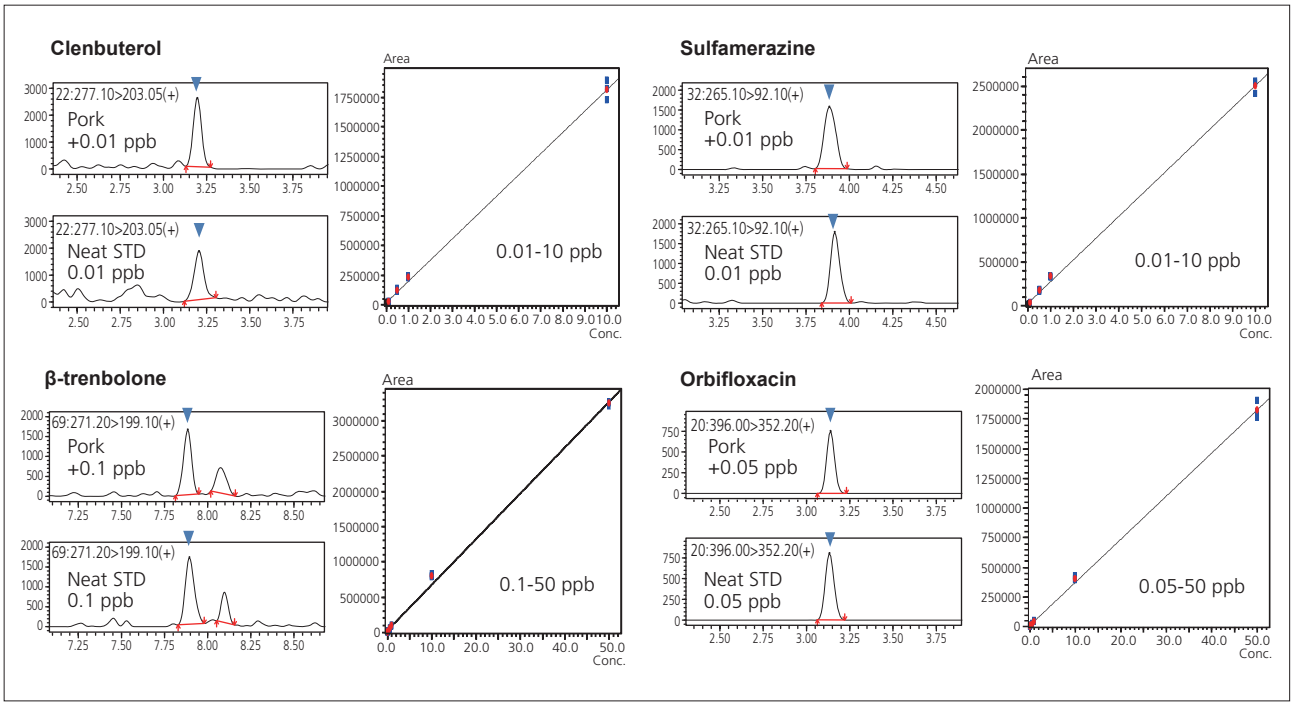

Fig. 4 MRM Chromatograms in the Vicinity of the LOQ and Calibration Curves of Typical Compounds

Recoveries of Veterinary Drugs in Crude Extracts from Livestock and Fishery Products (Matrix Effect Verification)

We examined whether or not the matrix affected measurement of actual samples. This time, four types of food product samples were used, including shrimp, chicken meat, pork, and salmon. Standard solution was added to the acetonitrile extraction solution of each of these to obtain a final concentration of 10 µg/L, after which the rates of recovery were determined. The results indicated that 90 % of the compounds were recovered at rates of 70 to 120 % and measurement was accomplished without any adverse matrix effects even though the crude extract solution was subjected only to acetonitrile extraction.

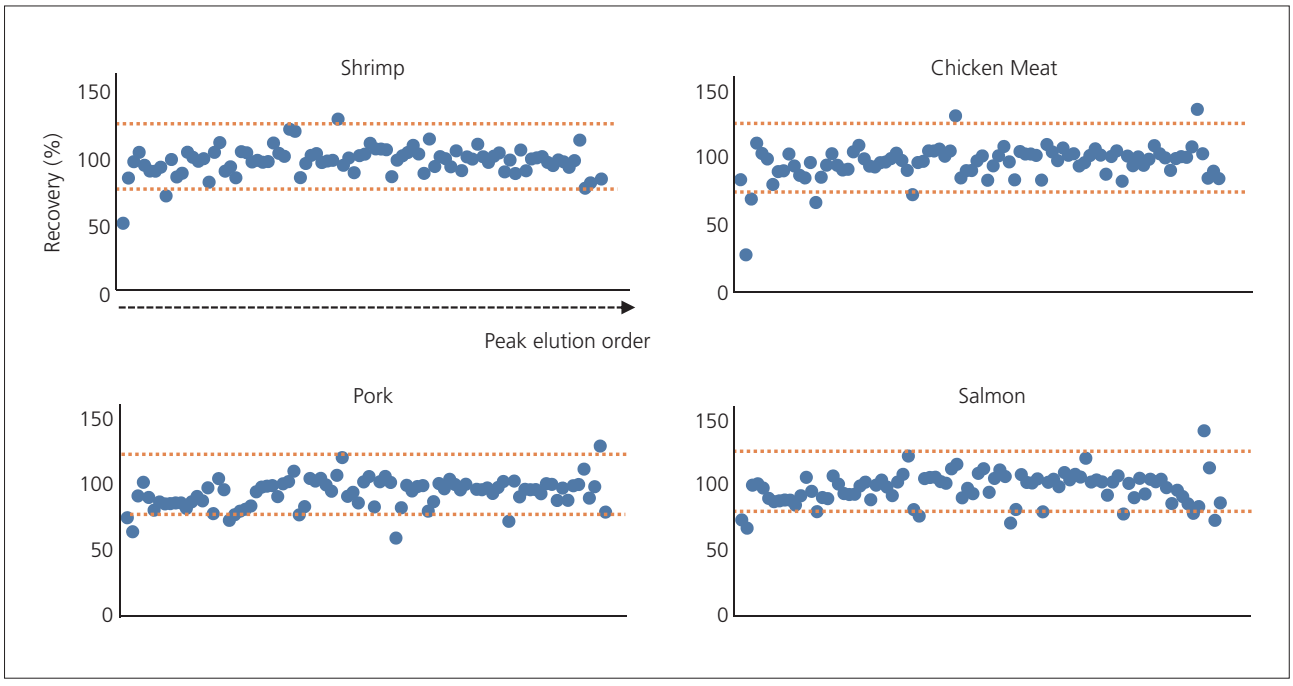

Fig. 5 Recoveries of Veterinary Drugs in Each of the Matrices

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Acetonitrile Extraction Efficiency Using QuEChERS Method

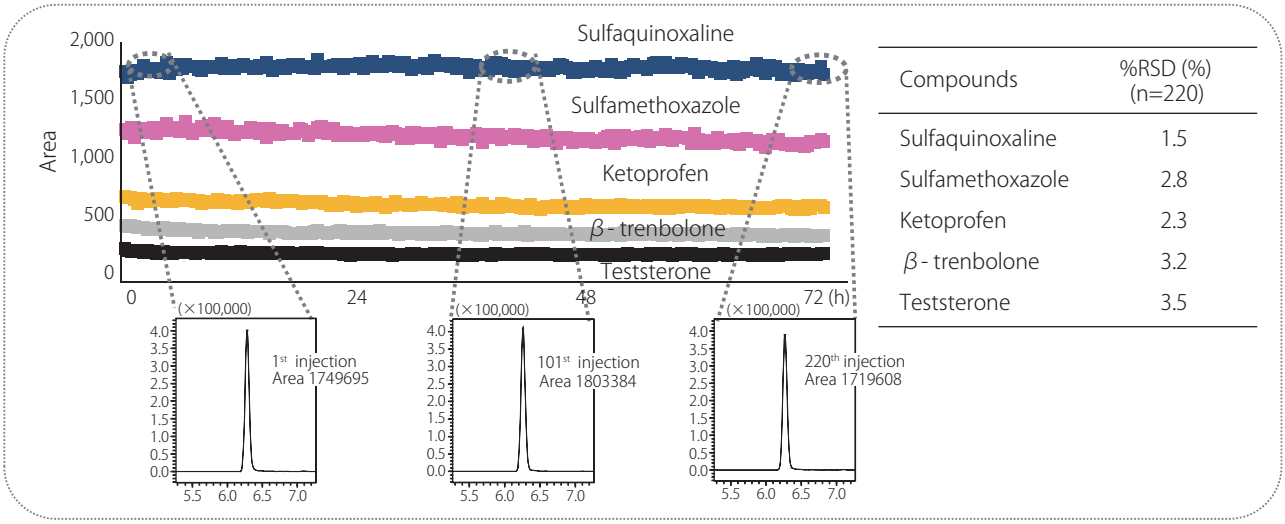
To check the efficiency of acetonitrile extraction by the QuEChERS method, standard solution was added at stage (2) of Fig. 1 to obtain a concentration of 10 µg/L, and the recoveries were determined. Good recoveries of approximately 80 % were obtained in cases both with and without the addition of matrix. However, relatively poor recoveries were seen for highly polar compounds such as tetracycline and quinolone. For these compounds, it is necessary to examine the use of a separate extraction solvent and extraction reagent.

Table 2 Recoveries (Pre-Spike)

Recovery	Without Matrix	With Matrix (Pork)	Compounds with Poor Recovery
< 50 %	17 (19 %)	13 (15 %)	Tetracyclines Quinolones
50 % - 70 %	1 (1 %)	8 (9 %)	
> 70 %	71 (80 %)	68 (76 %)	

Robustness

We checked the long-term stability of the instrument using a solution of pork crude extract (spiked with 10 µg/L standard solution). Even after continuous measurement of an extremely complex matrix over a period of 3 days, we were able to obtain stable data.



Compounds	%RSD (%) (n=220)
Sulfaquinoxaline	1.5
Sulfamethoxazole	2.8
Ketoprofen	2.3
β - trenbolone	3.2
Teststerone	3.5

Fig. 6 Area Plot and %RSD of Typical Compounds with Continuous Analysis

Table 3 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅱ (75 mm × 2.0 mm I.D., 2.2 µm)
Mobile Phase A	: 0.1 % Formic Acid - Water
Mobile Phase B	: Acetonitrile
Time Program	: 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10-13 min) → 1 %B (13.01-16 min)
Flowrate	: 0.2 mL/min.
Injection Volume	: 2 µL (2 µL sample solution + 10 µL water)
Oven Temperature	: 40 °C
Ionization Mode	: ESI (Positive / Negative)
Probe Voltage	: +2.0 kV / -1.0 kV
Neburizing Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 10.0 L/min.
Heating Gas Flow	: 10.0 L/min.
Interface Temperature	: 400 °C
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C

First Edition: Jan. 2015

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Application News

No. C161

Liquid Chromatograph Mass Spectrometry

Multi-Residue Veterinary Drug Analysis of >200 Compounds using MRM Spectrum Mode by LC-MS/MS

Veterinary drugs are used for therapeutic, metaphylactic, prophylactic and growth promotion purposes. To provide an assurance that food from animals is safe with regards to residues of veterinary medicines, regulatory authorities have established Maximum Residue Limits (MRL's) for certain drugs in target tissues and animal species. Some pharmacologically active compounds identified by regulatory authorities have been prohibited and their hazardousness at all levels are being considered (EU regulation EC 37/2010; Commission Decision 2003/181/EC; 21CFR Part 556 Tolerances for Residues of New Animal Drugs in Food). In this article, we describe how a triple quadrupole mass spectrometer, which is both highly sensitive and selective, contributes to reducing false positive and false negative reporting when using a measurement mode called MRM Spectrum mode. MRM Spectrum mode acquires a high number of fragment ion transitions for each target compound and generates fragmentation spectra that can be used in routine library searching and compound verification using reference library match scores.

David Baker *1, Laetitia Fages *2, Eric Capodanno *2, Neil Loftus *1
*1 : Shimadzu, Manchester, UK
*2 : Phytocontrol, Nimes, France

Samples and Analysis Conditions

Samples of beef, egg, honey, milk and salmon were extracted and spiked with veterinary drugs in the calibration range of 0.001 to 0.1 mg/kg. Repeatability was assessed at low and high concentrations. Samples were measured using Shimadzu's Nexera X2 UHPLC and LCMS-8060 triple quadrupole mass spectrometer (Table 1 and 2). Over 200 veterinary drugs were targeted and over 2,000 MRM transitions in both ESI +/- were monitored during a gradient elution time of 12 minutes.

Table 1 UHPLC Conditions

Liquid chromatography				
UHPLC	Nexera LC system			
Analytical column	Restek Biphenyl (100 × 2.1, 2.7 µm)			
Column temperature	40 °C			
Flow rate	0.4 mL/minute			
Solvent A	0.1 % formic acid 0.5 mM ammonium formate solution			
Solvent B	0.1 % formic acid in methanol			
Binary Gradient	Time (mins)	%B	Time (mins)	%B
	0.00	2	14.60	2
	12.50	100	17.50	Stop
	14.50	100		

Table 2 MS/MS Acquisition Parameters

Mass spectrometry	
Mass spectrometer	Shimadzu LCMS-8060
Pause time/dwell time	1 msec/3 msec
Polarity switching time	Pos/neg switching time set to 5 msec
Scope	218 drugs in positive ion mode (including internal standards)
	11 drugs in negative ion mode
	Structure Analytics (in house development tool)
Source temperatures (interface; heat block; DL)	350 °C; 300 °C; 150 °C
Gas flows (nebulising; heating; drying)	3 L/min; 10 L/min; 10 L/min

Advantages of MRM Spectrum Mode

The measurement method can be easily set using the MRM optimization tool and measurement window (MRM Synchronization) settings of LabSolutions LCMS. The method achieves high data densities and a high data sampling rate across each elution peak. This approach generates a consistent loop time and sampling rate producing reliable quantitation and peak integration. It also provides great operator-friendliness in routine simultaneous analysis of veterinary drugs by enhancing flexibility in qualifier and quantifier ion selection. The number of fragment ion transitions generated from a single precursor ion is limited only by the chemical structure of the veterinary drug.

Results

MRM Spectrum mode was used to acquire a high number of fragment ion transitions for each veterinary drug target. For chlortetracycline, 11 precursor-fragment ion transitions were acquired using optimized collision energies (Fig. 1). Acquiring a high number of fragment ion transitions enables generation of fragmentation spectra which can be used in library searching and compound verification for each veterinary drug. (Chlortetratcycline is a tetracycline class of antimicrobials. According to the Sixth ESVAC report published in 2016, of the overall sales of antimicrobials in the 29 EU countries in 2014, the largest amount, expressed as a proportion of mg/PCU, was accounted for by tetracyclines (33.4 %). This is followed by penicillins (25.5 %) and sulfonamides (11.0 %). Chlortetracycline was selected as a representative target).

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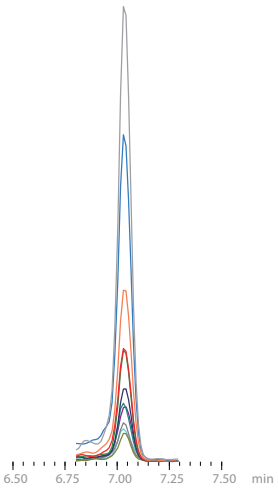
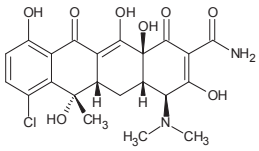


Compound name Chlortetracycline
Accurate mass 479.1216 [M+H]+
Formula C22H23ClN2O8
CAS 57-62-5



MRM Spectrum Mode
11 MRM's acquired for chlortetracycline at 10pg/uL in egg.

1:479.10>444.00 (+)	CE: -23V	7:479.10>300.80(+)	CE: -45V
2:479.10>461.95 (+)	CE: -35V	8:479.10>287.90(+)	CE: -53V
3:479.10>154.00 (+)	CE: -34V	9:479.10>274.95(+)	CE: -44V
4:479.10>98.05(+)	CE: -45V	10:479.10>370.95(+)	CE: -31V
5:479.10>260.05(+)	CE: -60V	11:479.10>285.85(+)	CE: -56V
6:479.10>303.05(+)	CE: -37V		

MRM Spectrum mode
Higher specificity
Higher reporting confidence
Library searchable fragment data.
The number of precursor-fragment ion transitions monitored is limited only by the structural chemistry of the molecule. Typically more than 10 precursor-fragment ion transitions were monitored for each veterinary drug.

Fig. 1 Utilization of MRM Spectrum Mode (Chlortetracycline)

Fig. 2 shows the MRM reference spectrum for chlortetracycline with assigned fragment structures. The MRM Spectrum mode is a measurement mode which combines MRM with the generation of a product ion spectrum. The product ion spectrum can be used for compound identification by searching a library.

As the collision energy was optimized for each fragment ion to generate a product ion spectrum, the library spectrum is highly specific and selective.

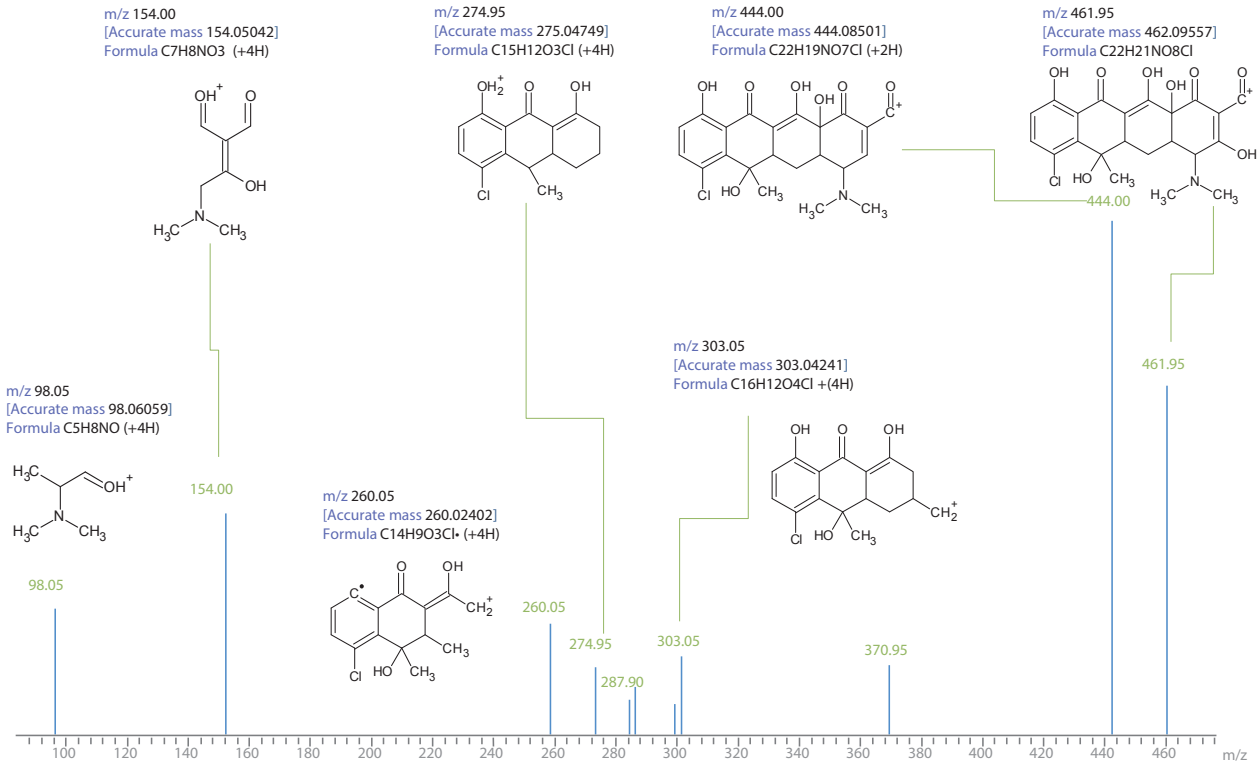


Fig. 2 MRM Reference Spectrum with Assigned Fragment Structures (Chlortetracycline)

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Library Identification using MRM Spectrum Mode

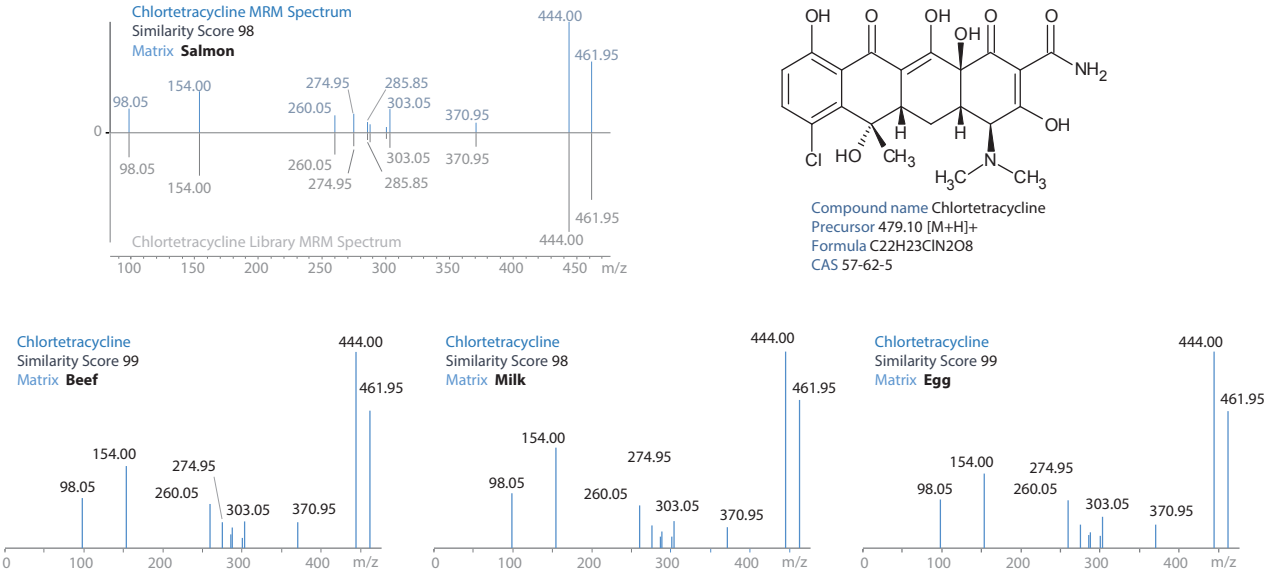
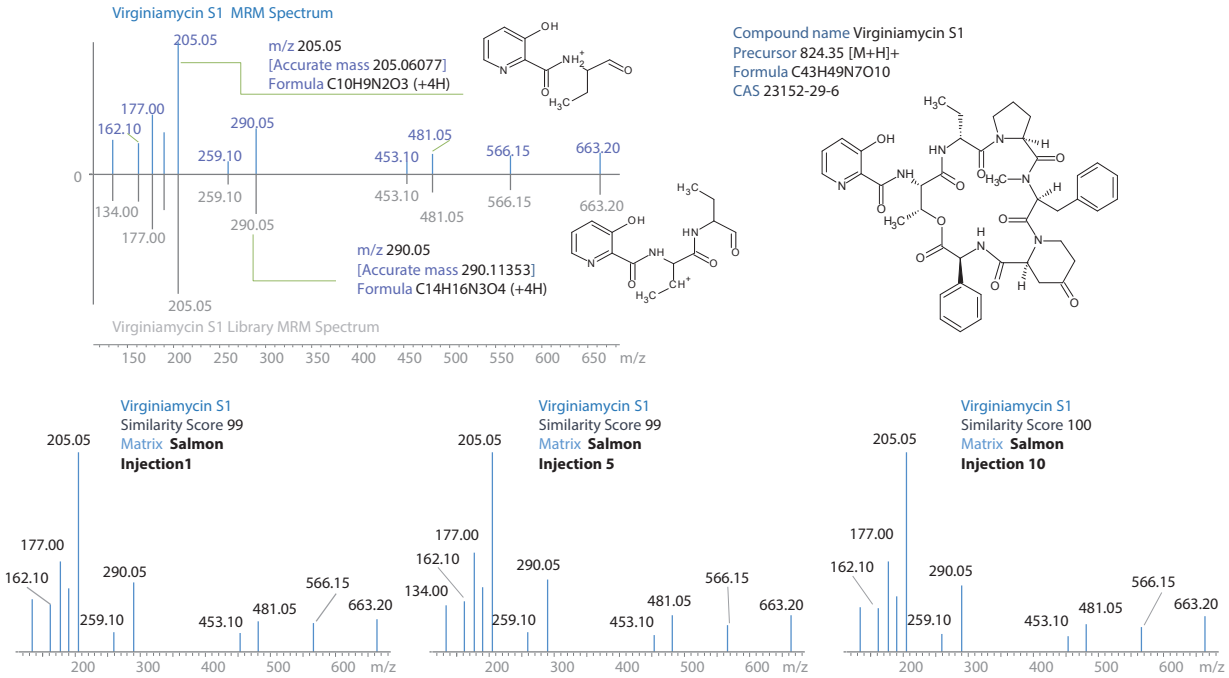


Fig. 3 Library Searchable MRM Spectra in Different Matrices Spiked at 10 pg/µL (Chlortetracycline)

Fig. 4 shows the MRM spectra and the n=10 measurement results of four compounds for salmon extract spiked with virginiamycin S1 at a concentration of 10 pg/µL. The library match score was above 99 in all injections (MRM spectra of injections 1, 5 and 10 are indicated). Also, the %RSD for oxytetracycline, sulfadimethoxine, ormetoprim, and virginiamycin spiked into salmon extract (n=10; 10 pg/uL) acquired using a conventional 2-MRM method was compared with that of the MRM spectrum method.



Compound name	Oxytetracycline		Sulfadimethoxine		Ormetoprim		Virginiamycin	
Number of MRM's	2MRM's	8MRMs	2MRM's	11MRMs	2MRM's	11MRMs	2MRM's	11MRMs
Mean peak area								
Quantitation ion	1890170	1729171	7809989	7227748	8291171	8160952	2232967	1956045
%RSD	**3.74**	**3.04**	**1.49**	**1.46**	**1.54**	**1.18**	**0.91**	**1.65**

Fig. 4 MRM Spectra and n=10 Results of Salmon Extract Spiked with Virginiamycin S1 at 10 pg/µL

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Quantitation Results using MRM Spectrum Mode

To assess the robustness of the MRM Spectrum mode, the same sample was repeatedly injected. The method used complies with the identification criteria set out in the EU guidelines SANTE/11945/2015 that require the retention time and the ion ratio from at least 2 MRM ion ratios to be within acceptable tolerance limits. The absolute response and signal variability were compared to those of the MRM Spectrum mode (Fig. 4). Both methods resulted in a variance of less than 4 %RSD (n=10 for each method; 10 pg/uL spiked into salmon matrix). Fig. 5 indicates MRM spectra and the calibration curve obtained for sulfamerazine as an example of quantitation results.

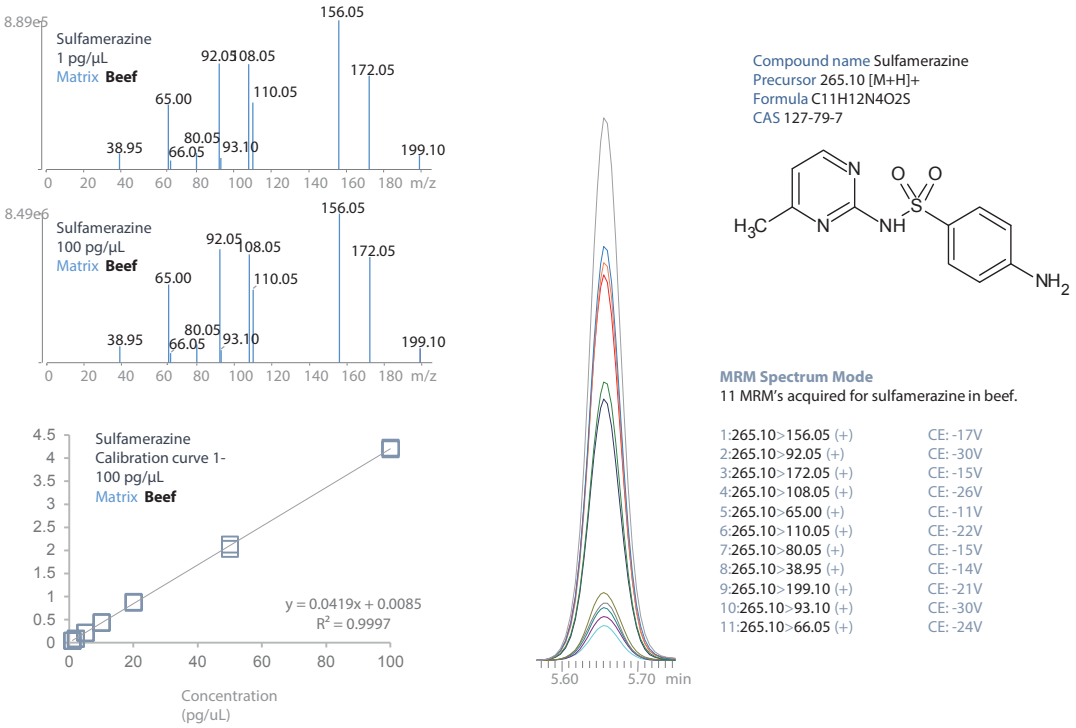

Fig. 5 MRM Spectra and Calibration Curve of Sulfamerazine (1 pg/µL to 100 pg/µL)

Conclusion

The level of confidence in compound identification and verification was increased by using a higher number of MRM transitions for each veterinary drug target and thereby reducing false negative and false positive reporting. Although the number of transitions for each target is dependent upon the chemical structure of the target, typically more than 10 transitions can be monitored for each compound. MRM Spectrum mode combines conventional quantitation with the generation of a high quality product ion spectrum which can be used to achieve highly reliable compound identification and verification by library searching. In this research, use of the MRM Spectrum mode was examined by quantifying and identifying 212 veterinary drugs (the method included 2,009 MRM transitions). Limits of detection, linearity or repeatability were not compromised compared to a conventional 2-MRM method.

First Edition: Aug. 2017

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Application News

No. C175

LC/MS

Fast Quantitative Analysis of Aminoglycoside Antibiotic Residues in Meat, Eggs and Milk and Identity Confirmation with MRM Spectrum Mode

Aminoglycoside (AGs) are an antibiotic family widely used for the treatment of bacterial infections in cattle, sheep, pigs and poultry. They have a broad-spectrum activity and are used against Gram-positive and Gram-negative bacteria.

AGs possess oto- and nephrotoxicity which did not hinder the widespread use of AGs in veterinary applications because of their low cost.

Due to their high affinity for tissues, They may occur in meat, milk or eggs if the withholding period has not been observed or if used improperly. Therefore, eating food containing aminoglycosides can be potentially hazardous for human health.

Regulatory agencies have set maximum residue limits (MRL) for these compounds with veterinary use.

Aminoglycosides are very polar compounds poorly retained by reversed-phase liquid chromatography. Ion-pairing reagents are not desirable as they can easily contaminate the analytical system and interfere in other methods.

A Method Package has been developed to overcome these problems. It comprises a protocol to generate clean extracts in a variety of commodities and a rapid quantitative method using hydrophilic interaction liquid chromatography (HILiC) combined with triple quadrupole mass spectrometry detection. When necessary, a second method for formal peak identification using MRM Spectrum Mode can be applied without changing reagents.

In this document, we report the use of the method package to assess the safety level of several meat samples and milk.

Mikaël Levi, Shimadzu Corporation, Kyoto, Japan.

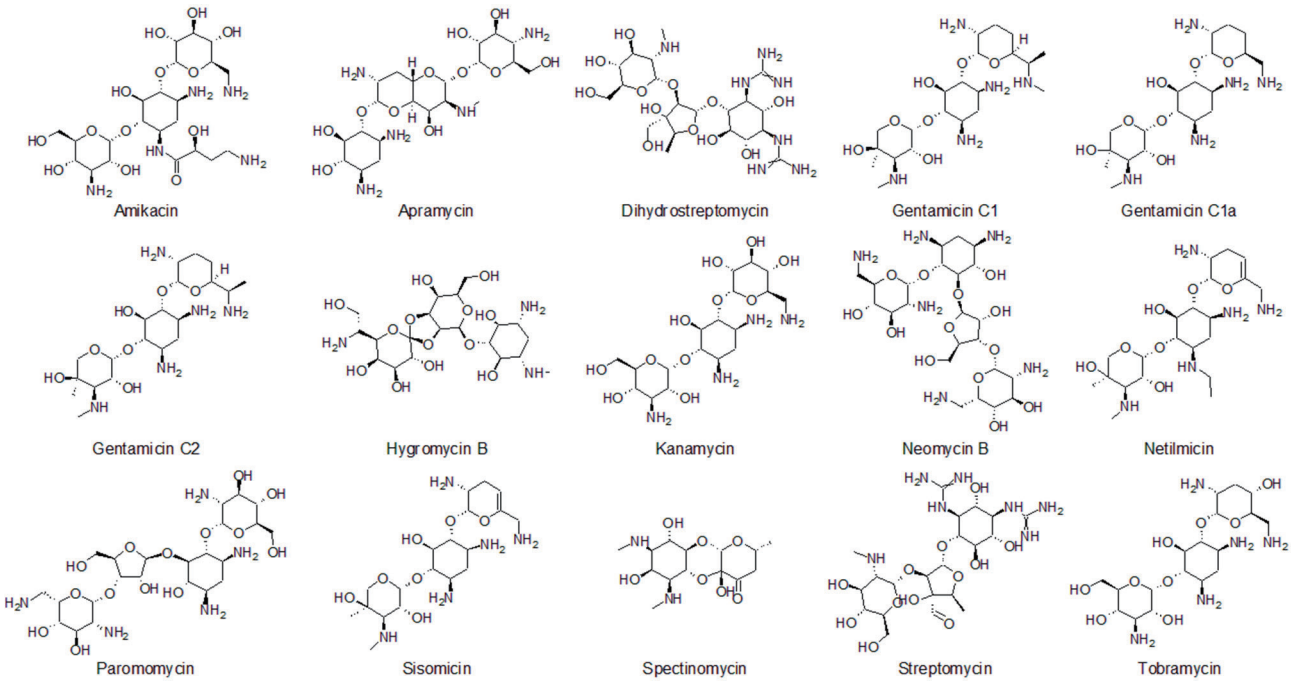

Fig. 1 Targeted aminoglycosides

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Sample Preparation

Meat samples (Kobe style beef muscle, chicken breast and liver, pork cutlet) and cow milk were purchased from local supermarket. After grinding, 5 g of sample were treated as described in Method Package. Briefly, after addition of internal standard (Ribostamycin), compounds were extracted twice with acidic buffer. Extracts were then purified by weak-cation exchange and diluted by a factor of 5 before injection (5 µL). Each sample was also spiked at 0.5 times and 1.5 times the MRL defined by Japanese Ministry of Health, Labour and Welfare.

All samples were prepared once except the beef sample spiked at 0.5 × MRL, which was prepared in 6 replicates.

LC-MS/MS Analysis

Purified extracts were assayed using LC-MS/MS conditions and ready-to-use methods included in the Method Package. A calibration curve prepared in mobile phase was used to quantify samples.

Samples were first assayed using a fast quantitative method. This method use HILiC conditions to elute compounds with a gradient of acetonitrile and a formate buffer. Cycle time for analysis is 4.5 minutes. Detection was performed in Multiple Reaction Monitoring (MRM) mode with 2 transitions acquired per compound.

For positive samples (i.e. over the MRL), a second injection of purified extracts was performed to assess peak identity. For this purpose, a second method with same column and mobile phases but alternative gradient and 15 MRM per compound (except ISTD) was used.

The analytical system was a Nexera™ X2 UHPLC coupled with LCMS-8060 triple quadrupole mass spectrometer. Data processing was made with LabSolutions Insight™ v.3.1 with Screening option.

Results

Depending on the species and commodities, MRL are different. According to current rule in Japan, if no MRL has been officially defined for a veterinary drug residue, a 'default' MRL of 10 µg/kg should be considered for any chemical tested. Then, for Apramycin, Dihydrostreptomycin, Gentamicin, Kanamycin, Neomycin, Spectinomycin and Streptomycin, the calibration range was set to cover from 10 % of the lowest MRL to 150 % of the highest one. For other compounds without official MRL, the calibration range was set from 20 % to 150 % of 10 µg/kg. Calibration values can be found in Table 1. Seven calibration levels, regularly dispatched within the range were prepared. Calibration standards with an accuracy within 85 - 115 % were selected. Representative calibration curves are shown in Fig. 2.

Samples without spiking revealed to be free of aminoglycoside residues. Then recovery was calculated in spiked samples using the calculated concentrations. Results can be seen in Table 2. Recoveries were in the acceptable range of 70 - 120 % for all compounds and all type of samples. Repeatability have been assessed in beef sample spiked at 0.5 × MRL. Results are presented in Table 3. The % RSD was less than 20 % which is suitable for such application.

Mass chromatograms example is presented in Fig. 3.

Table 1 Maximum residue limits in Japan for the selected samples and corresponding calibration ranges

			Calibration Range			
	Low MRL (µg/kg)	High MRL (µg/kg)	LLOQ (µg/kg)	LLOQ (ng/mL)	ULOQ (µg/kg)	ULOQ (ng/mL)
Amikacin	No value	Default (10)	2	0.1	15	0.75
Apramycin	60	500	6	0.3	750	37.5
Dihydrostreptomycin	200	600	20	1.0	900	45.0
Gentamicin (sum)	100	200	10	0.5	300	15.0
Hygromycin	No MRL	Default (10)	2	0.1	15	0.75
Kanamycin	40	500	4	0.2	750	37.5
Neomycin	500	500	50	2.5	750	37.5
Netilmicin	No MRL	Default (10)	2	0.1	15	0.75
Paromomycin	No MRL	Default (10)	2	0.1	15	0.75
Sisomicin	No MRL	Default (10)	2	0.1	15	0.75
Spectinomycin	200	2000	20	1.0	3000	150.0
Streptomycin	200	600	20	1.0	900	45.0
Tobramycin	No MRL	Default (10)	2	0.1	15	0.75

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Table 2 Calculated recoveries in spiked samples

		AMI	APRA	DHSTP	GENT C1a	GENT C1	GENT C2/C2a	HYGRO	KANA
Recovery at 0.5 × MRL	Milk	91.9 %	88.7 %	108 %	76.6 %	89.4 %	83.3 %	94.3 %	100 %
	Beef	107 %	89.0 %	117 %	90.4 %	94.2 %	95.2 %	107 %	102 %
	Pork	88.3 %	98.9 %	114 %	80.4 %	86.3 %	87.6 %	96.5 %	88.7 %
	Chicken Breast	82.2 %	90.3 %	97.4 %	98.7 %	92.4 %	90.3 %	105 %	94.8 %
	Chicken Liver	70.9 %	91.5 %	103 %	91.3 %	80.8 %	86.1 %	99.4 %	101 %
Recovery at 1.5× MRL	Milk	83.0 %	99.0 %	106 %	85.8 %	91.0 %	101 %	91.8 %	98.1 %
	Beef	89.9 %	95.9 %	96.9 %	98.8 %	91.2 %	95.5 %	104 %	96.1 %
	Pork	86.3 %	89.5 %	98.5 %	95.1 %	102 %	96.9 %	112 %	97.2 %
	Chicken Breast	82.2 %	90.3 %	97.4 %	98.7 %	92.4 %	90.3 %	105 %	94.8 %
	Chicken Liver	87.8 %	90.7 %	90.7 %	99.5 %	85.5 %	88.8 %	91.6 %	83.8 %

		NEO	NETIL	PARO	SISO	SPC	STP	TOB
Recovery at 0.5 × MRL	Milk	81.2 %	101 %	73.3 %	75.3 %	94.0 %	111 %	91.0 %
	Beef	91.4 %	101 %	88.1 %	88.4 %	110 %	114 %	91.5 %
	Pork	85.7 %	91.0 %	90.7 %	76.4 %	101 %	111 %	85.8 %
	Chicken Breast	94.1 %	90.5 %	78.4 %	84.9 %	92.7 %	102 %	107 %
	Chicken Liver	78.6 %	90.8 %	76.5 %	78.8 %	101 %	108 %	92.5 %
Recovery at 1.5 × MRL	Milk	96.7 %	93.6 %	86.9 %	99.4 %	94.8 %	105 %	102 %
	Beef	113 %	91.1 %	103 %	106 %	86.9 %	93.1 %	105 %
	Pork	106 %	90.4 %	94.8 %	94.3 %	95.2 %	105 %	108 %
	Chicken Breast	94.1 %	90.5 %	78.4 %	84.9 %	92.7 %	102 %	107 %
	Chicken Liver	109 %	82.4 %	89.5 %	95.3 %	75.3 %	90.0 %	98.1 %

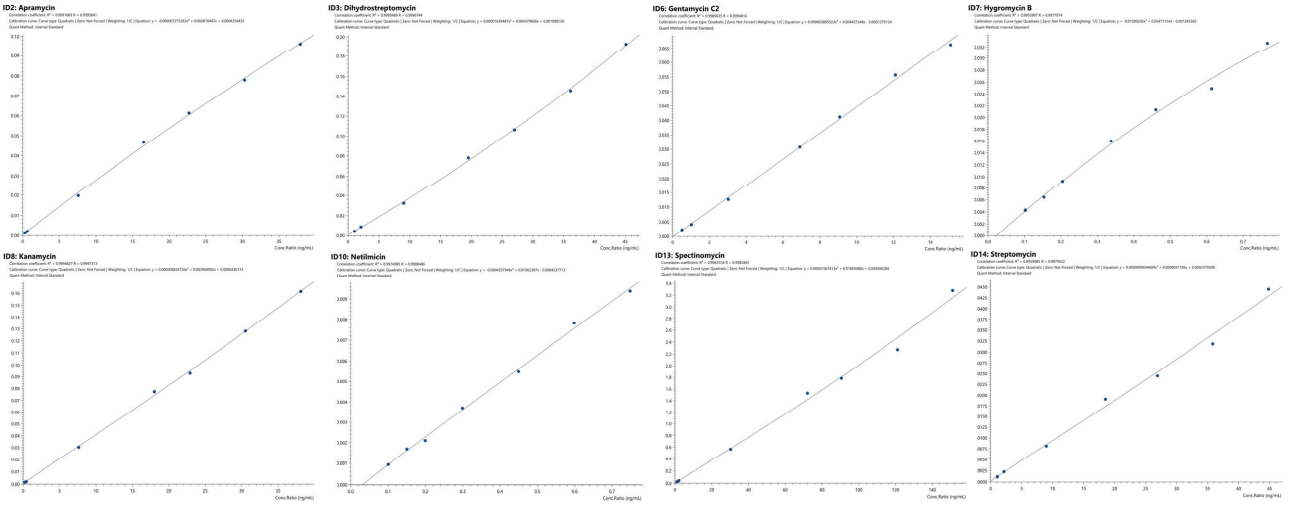

Fig. 2 Representative calibration curves

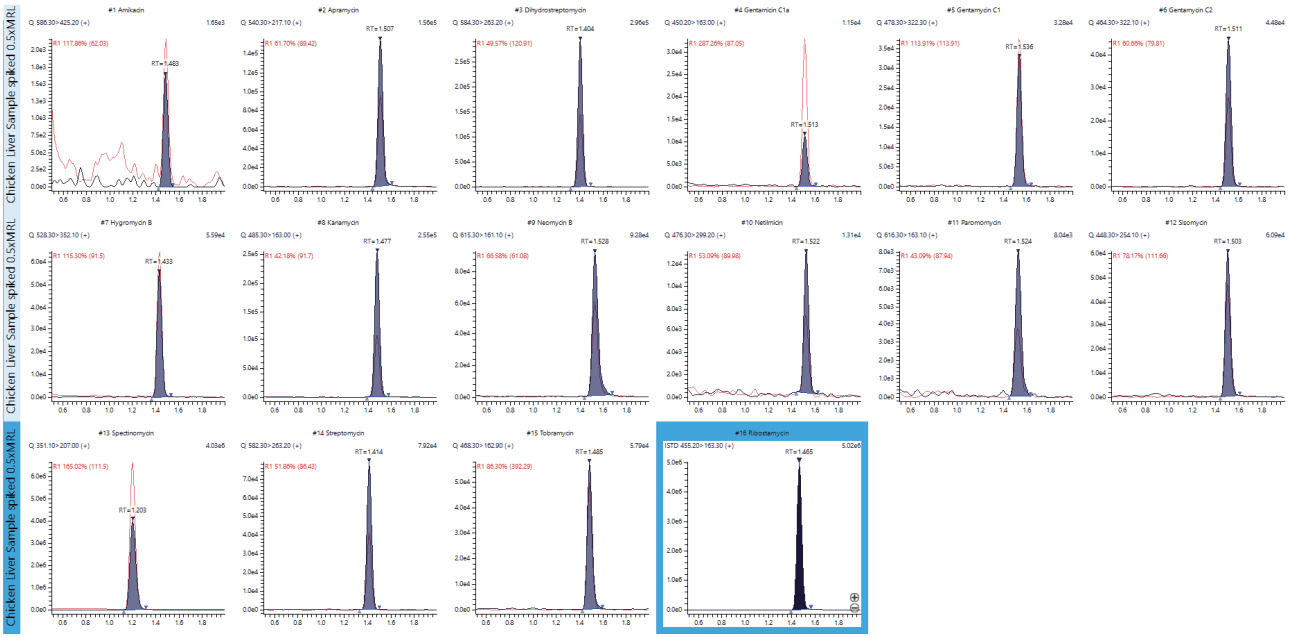

Fig. 3 Chicken liver sample spiked at 50 % of the MRL for each compound

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Table 3 Repeatability in beef sample at 0.5× MRL

	AMI	APRA	DHSTP	GENT C1a	GENT C1	GENT C2/C2a	HYGRO	KANA
Mean Conc. (µg/kg)	5.38	225	350	45.6	47.5	48.0	5.32	21.1
Recovery	107 %	89.0 %	117 %	90.4 %	94.2 %	95.2 %	107 %	102 %
%RSD	19.9 %	7.7 %	10.0 %	10.8 %	10.2 %	6.9 %	7.1 %	12.0 %

	NEO	NETIL	PARO	SISO	SPC	STP	TOB
Mean Conc. (µg/kg)	228	5.03	4.39	4.47	275	348	4.66
Recovery	91.4 %	101 %	88.1 %	88.4 %	110 %	114 %	91.5 %
%RSD	8.8 %	10.0 %	8.1 %	4.4 %	11.0 %	11.9 %	6.2 %

Results (continued)

For increased confidence in identification of compounds exceeding the MRL, additional injection of the extracts can be done using a second method with elongated gradient time and acquisition of 15 MRM transitions per compound. MRM signals are then merged to create a spectrum in which every fragment is acquired at optimum collision energy.

An example of search result by LabSolutions Insight with Screening option was illustrated below (Fig. 4). The samples can be processed and the library search can be automatically done in batch mode. In this case, high identification score can be obtained. Dihydrostreptomycin got a score of 95 while the second hit (Streptomycin, a very close compound) got a score of only 51.

Conclusion

A newly developped Method Package was succesfully applied to real meat and milk samples. The quantitative method gave good recoveries and accuracies, even for non-regulated compounds at trace levels. It can be applied to a variety of samples without using matrix-matched calibration curves.

A complementary method gives increased confidence in identification for over-the-limit compounds using MRM Spectum mode.

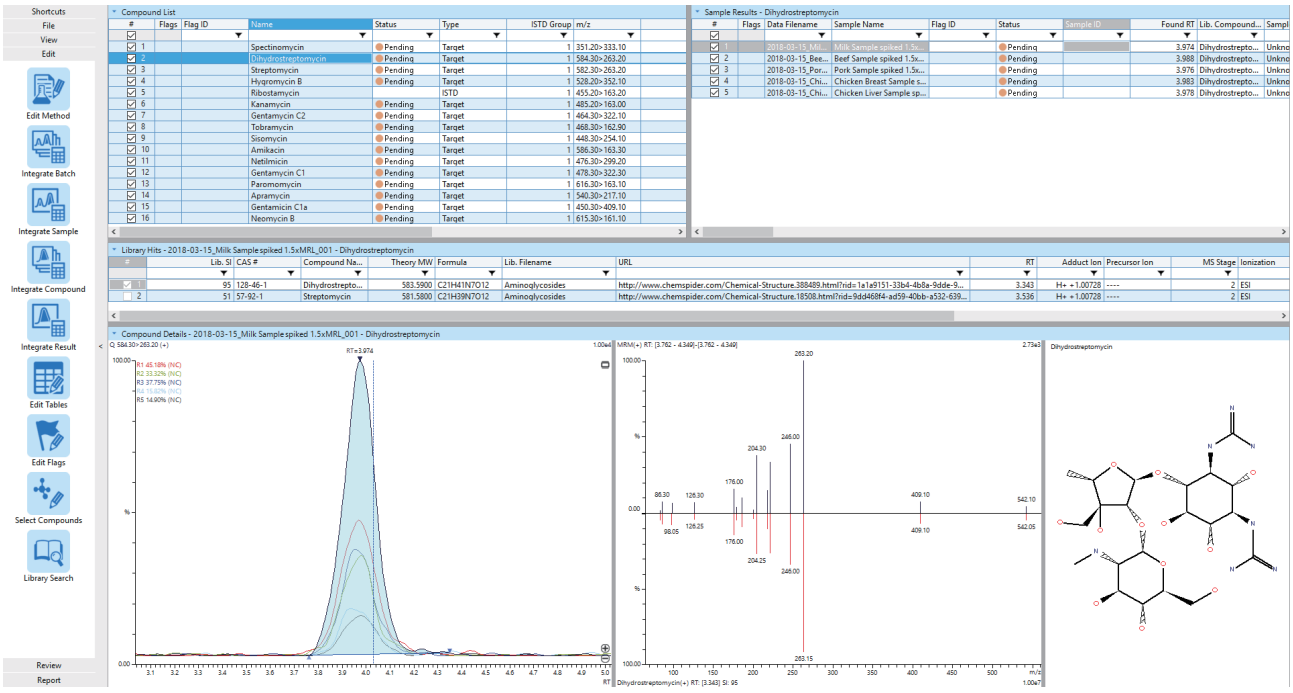

Fig. 4 Library search result of dihydrostreptomycin MRM spectrum in milk sample spiked at 1.5× MRL

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Application News

No. C199

Liquid Chromatography Mass Spectrometry

Analysis of Chloramphenicol in Shrimp and Chicken Egg Extracts Using Triple Quadrupole LC/MS/MS

Chloramphenicol is an antibiotic with a broad antimicrobial spectrum and is widely used as a veterinary medicine for the prevention and treatment of livestock diseases.

When the positive list system was introduced, chloramphenicol was set as a component that was not to be detectable in food. During the 2014 review, it could not be denied that it is genotoxic and possibly carcinogenic, so it was reevaluated as a component that should not have a set acceptable daily intake (ADI) which must not be contained in food continuously.

In addition, since it has been confirmed that chloramphenicol glucuronide conjugates are hydrolyzed in vivo, generating chloramphenicol, the test method for chloramphenicol was revised in 2017 (Notification No. 49 of the Ministry of Health, Labour and Welfare, 2017), adding chloramphenicol glucuronide conjugates as a target of measurement. In this study, we present an example analysis of quantified chloramphenicol in shrimp and chicken eggs in accordance with the revised test method.

H.Horiike

Sample Pretreatment

The shrimp was shredded and homogenized, and 10 g was weighed and taken. In addition, the chicken eggs were well mixed and homogenized and 10 g was weighed and taken.

Methanol was added to each sample, and after fine homogenization they were centrifuged twice to remove the supernatant, then made up to the fixed volume of 100 mL with methanol. 4 mL was collected, the solvent was removed, then after hydrolyzation by adding 9 mL of phosphate buffer and 1 mL of β-glucuronidase solution, ethyl acetate was added and the ethyl acetate layer was removed by centrifugation. Two extractions with ethyl acetate were followed by purification using a divinylbenzene-N-vinylpyrrolidone copolymer column to achieve the sample for measurement.

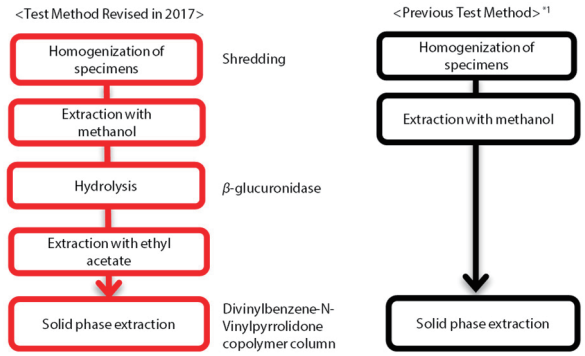

Fig. 1 Pretreatment Operation

*1 Notification No. 499 of the Ministry of Health, Labor and Welfare, 2005
Notification No. 370 of the Ministry of Health and Welfare, 1959

Analysis of Chloramphenicol and Chloramphenicol Glucuronide Conjugate Mixed Standard Solution

The MRM chromatograms obtained by measuring the concentration of chloramphenicol and chloramphenicol glucuronide conjugates in the 1 µg/L mixed standard solution are shown in Fig. 2. The analysis conditions were set such that the retention time for chloramphenicol was 4 min.

Concurrently with the revision of the test method, notice was given of points requiring attention: it is necessary to confirm in advance that interference peaks derived from enzymes do not affect the quantification, and that the chloramphenicol glucuronide conjugates are sufficiently hydrolyzed in pretreatment (Notification 0223-3, February 23, 2017).

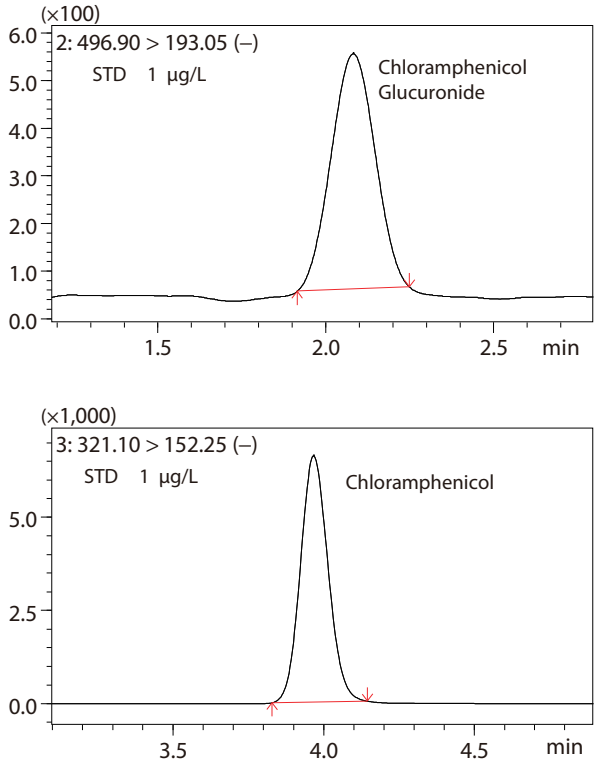

Fig. 2 MRM Chromatograms for Chloramphenicol and Chloramphenicol Glucuronide Conjugates in Mixed Standard Solution

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Table 1 Analysis Conditions

Column	:	Shim-pack™ HR-ODS (150 mmL. × 2.1 mm i.d., 3 µm, Shimadzu Corp.)
Mobile phases	:	10 mmol/L ammonium acetate water / Acetonitrile = 70 / 30 (v/v)
Flow rate	:	0.35 mL/min
Column temperature	:	40 °C
Injection volume	:	5 µL
Probe voltage	:	−1.0 kV (ESI-Negative)
DL temperature	:	300 °C
Block heater temperature	:	500 °C
Interface temperature	:	400 °C
Nebulizing gas flow	:	3 L/min
Drying gas flow	:	10 L/min
Heating gas flow	:	10 L/min
MRM transition	:	Chloramphenicol *m/z* 321.10 > 152.25 (Quantifier ion) 321.10 > 257.05 (Qualifier ion)
	:	Chloramphenicol glucuronide *m/z* 496.90 > 193.05 (Quantifier ion) 496.90 > 113.00 (Qualifier ion)

Linearity of Calibration Curve

A 5-point calibration curve was created in the concentration range of 0.1 to 2 µg/L for chloramphenicol and 0.5 to 10 µg/L for chloramphenicol glucuronide conjugates.

With the LC/MS method, the lower limit of detection of chloramphenicol in livestock produce is 0.005 mg/kg, and as shown in Fig. 3, good linearity was obtained from 0.1 µg/L as the quantitative lower limit.

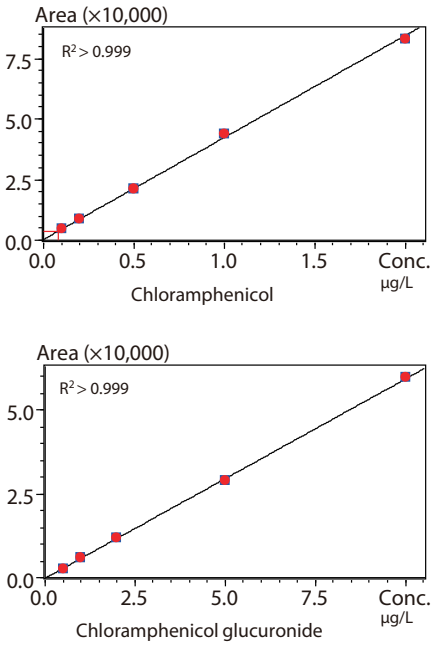
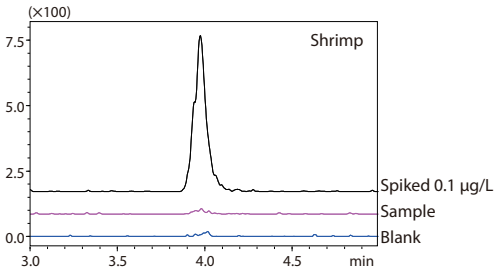


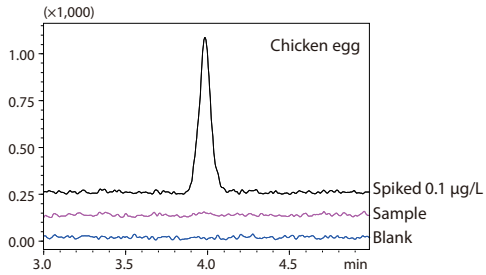
Fig. 3 Calibration Curves

Analysis of Shrimp and Chicken Eggs



Chloramphenicol	0.1 µg/L added
Recovery factor	87.9%
%RSD	7.6%

Fig. 4 Results of Spike and Recovery Test (n=3, Shrimp)



Chloramphenicol	0.1 µg/L added
Recovery factor	95.5%
%RSD	13.5%

Fig. 5 Results of Spike and Recovery Test (n=3, Chicken Egg)

The results of the measurements were that chloramphenicol was not detected in either commercially available shrimp (Indian black tiger) or chicken eggs (domestically produced in Japan). Therefore, only chloramphenicol standard solution was added to both blank samples to achieve a concentration of 0.1 µg/L, measurements were performed and it was confirmed that a recovery rate of 85% or greater can be obtained.

Further, the result of adding only chloramphenicol glucuronide conjugates to the blank solvent and measuring after the same pretreatment was that the chloramphenicol glucuronide conjugates were not detectable, while chloramphenicol was detected, which confirmed that the pretreatment implemented in this study achieved sufficient hydrolyzation.

Using the LCMS™-8050 allows accurate measurement from a concentration of 0.1 µg/L.

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Application News

No. C202

Liquid Chromatography Mass Spectrometry

Analysis of Chlorpromazine in Milk and Chicken Egg Extracts using Triple Quadrupole LC/MS/MS

Chlorpromazine hydrochloride is used as a tranquilizer (pharmaceutical). At the same time, the use of veterinary medicines that have chlorpromazine as an active constituent is prohibited on animals to be used for food, and those which produce milk, eggs, etc. to be shipped for food. (Ministry of Agriculture, Forestry and Fisheries Ordinance No. 44, 2013)

In addition, in the Positive List system, chlorpromazine is classified as a substance which must not be contained in food, and the LC/MS method has been cited as the method for testing for it in the "Standards for Food, Food Additives, etc." (Ministry of Health and Welfare Notification No. 370, 1959).

However, this test method cannot be applied to all livestock and seafood, and it is being reviewed because it may not be possible to obtain good analysis results depending on the food.

In March 2019, the Pharmaceutical Affairs and Food Sanitation Council (food sanitation subcommittee, agricultural chemicals and veterinary medicines group) reported a consultation document (Ministry of Health, Labour and Welfare Notification 0220-4) on a new chlorpromazine test method whose development has been completed.

In this article, we present an example analysis of chlorpromazine in milk and chicken eggs in accordance with the test method described in the consultation document.

H.Horiike

Sample Pretreatment

In accordance with the draft report on the test method, 10 g of milk or chicken egg was weighed out, subjected to extraction twice using acetone, then made up to the fixed volume of 100 mL.

A volume of 10 mL was collected, ultrapure water and formic acid were added, and solid phase extraction was performed using a sulfonate-modified methacrylate copolymer mini-column.

After concentrating the eluate to about 1 mL at 40 °C, it was accurately made up to the fixed volume of 5 mL with a mixture of 0.1% formic acid solution and 0.1% formic acid acetonitrile (3:2), which was used as the sample for measurement.

Although the sample coverage has broadened, there are fewer treatment processes than those with the conventional test method, making the pretreatment easier.

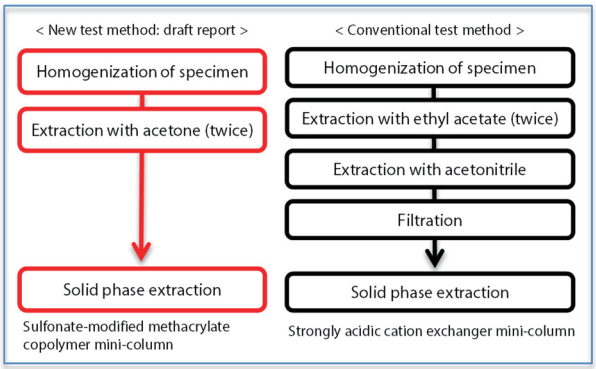

Fig. 1 Pretreatment Operations

Linearity of MRM Chromatograms and Calibration Curves of Chlorpromazine Standard Solution

The chlorpromazine standard solution (10 ng/L) was analyzed and the resulting MRM chromatogram is shown in Fig. 2. The lower limit of detection for the test method being reported is taken to be 20 ng/L when an injection volume is 5 µL, but if the LCMS™-8050 is used, it is possible to measure from 10 ng/L as a quantitative lower limit concentration even if the injection volume is reduced to 2 µL.

Fig. 3 shows the calibration curve from 10 to 1,000 ng/L; good linearity was obtained with a coefficient of determination of R^2>0.9998. The analysis conditions for this are shown in Table 1.

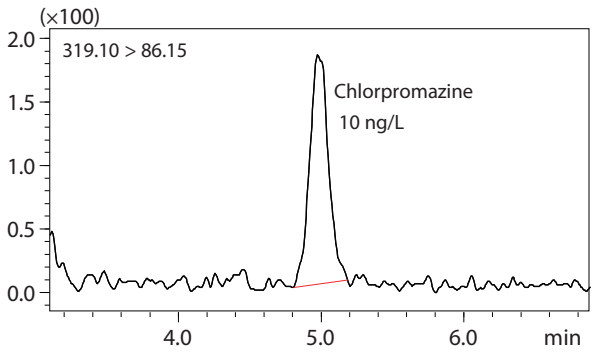

Fig. 2 MRM Chromatogram of Chlorpromazine Standard Solution

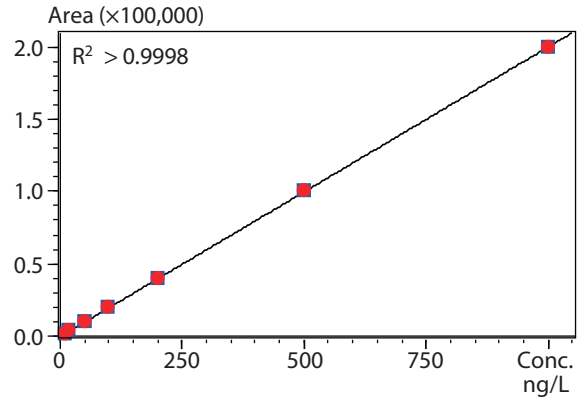

Fig. 3 Calibration Curves

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Table 1 Measurement Conditions

Column	:	Shim-pack™ HR-ODS (150 mmL. × 2.1 mm i.d., 3 µm, Shimadzu Corp.)
Mobile phases	:	0.1% formic acid water / 0.1% formic acid acetonitrile = 72 / 28 (v/v)
Flow rate	:	0.20 mL/min
Column temperature	:	40 °C
Injection volume	:	2 µL
Probe voltage	:	+1.0 kV (ESI-positive)
DL temperature	:	250 °C
Block heater temperature	:	350 °C
Interface temperature	:	300 °C
Nebulizing gas flow	:	2 L/min
Drying gas flow	:	5 L/min
Heating gas flow	:	15 L/min
MRM transition	:	*m/z* 319.10 > 86.15 (quantifier ion) 321.10 > 58.10 (qualifier ion)

Milk and Egg Analysis

A blank, including pretreatment, was analyzed to ensure that no analytes were detected. (See Fig. 4)

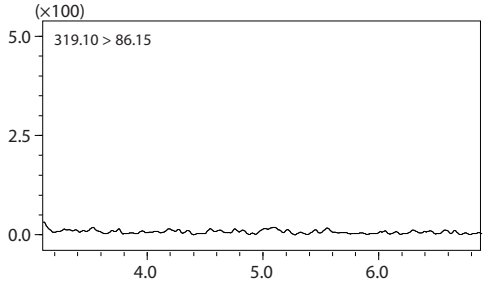

Fig. 4 Blank MRM Chromatogram

Store-bought milk and chicken eggs produced in Japan were pretreated, and the MRM chromatograms obtained for each by analyzing their extracts are shown in the upper figures in Fig. 5 and Fig. 6. With milk, minor peaks were detected, but they were generally calculated to be less than 1/5th of the quantitative lower limit, and were not detected with chicken eggs.

In addition, the chlorpromazine standard solution was added to milk and chicken eggs to achieve the equivalent of 0.0001 mg/kg, then pretreated test solutions were prepared by following the procedure shown in Fig. 1.

The MRM chromatograms obtained by analyzing them are shown in the lower figures in Fig. 5 and Fig. 6 respectively. The concentration of the test solution equivalent to 0.0001 mg/kg in the sample is 20 ng/L. As shown in Table 2 and Table 3, the recovery factors (trueness) were very good, at 103% for milk extract and 102% for chicken egg extract. Using the LCMS-8050 in this way makes it possible to accurately measure chlorpromazine.

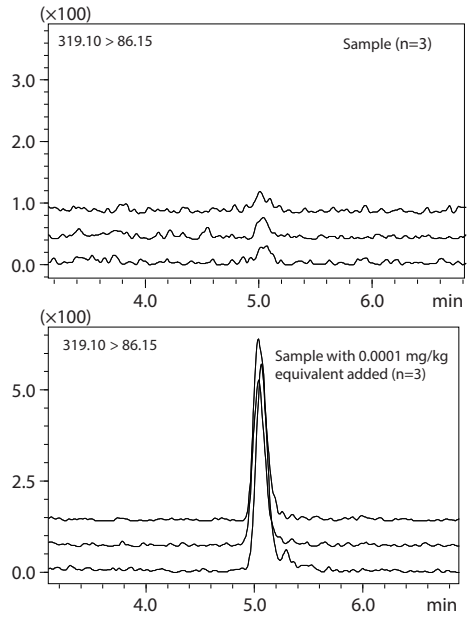

Fig. 5 MRM Chromatogram (n=3, milk)

Table 2 Spike and Recovery Test Results (n=3, milk)

	Average concentration	Average recovery	Area %RSD
Spiked sample	20.69 ng/L	103%	5.71

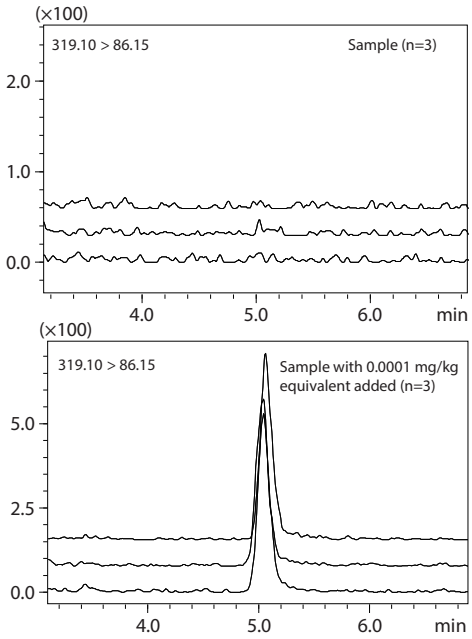

Fig. 6 MRM Chromatogram (n=3, chicken egg)

Table 3 Spike and Recovery Test Results (n=3, chicken eggs)

	Average concentration	Average recovery	Area %RSD
Spiked sample	20.50 ng/L	102%	2.45

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Application News

No.C103

Liquid Chromatography Mass Spectrometry

Analysis of Nivalenol, Deoxynivalenol, 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol Using Triple Quadrupole LC/MS/MS (LCMS-8050)

Nivalenol and deoxynivalenol are mycotoxins which are produced by the fusarium fungi. A provisional reference value of 1.1 ppm was established in Japan for deoxynivalenol (Notification No. 0521001 issued by the Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on May 21, 2002). The test methods specified for deoxynivalenol are HPLC for both qualitative and quantitative analysis, and LC/MS for verification testing (Notification No. 0717001 issued by the Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on July 17, 2003).

This paper describes an LC-MS/MS method for high-sensitivity simultaneous analysis of the four compounds, nivalenol, deoxynivalenol and the deoxynivalenol metabolytes, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol.

Analysis of a Standard Mixture

Fig. 1 shows the chromatograms obtained using a 2 µL injection of the four-component standard mixture (each 10 ppb), and Table 1 shows repeatability of retention time and peak areas for the four substances, respectively, using six repeat measurements.

Nivalenols are detected using the heated electrospray ionization (hESI) method in negative mode. Although water and acetonitrile alone can be used as the LC eluent for LC/MS analysis, higher sensitivity was obtained for each compound by adding low-concentration ammonium acetate (in this case, 0.5 mmol/L) to eluent A. Fig. 1 shows the mass chromatograms for the highest sensitivity MRM transitions for each compound. The analytical conditions are shown in Table 2.

Next, six repeat analyses of a 10 ppb standard solution were conducted, corresponding to approximately 1/100 the concentration of the provisional reference value. The relative standard deviations (%RSD) for the measured retention times and peak areas are shown in Table 1. Good repeatability was obtained for both retention time and peak area.

Table 1 Repeatability (10 ppb, n=6)

	R.T. %RSD	Area %RSD
Nivalenol	0.04	2.57
Deoxynivalenol	0.04	6.52
15-Acetyldeoxynivalenol	0.06	4.09
3-Acetyldeoxynivalenol	0.05	2.58

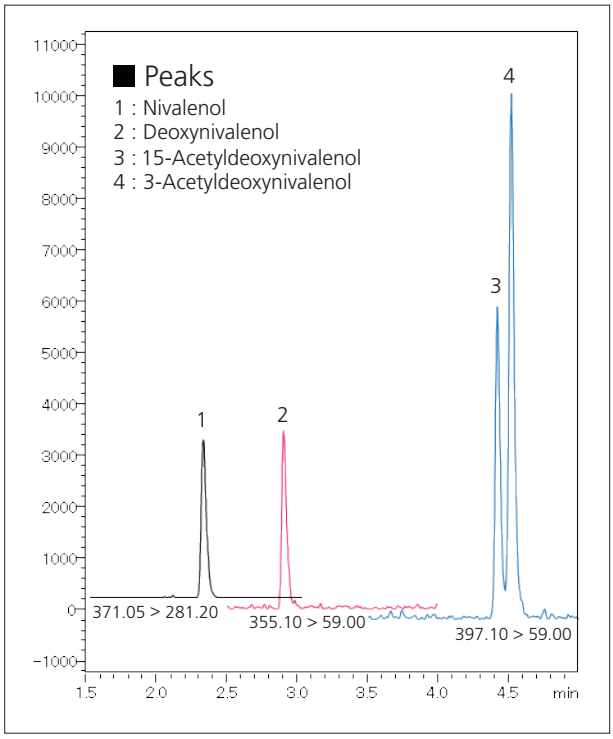

Fig. 1 MRM Chromatograms of a Standard Mixture (10 ppb each)

Linearity of Calibration Curves

Fig. 2 shows the calibration curves generated using the analytical conditions of Table 2. Excellent linearity with a coefficient of determination greater than $R^2 = 0.999$ was obtained for calibration curves using a concentration range from 1 to 250 ppb for each component.

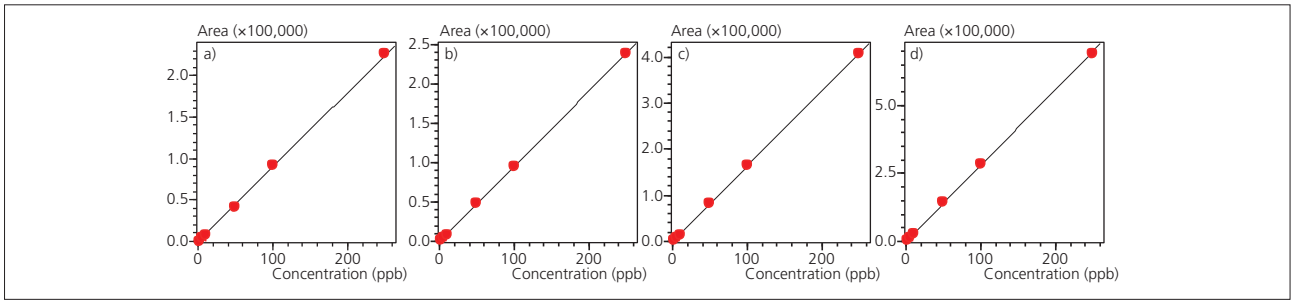

Fig. 2 Linearity of Calibration Curves: a) Nivalenol b) Deoxynivalenol c)15-Acetyldeoxynivalenol d) 3-Acetyldeoxynivalenol

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Analysis of Wheat

Fig. 3 describes the sample pretreatment procedure for wheat. The wheat extract solution was purified using either the MultiSep #227 multi-function column (Romer Labs) or the Autoprep MF-T column (Showa Denko K.K.). The chromatograms generated using the samples prepared using the MultiSep #227 (unspiked samples) and the standard-spiked samples, respectively, are shown in Fig. 4. The standard mixture was added to obtain a final concentration of 25 ppb for the four components (about 1/40 of the provisional reference value), respectively. No large contaminant peaks were detected in the chromatograms of the pretreated samples. Furthermore, although deoxynivalenol was detected, it was at a level below that of the provisional reference value. The spike-and-recovery rates for the four components were excellent, from 101 to 107 %, without any particular matrix effects. Even in samples pretreated using Autoprep MF-T, comparable spike-and-recovery test results were obtained.

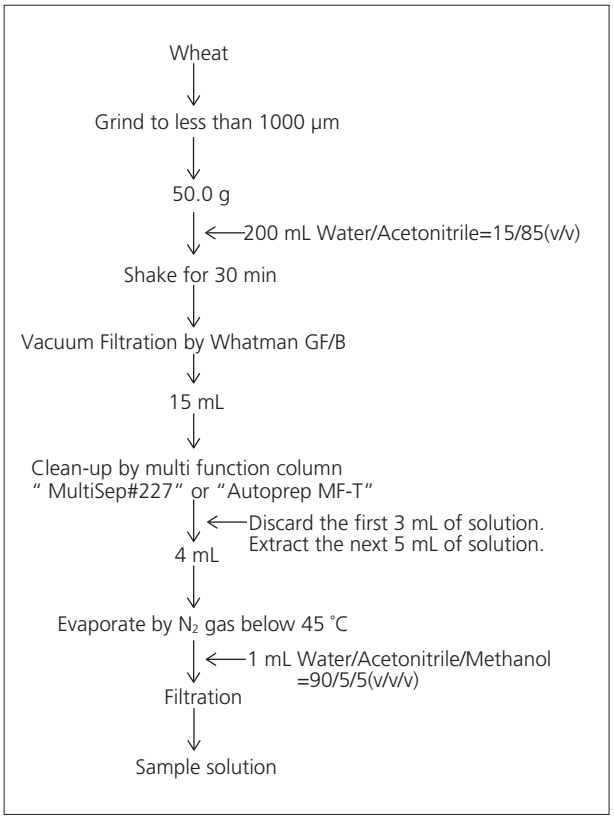

Fig. 3 Pretreatment

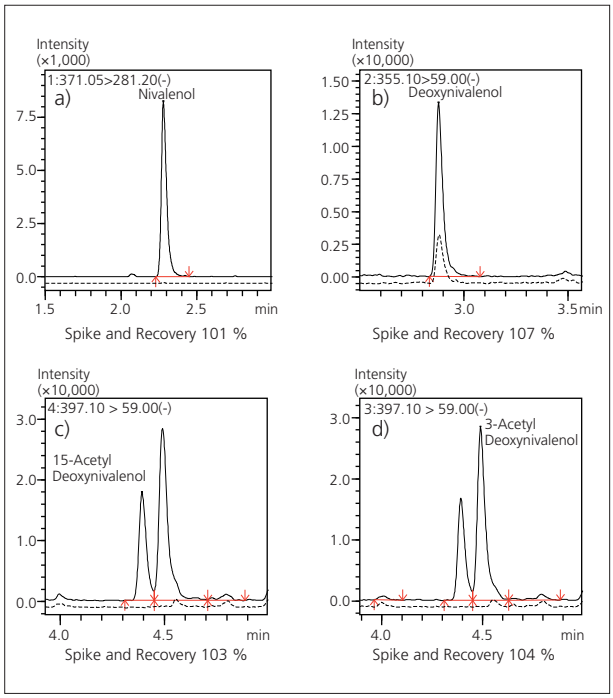

Fig. 4 Chromatograms of Wheat
(Dotted line: Unspiked Sample, Solid line: Spiked Sample, Spiked at 25 ppb each)
a) Nivalenol b) Deoxynivalenol
c) 15-Acetyldeoxynivalenol d) 3-Acetyldeoxynivalenol

Table 2 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅲ (150 mm L. × 2.0 mm I.D., 2.2 µm)		
Mobile Phases	: A 0.5 mmol/L Ammonium Acetate - Water		
	: B Acetonitrile		
Time Program	: 5 %B (0 min) → 45 %B (5.0 min) → 95 %B (5.01-7.0 min) → 5 %B (7.01 min) → STOP (12 min)		
Flowrate	: 0.3 mL/min		
Column Temperature	: 40 ˚C		
Injection Volume	: 2 µL		
Probe Voltage	: -3.0 kV (ESI-negative mode)		
DL Temperature	: 100 ˚C		
Block Heater Temperature	: 200 ˚C		
Interface Temperature	: 200 ˚C		
Nebulizing Gas Flow	: 2 L/min		
Drying Gas Flow	: 10 L/min		
Heating Gas Flow	: 10 L/min		
MRM Transition	: Nivalenol	371.05 > 281.20	CE: 16.0 V
	: Deoxynivalenol	355.10 > 59.00	CE: 22.0 V
	: 15-Acetyldeoxynivalenol	397.10 > 59.00	CE: 22.0 V
	: 3-Acetyldeoxynivalenol	397.10 > 59.00	CE: 26.0 V

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Application News

No.C104

Liquid Chromatography Mass Spectrometry

Analysis of Diarrhetic Shellfish Toxin Using Triple Quadrupole LC/MS/MS (LCMS-8050)

The Japanese Ministry of Health, Labour and Welfare (JMHLW) specified in July, 1980 that the mouse bioassy (MBA) be used as the official method for diarrhetic shellfish toxin, and that the permissible exposure limit be 0.05 MU per gram of edible shellfish*). Shellfish in which the toxin exceeds this limit are prohibited from being sold at market according to the Japanese Food Sanitation Law Article 6, Item 2.

Due to significant technological advances since 1980, the sensitivity and accuracy obtained using the MBA method are significantly inferior compared to the high-precision, high-sensitivity possible using liquid chromatography mass spectrometry analytical instrumentation, which is currently used for this application. A complete transition to instrumental analysis for lipophilic marine biotoxins is scheduled to be implemented by January 2015 throughout the EU.

Based on this international trend, the JMHLW is currently considering migration to an instrumental analysis assay and setting new reference values to be used with instrumental analysis, in addition to the introduction of the Codex standard for okadaic acids (OA, Reference 1).

Table 1 CODEX Standard 292-2008

	Reference Value
OA Acids (OA and DTX group)	Permissible ingestion limit of 0.16 mg OA per kg of edible shellfish

Fig. 1 shows examples of LC/MS/MS high-sensitivity analysis of okadaic acid (OA), dinophysistoxin 1 (DTX1) and pectenotoxins (PTX1, 2, 6) and yessotoxin 1 (YTX1). Thus, it is possible to conduct high-sensitivity, high-separation analysis of each component.

Fig. 2 and Fig. 3 show MRM chromatograms of standard samples of OA and DTX1, respectively.

* The amount of toxin resulting in the death of two out of three mice following intraperitoneal administration of the equivalent of 20 g per edible shellfish.

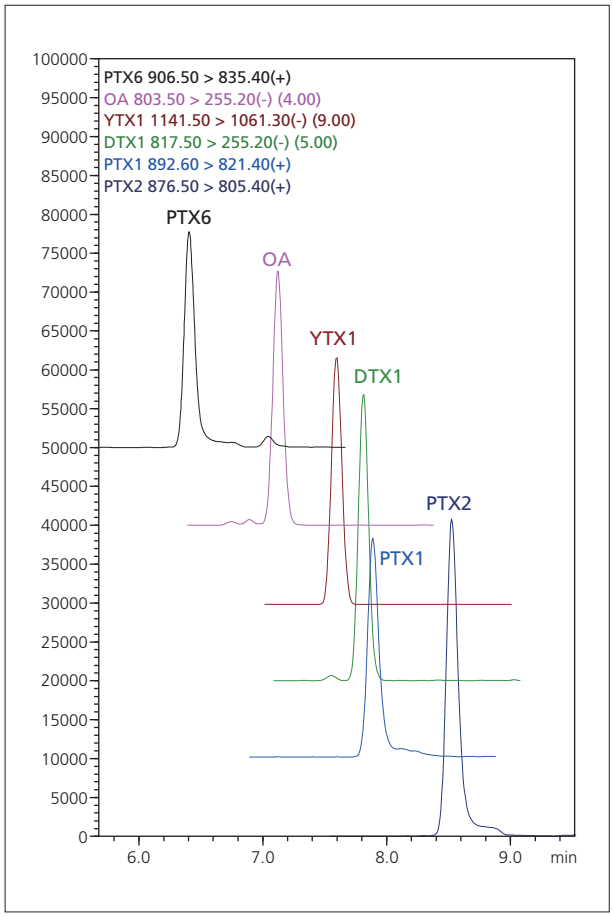

Fig. 1 MRM Chromatograms of Diarrhetic Shellfish Toxin (1 ng/mL)

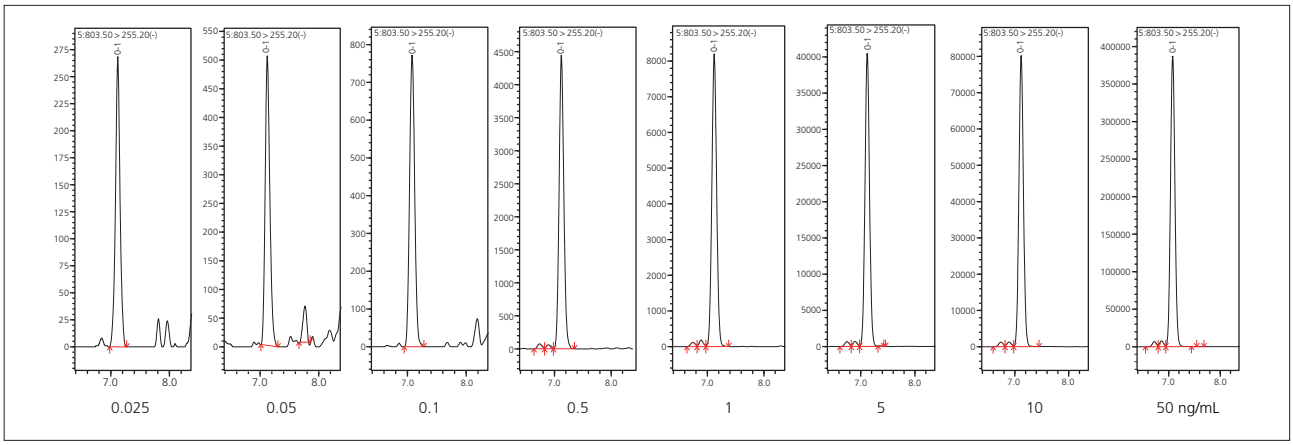

Fig. 2 MRM Chromatograms of Okadaic Acid (OA)

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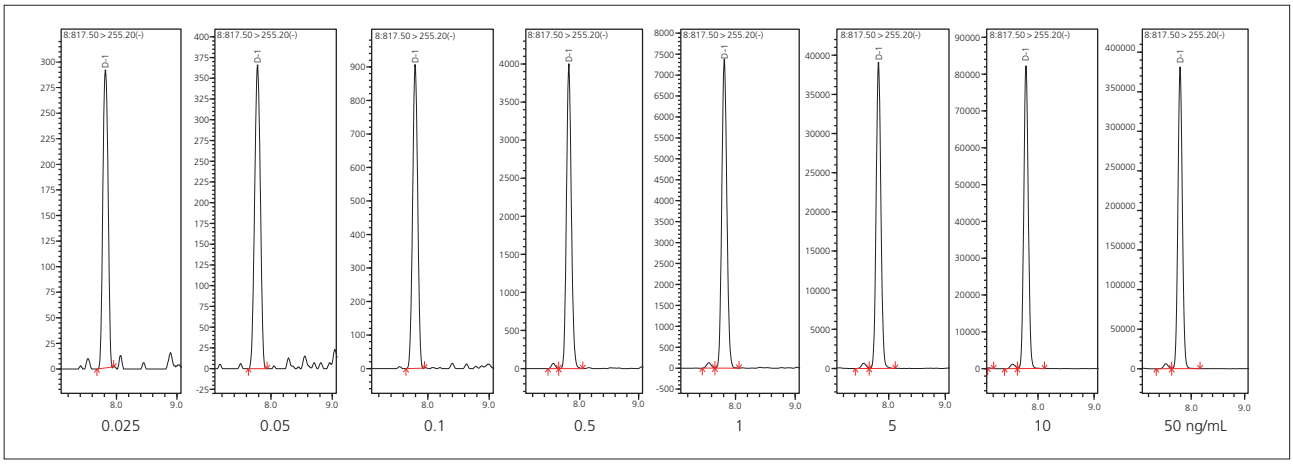

Fig. 3 MRM Chromatograms of Dinophysistoxin 1 (DTX1)

In addition, the calibration curves of OA and DTX1 are shown in Fig. 4. In both cases, the coefficient of determination R^2 was greater than 0.9999, indicating excellent linearity. Comparable linearity was also obtained for the other four substances.
Thus, instrumental analysis of shellfish by LC/MS/MS offers high sensitivity and accuracy, making it a highly effective analytical method. For this reason it is attracting attention as an alternative to the traditional MBA method.

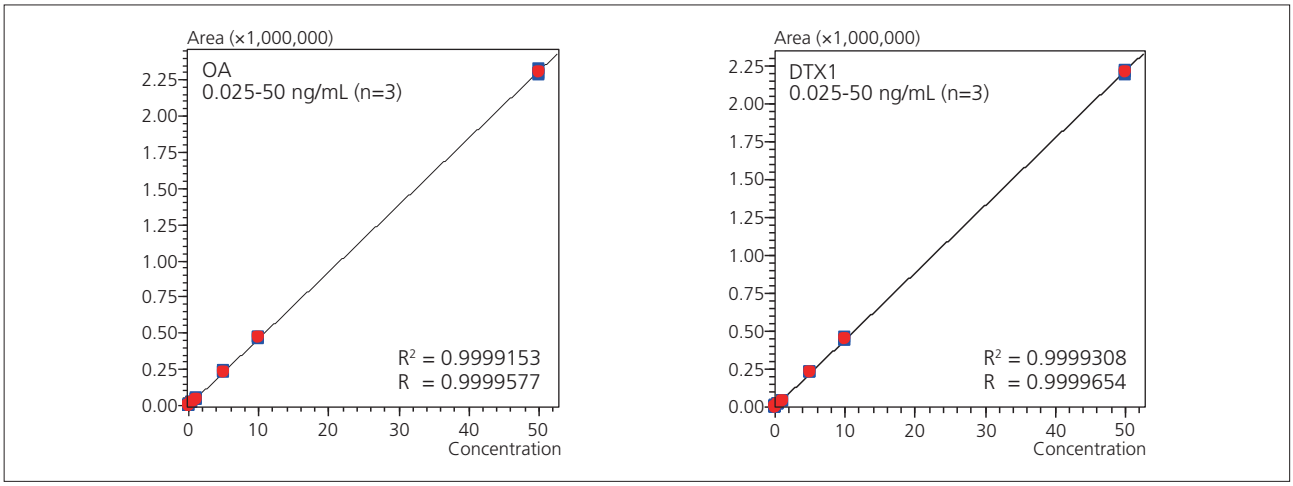

Fig. 4 Calibration Curves of OA and DTX1

Table 2 Analytical Conditions

Column	: InertSustain C8 (50 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phases	: A 2 mmol/L Ammonium Formate – Water (pH adjusted to 8.5 with ammonia water)
	: B Methanol
Time Program	: 20 %B (0 min) – 100 %B (10 min) – 20 %B (10.01 min) – STOP (15 min)
Flowrate	: 0.2 mL/min
Column Temperature	: 40 ˚C
Injection Volume	: 10 µL
Probe Voltage	: +4.0 kV/-3.0 kV (ESI-positive / negative mode)
DL Temperature	: 200 ˚C
Block Heater Temperature	: 400 ˚C
Interface Temperature	: 350 ˚C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
MRM Transition	: (+) PTX6 906.50 > 835.40, PTX1 892.60 > 821.40, PTX2 876.50 > 805.40
	: (-) OA 803.50 > 255.20, YTX1 1141.50 > 1061.30, DTX1 817.50 > 255.20

The diarrhetic shellfish toxin standards were provided courtesy of Dr. Toshiyuki Suzuki of the Japanese National Research Institute of Fisheries Science.

Reference 1: July, 2014, Food Safety Commission of Japan "Natural Poison Evaluation Report – Okadaic Acid Group Among Bivalves"
http://www.fsc.go.jp/fsciis/evaluationDocument/list?itemCategory=009

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Application News

No.C138

Liquid Chromatography Mass Spectrometry

Multi-Residue Analysis of 18 Regulated Mycotoxins by LC/MS/MS

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[1] Shimadzu UK, [2] Scientific Analysis Laboratories

Mycotoxins are one of the most important contaminants in food and feed due to their widespread distribution in the environment and toxic effects on humans and animals.[1)] Structurally, mycotoxins are a very diverse group with a wide range of physicochemical properties and low molecular weights.[2)] They are produced by fungi (mould) frequently found on agricultural produce, and are often not visible to the naked eye.[3)] Some of the most commonly contaminated food stuffs include wheat, oats, rye, corn, barley, rice, nuts and milk.[4)]

Due to the risks posed by mycotoxins in food they are regulated globally, including, the EU, US, China, Singapore and Brazil.[5)] In the EU, reporting limits are harmonised in Regulation (EC) No 1886/2006 (amended by (EC) No 1126/2007) and sampling and analysis in Regulation (EC) No 401/2006.

LC/MS/MS is the technique most commonly employed for mycotoxin quantitation in order to achieve the necessary low reporting limits in complex food and feed matrices.

Experimental

Solvent extracts were provided by Scientific Analysis Laboratories (SAL, UK) following validated extraction protocols. Samples were analysed using the Nexera UHPLC and the LCMS-8060 triple quadrupole detector (Table 1) . Calibration was performed using ^{13}C internal standards spiked during sample extraction. All MRM transitions and associated internal standards for each compound are listed in Table 2. All solvents used during analysis were LCMS quality from Sigma-Aldrich.

Due to the wide range of physicaland chemical properties of mycotoxins, different LC/MS/MS methods are typically developed for small groups of compounds with similar properties.

In this application paper a single LC/MS/MS method has been developed for the determination of 18 mycotoxins in food safety. Limits of quantification were at or below the maximum levels set in the EC/1886/2006 document. The scope of the method included Aflatoxins (B1, B2, G1, G2), Fumonisins (B1, B2, B3), Ochratoxin A (OTA) and Trichothecenes (3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON), Deoxynivalenol (DON), Diaceteoxyscripanol (DAS), Fusarenon-X (FUS X), HT-2, Neosolaninol (NEO), Nivalenol (NIV), T2, Zearalenone (ZON)) with an analysis cycle time of 12.5 minutes.

Table 1 Analytical Conditions

UHPLC	: Nexera LC System
Mobile Phase	: A; Water with additives B; Methanol with additives
Column	: Reversed phase column (100 mm L.× 2.1 mm I.D.)
Column Temperature	: 40 ˚C
Flowrate	: 0.4 mL/minute
Gradient	: B. Conc 15 % (0 min) → 25 % (1 min) → 40 % (2 min) → 41 % (4.5 min) → 100 % (7.5 - 10.0 min) → 15 % (10.10 min) → Stop (12.5 min)
LC-MS/MS	: LCMS-8060
Dwell Time	: 10 to 40 msec.
Pause Time	: 1 msec.
Ionisation Mode	: ESI +/-
Polarity Switching	: 5 msec.

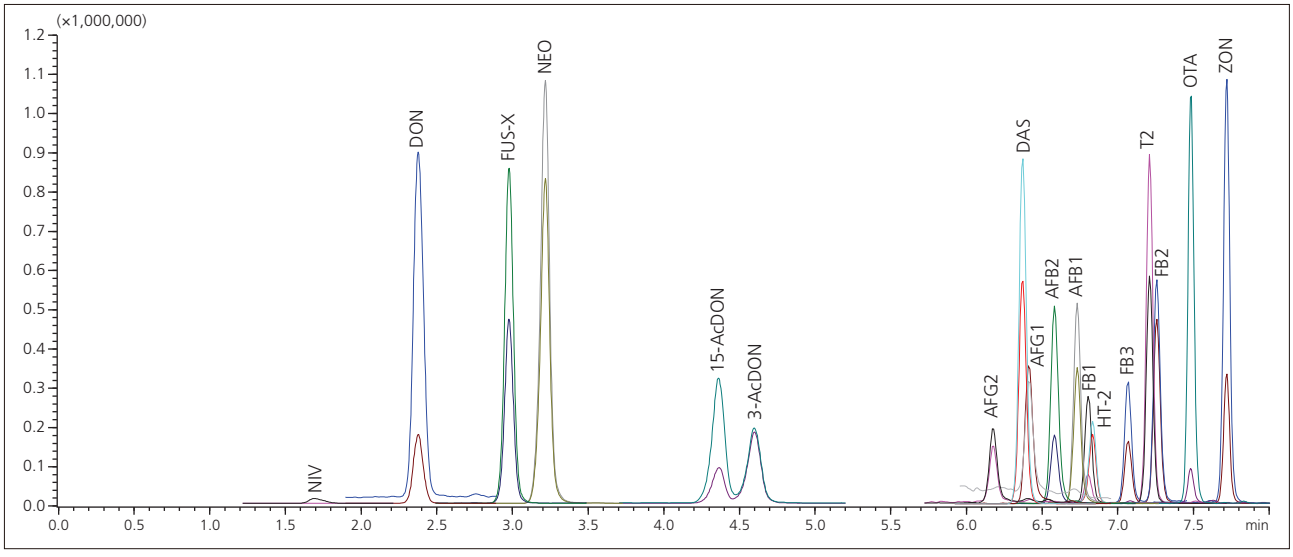

Fig. 1 MRM Chromatograms of 18 Mycotoxins

AFB1 (aflatoxin B1; 1 µg/kg), AFB2 (aflatoxin B2; 1 µg/kg), AFG1 (aflatoxin G1; 1 µg/kg), AFG2 (aflatoxin G2; 1 µg/kg), OTA (ochratoxin A; 4 µg/kg), FB1 (fumonisin B1; 100 µg/kg), FB2 (fumonisin B2; 100 µg/kg), FB3 (fumonisin B3; 100 µg/kg), 15-AcDON (15-acetyldeoxynivalenol; 100 µg/kg), 3-AcDON (3-acetyldeoxynivalenol; 100 µg/kg), DON (deoxynivalenol; 100 µg/kg), DAS (diaceteoxyscripanol; 100 µg/kg), FUS-X (fusarenon-X; 100 µg/kg), HT-2 (100 µg/kg), T-2 (100 µg/kg), NEO (neosolaninol; 100 µg/kg), NIV (nivalenol; 100 µg/kg), ZON (zearalenone; 100 µg/kg).
For clarity only 2 MRM transitions are displayed per compound and the following MRM chromatograms were changed; neosolaniol (x0.3), T2 (x0.3), aflatoxins (x3), fumonisins (x2).

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Table 2 All MRM's Measured in the Mycotoxin Method and Corresponding Calibration Range and R^2 Result

	Compound name	Parent ion	Ret. Time (mins)	MRM 1	MRM 2	MRM 3	ISTD	Calibration range µg/kg	R^2
1	Aflatoxin B1	$[M+H]^+$	6.773	313 > 241	313 > 285	313 > 269	^{13}C Aflatoxin B1	0.1 - 10	0.9988
2	Aflatoxin B2	$[M+H]^+$	6.621	315 > 259	315 > 287	315 > 243	^{13}C Aflatoxin B2	0.1 - 10	0.9995
3	Aflatoxin G1	$[M+H]^+$	6.453	329 > 243	329 > 200		^{13}C Aflatoxin G1	0.1 - 10	0.9998
4	Aflatoxin G2	$[M+H]^+$	6.219	331 > 245	331 > 285		^{13}C Aflatoxin G2	0.1 - 10	0.9965
5	Ochratoxin A	$[M+H]^+$	7.509	404 > 239	404 > 221	404 > 358	^{13}C Ochratoxin A	0.4 - 40	0.9969
6	Fumonisin B1	$[M+H]^+$	6.811	722 > 352	722 > 334	722 > 704	^{13}C Aflatoxin B2	10 - 1000	0.9937
7	Fumonisin B2	$[M+H]^+$	7.260	706 > 318	706 > 354	706 > 688	^{13}C Aflatoxin B2	10 - 1000	0.9998
8	Fumonisin B3	$[M+H]^+$	7.073	706 > 318	706 > 354	706 > 688	^{13}C Aflatoxin B2	10 - 1000	0.9991
9	Deoxynivalenol	$[M+H]^+$	2.372	297 > 279	297 > 249		^{13}C Deoxynivalenol	10 - 1000	0.9992
10	Diacetoxyscirpenol	$[M+NH_4]^+$	6.349	384 > 229	384 > 307	384 > 247	^{13}C T2 Toxin	10 - 1000	0.9994
11	T2	$[M+NH_4]^+$	7.206	484 > 185	484 > 215	484 > 245	^{13}C T2 Toxin	10 - 1000	0.9989
12	HT-2	$[M+Na]^+$	6.822	447 > 345	447 > 285		^{13}C T2 Toxin	10 - 1000	1.0000
13	Nivalenol	$[M-CH_3COO]^-$	1.684	371 > 281	371 > 311		^{13}C HT-2	10 - 1000	0.9991
14	Neosolaniol	$[M+NH_4]^+$	3.227	400 > 215	400 > 305	400 > 185	^{13}C Deoxynivalenol	10 - 1000	0.9995
15	Fusarenon X	$[M+H]^+$	2.986	355 > 247	355 > 277		^{13}C Deoxynivalenol	10 - 1000	0.9987
16	Zearalenone	$[M-H]^-$	7.711	317 > 175	317 > 131	317 > 273	^{13}C T2 Toxin	10 - 1000	0.9985
17	15-Acetyldeoxynivalenol	$[M+H]^+$	4.406	339 > 261	339 > 297		^{13}C Deoxynivalenol	10 - 1000	1.0000
18	3-Acetyldeoxynivalenol	$[M+H]^+$	4.618	339 > 261	339 > 297		^{13}C Deoxynivalenol	10 - 1000	0.9986
19	^{13}C HT-2	$[M+NH_4]^+$	6.844	464 > 278					
20	^{13}C T2	$[M+NH_4]^+$	7.228	508 > 322					
21	^{13}C Aflatoxin B1	$[M+H]^+$	6.754	330 > 301					
22	^{13}C Aflatoxin B2	$[M+H]^+$	6.614	332 > 303					
23	^{13}C Aflatoxin G1	$[M+H]^+$	6.435	346 > 212					
24	^{13}C Aflatoxin G2	$[M+H]^+$	6.219	348 > 259					
25	^{13}C Ochratoxin A	$[M+H]^+$	7.516	424 > 250					

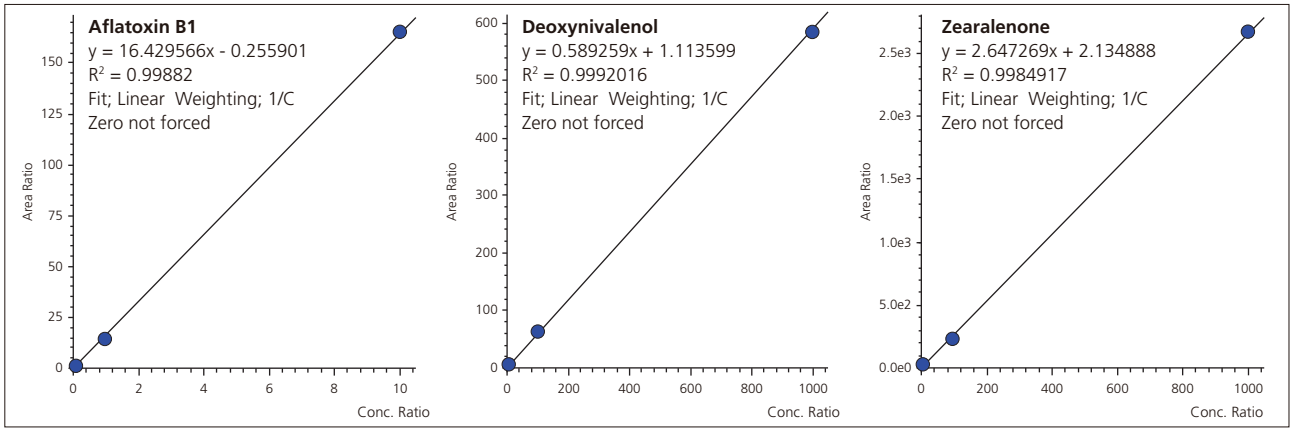

Fig. 2 Calibration Curves for Selected Compounds
Calibration Curves for Aflatoxin (0.1 – 10 µg/kg), Deoxynivalenol (10 – 1000 µg/kg), and Zearalenone (10 – 1000 µg/kg).

Conclusions

In this study a single method has been developed for the analysis of 18 regulated mycotoxins with an injection to injection cycle time of 12.5 minutes. This method achieves the required EU reporting limits (between 0.1 -10 µg/kg) with linear regression coefficients R^2 typically greater than 0.998 (Fig. 2 and Table 1). The LC mobile phase, column and gradient were all optimised and provided chromatographic resolution of 15-acetyldeoxynivalenol and 3-acetyldeoxynivalenol.

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5) Mycotoxins regulations for Food. http://www.mycotoxins.info/myco_info/consum_regu.html Accessed 6th September 2016

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Application News

No. C165

LC-MS/MS

Multi-residue analysis of 18 regulated mycotoxins by LC-MS/MS (2)

Fusarium mycotoxins are a structurally diverse group of secondary metabolites known to contaminate a diverse array of food and feed resulting in a risk for human and animal health. European guidance legislation has set maximum levels for mycotoxins in food and feed to minimize the impact to human and animal health. The most toxicologically important Fusarium mycotoxins are trichothecenes (including deoxynivalenol (DON) and T-2 toxin (T-2)), zearalenone (ZON) and fumonisin B1 (FB1).

In this work, a single LC-MS/MS method has been developed for the determination of 18 mycotoxins in food safety. Limits of quantification were at or below the maximum levels set in the EC/1886/2006 document. The scope of the method included aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), ochratoxin A (OTA) and trichothecenes (3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), deoxynivalenol (DON), diasteoxyscripanol (DAS), fusarenon-X (FUS X), HT-2, neosolaninol (NEO), nivalenol (NIV), T2, zeareleonone (ZON)) with an analysis cycle time of 12.5 minutes.

Materials and Methods

Solvent extracts were provided by Concept Life Sciences following validated extraction protocols. Samples were measured using a Nexera UHPLC and the LCMS-8060 triple quadrupole detector (Table 1). To separate out the three pairs of regioisomers (3-AcDON/15-AcDON, FB2/FB3, and FA2/FA3) a pentafluorophenyl (PFP) column was used and compared against a C18 material. To enhance signal response a series of mobile phase additives were considered including ammonium acetate, ammonium fluoride, ammonium formate and acetic acid solutions.

In this work, ammonium fluoride solution and ammonium fluoride with acetic acid solution was the preferred solvent system as it resulted in a considerable enhancement of signal intensity in positive ion mode for all mycotoxins. Calibration was performed using ^{13}C internal standards spiked during sample extraction. All solvents used during analysis were LCMS quality from Sigma-Aldrich.

David Baker*1, Christopher Titman*1, Neil Loftus*1, Jonathan Horner*2
*1 : Shimadzu, Manchester, UK
*2 : Concept Life Sciences, Cambridge, UK

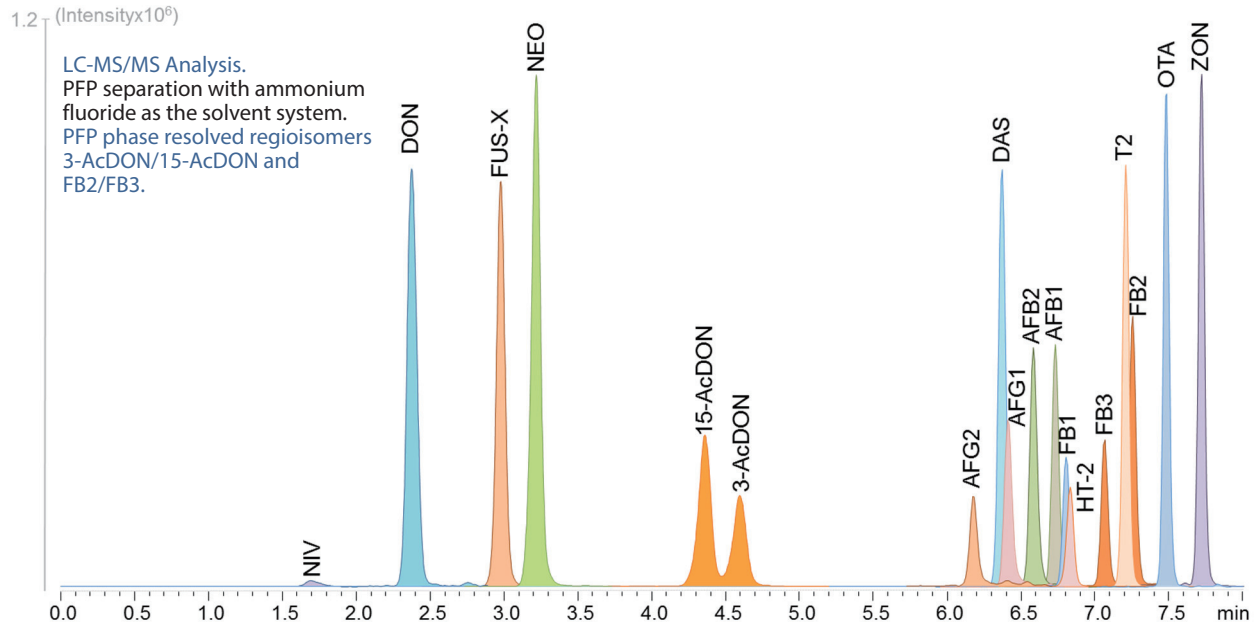

Fig. 1 MRM chromatograms of 18 mycotoxins using a PFP bonded phase.
AFB1 (aflatoxin B1; 1 µg/kg; rescaled x3), AFB2 (aflatoxin B2; 1 µg/kg; rescaled x3), AFG1 (aflatoxin G1; 1 µg/kg; rescaled x3), AFG2 (aflatoxin G2; 1 µg/kg; rescaled x3), OTA (ochratoxin A; 4 µg/kg), FB1 (fumonisin B1; 100 µg/kg; rescaled x2), FB2 (fumonisin B2; 100 µg/kg; rescaled x2), FB3 (fumonisin B3; 100 µg/kg; rescaled x2), 15-AcDON (15-acetyldeoxynivalenol; 100 µg/kg), 3-AcDON (3-acetyldeoxynivalenol; 100 µg/kg), DON (deoxynivalenol; 100 µg/kg), DAS (diasteoxyscripanol; 100 µg/kg), FUS-X (fusarenon-X; 100 µg/kg), HT-2 (100 µg/kg), T-2 (100 µg/kg; rescaled x0.3), NEO (neosolaniol; 100 µg/kg; rescaled x0.3), NIV (nivalenol; 100 µg/kg), ZON (zearalenone; 100 µg/kg)

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Influence of ammonium fluoride on ion signal intensity

Ammonium fluoride solution has a high gas-phase basicity and known to be effective in improving sensitivity for small molecules in negative mode LC-MS. However, ammonium fluoride has also been shown to enhance sensitivity in positive ion mode. Compared to standard mobile phases used for mycotoxin analysis the addition of ammonium fluoride has a positive impact on ion signal intensity.

Fig. 2 indicates that ammonium fluoride markedly increases ion signal intensity compared to other solvent systems. All chromatograms are normalized to the same signal intensity. Ammonium fluoride delivered higher ion signal response for mycotoxins in positive ion mode compared to other mobile phase solvent system (Fig. 2a).

Table 1 Analytical Conditions

UHPLC	Nexera X2 LC system
Analytical column	Mastro PFP (100 mmL. × 2.1 mm I.D., 3 µm)
Column temperature	40 °C
Flow rate	0.4 mL/min
Solvent A	0.15 mmol/L ammonium fluoride aqueous solution
Solvent B	0.15 mM ammonium fluoride methanol solution with 2 % acetic acid
Binary Gradient	B conc. 15 % (0 min) - 25 % (1 min) - 40 % (2 min) - 41 % (4.5 min) - 100 % (7.5 - 10 min) - 15 % (10.1 min) – Stop (12.5 min)

Mass spectrometer	Shimadzu LCMS-8060
Pause time/Dwell time	1 msec/10-40 msec
Polarity switching time	Pos/neg switching time set to 5 msec
Source temperatures (interface; heat block; DL)	300 °C; 400 °C; 250 °C
Gas flows (nebulising; heating; drying)	3 L/min; 10 L/min; 10 L/min

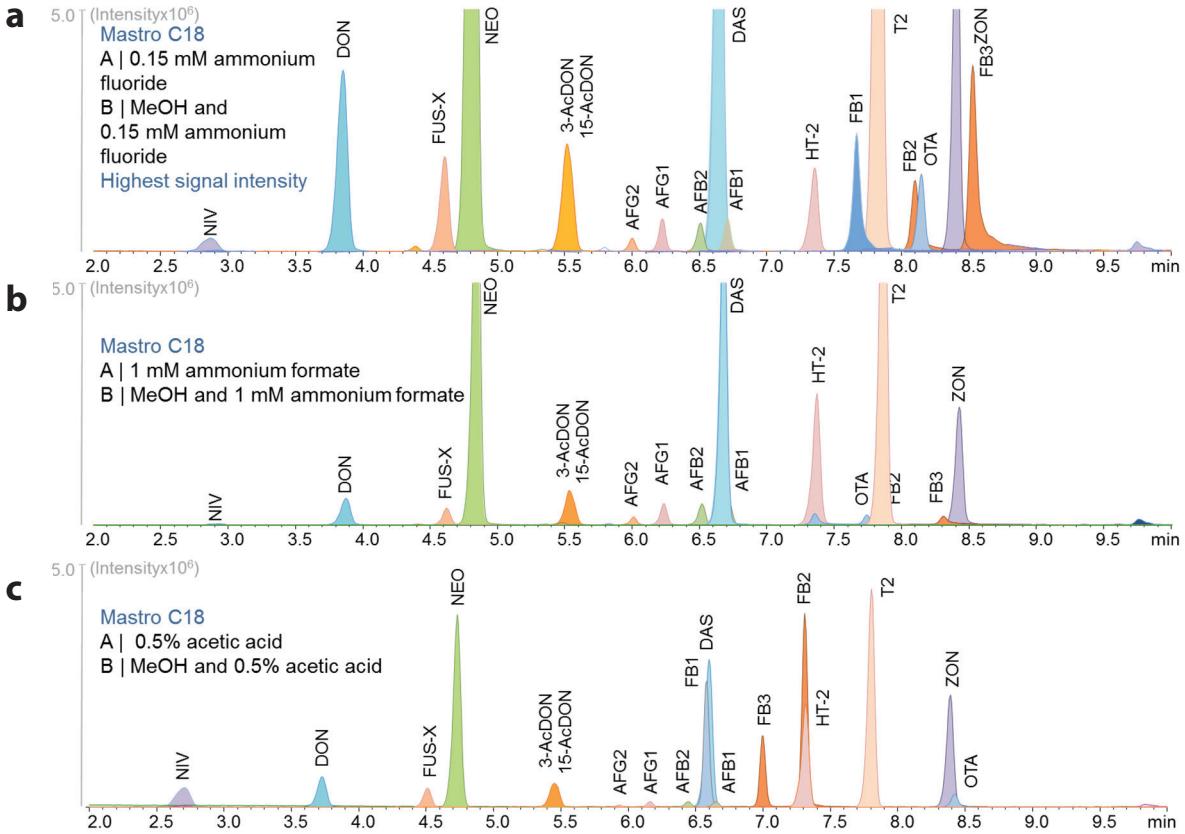

Fig. 2 Comparison of MRM Chromatograms of 18 Mycotoxins under the Different Mobile Phase Conditions (Mastro C18 Column)
a: Mobile Phase A = 0.15 mM Ammonium Fluoride Aqueous Solution, Mobile Phase B = 0.15 mM Ammonium Fluoride Methanol Solution
b: Mobile Phase A = 1 mM Ammonium Formate Aqueous Solution, Mobile Phase B = 1 mM Ammonium Formate Methanol Solution
c: Mobile Phase A = 0.5 % Acetic Acid Aqueous Solution, Mobile Phase B = 0.5 % Acetic Acid Methanol Solution

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Fig. 3 shows 18 mycotoxins separated on a PFP phase compared to a C18 bonded material using ammonium fluoride as the mobile phase. PFP phases delivered near baseline resolution of 3- and 15-acetyldeoxynivalenol which is not possible on a C18 phase (C18 material can still be used due to preferential ionisation of 3-AcDON in negative ion mode).

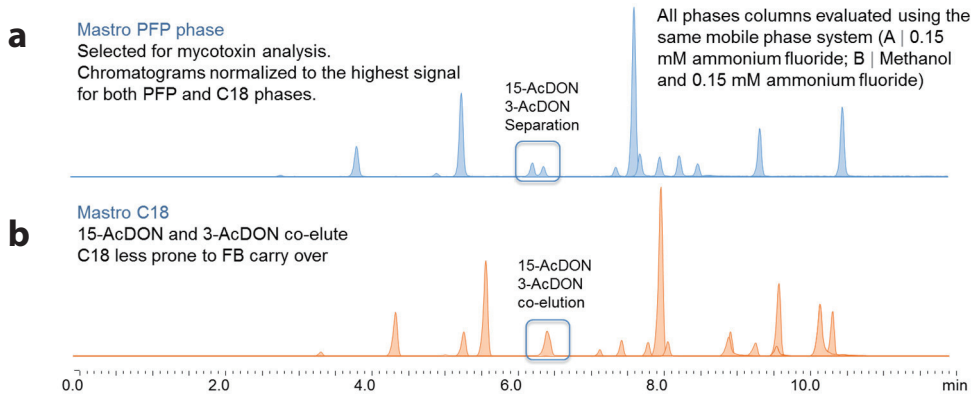

Fig. 3 Comparison of MRM Chromatograms of 18 Mycotoxins Using Different Columns
Mobile Phase A = 0.15 mM Ammonium Fluoride Aqueous Solution,
Mobile Phase B = 0.15 mM Ammonium Fluoride Methanol Solution (for Both Columns)
a: Mastro PFP Column, b: Mastro C18 Column

Analysis of sample matrices

To separate the regioisomers 3-AcDON/15-AcDON and FB2/FB3 several PFP phases were evaluated including Mastro PFP, Kinetix PFP, Discovery HS F5 PFP and ACE PFP. Compared to a C18 bonded phase, the PFP phases delivered near baseline resolution of the regioisomers 3-AcDON/15-AcDON and FB2/FB3 but required a modification of the mobile phase to reduce FB carry over (2 % acetic acid was added to the mobile phase to negate the effects of FB's carry over).

Fig. 4 shows the analysis of a mixed spice extract and a pepper extract spiked with Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg) using ammonium fluoride solution in the mobile phase. Repeatedly injecting the extracts resulted in a %RSD typically below 10 % (n=12) for Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg).

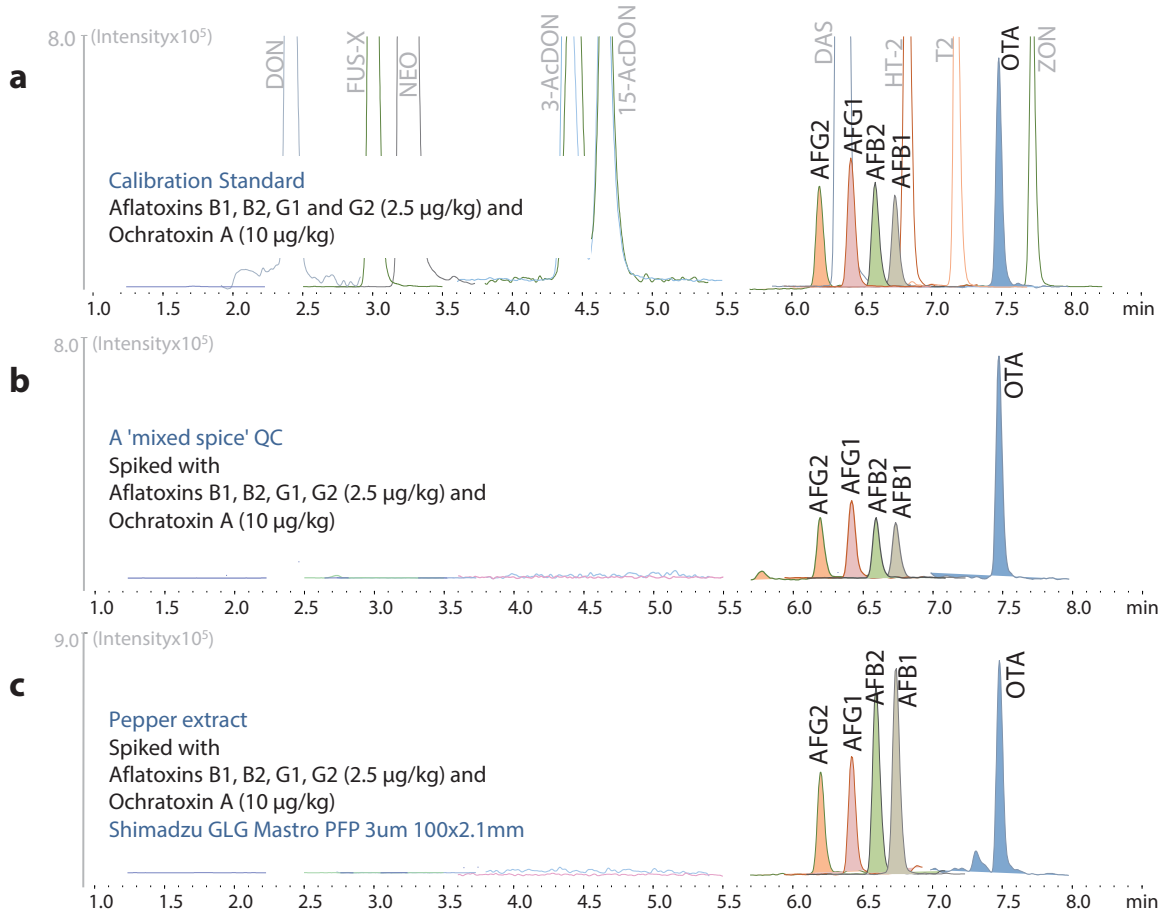

Fig. 4 Chromatograms of the Mycotoxin Standard Solution, Mixed Spice Extract, and Pepper Extract Spiked with Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg)
a: Mycotoxin Standard Solution, b: Mixed Spice Extract, c: Pepper Extract

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Table 2 MRM's of mycotoxins in positive and negative mode ionisation.

Compound name	Parent ion	RT	MRM 1	MRM 2	Internal Standard	Calibration range (µg/kg)	R^2
Aflatoxin B1	$[M+H]^+$	6.773	313 > 241	313 > 285	^{13}C Aflatoxin B1	0.1 - 10	0.9988
Aflatoxin B2	$[M+H]^+$	6.621	315 > 259	315 > 287	^{13}C Aflatoxin B2	0.1 - 10	0.9995
Aflatoxin G1	$[M+H]^+$	6.453	329 > 243	329 > 200	^{13}C Aflatoxin G1	0.1 - 10	0.9998
Aflatoxin G2	$[M+H]^+$	6.219	331 > 245	331 > 285	^{13}C Aflatoxin G2	0.1 - 10	0.9965
Ochratoxin A	$[M+H]^+$	7.509	404 > 239	404 > 221	^{13}C Ochratoxin A	0.4 - 40	0.9969
Fumonisin B1	$[M+H]^+$	6.811	722 > 352	722 > 334	^{13}C Aflatoxin B2	10 - 1000	0.9937
Fumonisin B2	$[M+H]^+$	7.26	706 > 318	706 > 354	^{13}C Aflatoxin B2	10 - 1000	0.9998
Fumonisin B3	$[M+H]^+$	7.073	706 > 318	706 > 354	^{13}C Aflatoxin B2	10 - 1000	0.9991
Deoxynivalenol	$[M+H]^+$	2.372	297 > 279	297 > 249	^{13}C Deoxynivalenol	10 - 1000	0.9992
Diacetoxyscirpenol	$[M+NH_4]^+$	6.349	384 > 229	384 > 307	^{13}C T-2 Toxin	10 - 1000	0.9994
T-2	$[M+NH_4]^+$	7.206	484 > 185	484 > 215	^{13}C T-2 Toxin	10 - 1000	0.9989
HT-2	$[M+Na]^+$	6.822	447 > 345	447 > 285	^{13}C T-2 Toxin	10 - 1000	1.0000
Nivalenol	$[M+CH_3COO]^-$	1.684	371 > 281	371 > 311	^{13}C HT-2	10 - 1000	0.9991
Neosolaniol	$[M+NH_4]^+$	3.227	400 > 215	400 > 305	^{13}C Deoxynivalenol	10 - 1000	0.9995
Fusarenon X	$[M+H]^+$	2.986	355 > 247	355 > 277	^{13}C Deoxynivalenol	10 - 1000	0.9987
Zearalenone	$[M-H]^-$	7.711	317 > 175	317 > 131	^{13}C T2 Toxin	10 - 1000	0.9985
15-Acetyldeoxynivalenol	$[M+H]^+$	4.406	339 > 261	339 > 297	^{13}C Deoxynivalenol	10 - 1000	1.0000
3-Acetyldeoxynivalenol	$[M+H]^+$	4.618	339 > 261	339 > 297	^{13}C Deoxynivalenol	10 - 1000	0.9986
^{13}C HT-2	$[M+NH_4]^+$	6.844	464 > 278				
^{13}C T-2	$[M+NH_4]^+$	7.228	508 > 322				
^{13}C Aflatoxin B1	$[M+H]^+$	6.754	330 > 301				
^{13}C Aflatoxin B2	$[M+H]^+$	6.614	332 > 303				
^{13}C Aflatoxin G1	$[M+H]^+$	6.435	346 > 212				
^{13}C Aflatoxin G2	$[M+H]^+$	6.219	348 > 259				
^{13}C Ochratoxin A	$[M+H]^+$	7.516	424 > 250				

Conclusions

Ammonium fluoride as a solvent system results in a higher signal response for mycotoxins in positive ion detection.

To negate any possible carry over effects with fumonisin's 2 % acetic acid was added to the mobile phase.

PFP bonded phases deliver a separation of mycotoxin regioisomers which can be applied routinely.

This method results in higher sensitivity for mycotoxins and can be applied to both PFP and C18 phases in routine quantitation with a cycle time of 12.5 minutes.

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Application News

No. C200

Liquid Chromatography Mass Spectrometry

Analysis of Diarrhetic Shellfish Toxins (Okadaic Acid Group) Using Triple Quadrupole LC/MS/MS

In regard to the handling of shellfish containing diarrhetic shellfish toxins, an instrumental analysis method is introduced based on "Handling of Shellfish Contaminated with Paralytic Shellfish Toxins, etc.", (Notice 0306 No. 2, dated March 6, 2015, issued by the Food Safety Manager, Pharmaceutical and Food Safety Bureau, MHLW). A regulatory value of 0.16 mg OA equivalent/kg has been set for the okadaic acid (abbreviated as OA) group, and selling shellfish that exceed the regulatory value is prohibited under the provisions of Chapter 6, Article 2 of the Food Sanitation Act.

Since April 2016, it has been possible to reliably obtain certified reference materials produced domestically in Japan. Accordingly, the mouse toxicity test in Notice No. 37 "Testing for Diarrhetic Shellfish Toxins (Okadaic Acid Group)" dated May 19, 1981 was superseded as of April 1, 2017 by Notice 0308 No. 2 and Notice 0308 No. 9 "Partial Revision of <Testing for Diarrhetic Shellfish Toxins (Okadaic Acid Group)>" dated March 8, 2017. In this revision, a regulatory value for the OA group, which is recognized as toxic to humans, was introduced and this group has become targeted in an instrumental analysis method. On the other hand, the PTX and YTX groups, which do not cause diarrhea, are not covered by the instrumental analysis method. In addition to OA, which is a toxin produced by phytoplankton, the OA group includes the dinophysistoxin group (DTX1, DTX2 and DTX3) as similar compounds. Because each of these compounds has a different strength of toxicity, the toxicity of each compound is calculated by converting it into an equivalent toxicity in terms of OA. For this purpose, a toxicity equivalence coefficient (TEF) has been defined, and with OA set as 1, DTX1 is 1 and DTX2 is 0.5. OA, DTX1, and DTX2 quantitative results are converted to OA equivalent values by multiplying them by their respective TEF values, then the sum is calculated. DTX3 is an esterified compound with a fatty acid compound, which is a metabolite of scallops, and no TEF value is set for it because it is converted to OA, DTX1 or DTX2 by the hydrolysis process in the pretreatment operation.

In this paper, we introduce an instrumental analysis method (LC/MS/MS) for the OA group.

M. Kobayashi

Analysis of Standards

For the OA and DTX1 standards, certified reference materials from the National Metrology Institute of Japan / National Institute of Advanced Industrial Science and Technology (NMIJ/AIST), which is a national metrology body, were used. For DTX2, CRM-DTX2 from the National Research Council Canada was used.

Fig. 1 shows the chromatogram when 5 µL of a three-compound mixed standard solution (1 ppb each) was injected, and Table 1 shows the repeatability of retention times and area values for each compound over five repetitions. OAs can be detected using the electrospray ionization (ESI) method in the negative ion mode. This analysis complies with the method specified in the Notice, and the details are shown in Table 2.

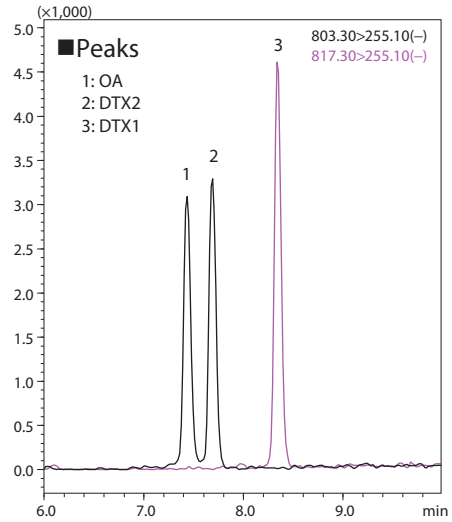

Fig. 1 MRM Chromatogram of the Standard Solution (1 ppb Each)

Table 1 Repeatability (1 ppb, n=5)

	R.T. %RSD	Area %RSD
OA	0.0419	2.03
DTX2	0.0401	2.98
DTX1	0.0385	2.08

Table 2 Analysis Conditions

Column	:	Shim-pack Scepter™ C18 (100 mm × 2.0 mm I.D., 1.9 µm)
Mobile Phases	:	A 2 mmol/L ammonium formate water with 50 mmol/L formic acid B Acetonitrile / Water: 95 / 5 (v/v) including 2 mmol/L ammonium formate with 50 mmol/L formic acid
Time Program	:	B conc. 40% (0 - 2.5 min) → 100% (7.5 - 12.5 min) → 40% (12.51 - 17.5 min) (Using the front cut valve, introduced into the MS only for 6 - 10 min)
Flow Rate	:	0.2 mL/min
Column Temperature	:	40 °C
Injection Volume	:	5 µL (2 µL when analyzing a scallop midgut gland certified reference material)
Probe Voltage	:	–3.0 kV (ESI-negative mode)
IF/DL/BH Temperature	:	350 / 150 / 450 °C
NG/HG/DG Flow	:	3 / 5 / 15 L/min
ESI probe position	:	+2 mm
MRM Transition	:	OA 803.30>255.10, 803.30>113.10 DTX2 803.30>255.10, 803.30>113.10 DTX1 817.30>255.10, 817.30>113.10

Linearity of Calibration Curve

Fig. 2 shows the calibration curves for each of the three compounds. When the calibration curve was created in the 0.1 to 10 ppb concentration range for each compound, favorable linearity was obtained with a coefficient of determination (r^2) of 0.999 or higher.

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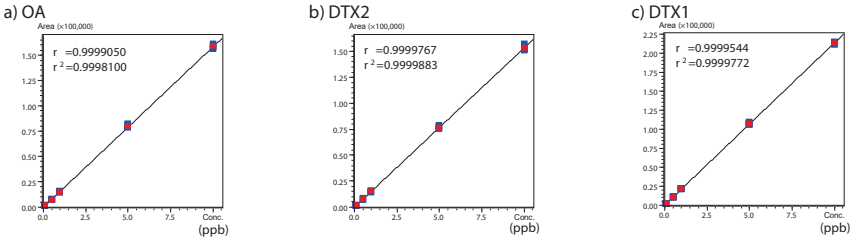

Fig. 2 Calibration Curve Linearity (0.1 to 10 ppb)

Analysis of Scallop Midgut Gland Certified Reference Material

Using a scallop midgut gland certified reference material, NMIJ CRM 7520-a[*1], extraction, hydrolysis, and purification were implemented in accordance with the method specified in the Notice (Fig. 3). 300 µL (250 µL according to the Notice) of 2.5 mol/L HCl was added for neutralization after hydrolysis. A reverse-phase polymer solid phase extraction column (200 mg, 6 cc) was used for the purification.

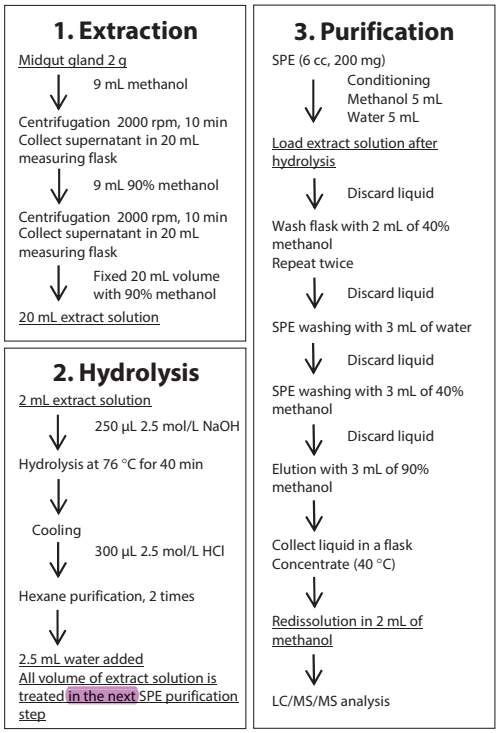

Fig. 3 Preparation

Table 3 Quantitative Value and Recovery Rate

Compounds	Certified Reference Materials		Extracted Samples				
	Certified Value (mg/Kg)	Expanded Uncertainty Mass Fraction (mg/Kg)	Quantitative Value (mg/Kg)	Recovery Rate (%)	Area Value Average (n=6)	Standard Deviation	Area Value %RSD (n=6)
OA	0.205	0.061	0.192	93	105424	2414.48761	2.29
DTX1	0.450	0.110	0.385	85	253677	1439.89408	0.57

It is generally known that the matrix effect of contaminants originating from midgut gland of scallops is large in LC/MS/MS analysis. Although it is possible to eliminate their influence by sufficiently diluting the sample, this time we introduce the standard addition method, which can be applied to various kinds of samples. Since the amount of OA standards purchased was small, standards were added to achieve concentrations at LC/MS/MS analysis of 10, 20, and 50 ppb for the extract before hydrolysis, and created a calibration point. Fig. 4 shows the chromatogram of the midgut gland extract after SPE purification (standard not added), Fig. 5 shows the calibration curves, Table 3 shows the quantitative value, recovery rate, and the area value repeatability of the certified reference material. The area repeatability %RSD of each peak, which is said to have an extensive matrix effect, is favorable with OA being 2.29 and DTX1 0.57 (n=6), the recovery rates of OA and DTX1 are 93% and 85%. It was shown that it is possible to quantify diarrhetic shellfish toxins according to the method specified in the Notice using LCMS™-8060.

*1 National Metrology Institute of Japan / National Institute of Advanced Industrial Science and Technology
Scallop midgut gland certified reference material, NMIJ CRM 7520-a No. 009 (for diarrhetic shellfish toxin analysis)
The uncertainty of certified values is the expanded uncertainty determined from the combined standard uncertainty and the coverage factor k = 2, representing half the width of the interval estimated to have a confidence level of approximately 95%.

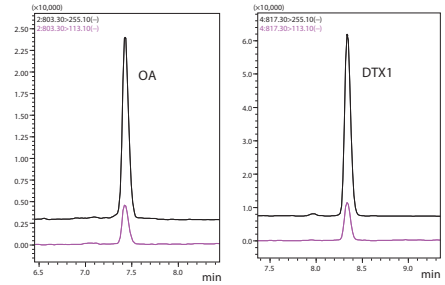

Fig. 4 Chromatograms of Certified Reference Materials

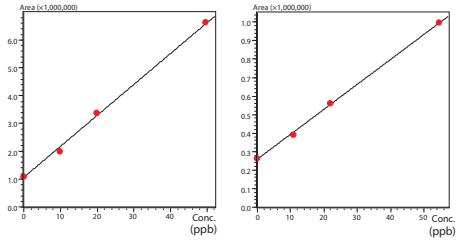

Fig. 5 Calibration Curves by Standard Addition Method

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Application News

No.C121

Liquid Chromatography Mass Spectrometry

Simultaneous Analysis of Nine Sweeteners Using Triple Quadrupole LC/MS/MS (LCMS-8040)

Artificial sweeteners such as saccharin sodium, aspartame, sucralose and acesulfame potassium fall under the category of specified additives in Japan's Food Sanitation Act, for which each specified criteria exist for their use in terms of eligible foods and amounts used.
Cyclamate, an artificial sweetener used in some regions of the world outside Japan, is an unspecified additive within Japan, for which inspection is required on specific imported foods.
In light of these situations, there is a demand for analyses of various different sweeteners, not only the quantitative testing of permitted sweeteners but also the testing of unspecified sweetener additives.
This article presents a simultaneous analysis of nine sweeteners including both specified additives and unspecified additives, using the LCMS-8040 high-performance liquid chromatograph-triple quadrupole mass spectrometer.

Analysis of a Standard Mixture

Fig. 1 shows chromatograms measured from a 5 µL injected sample of a 10 ng/mL standard mixture of nine sweeteners, analyzed with the analytical conditions shown in Table 1. Chromatograms at around the lower limit of quantitation (LLOQ) are shown in Fig. 2. The retention time, calibration curve range, and correlation coefficient for each compound are shown in Table 2.
A calibration point accuracy of within 100 ± 20 % and a percentage of area repeatability (%RSD) of within 20 % were employed. Good linearity was obtained for all compounds with a correlation coefficient of 0.997 or higher.

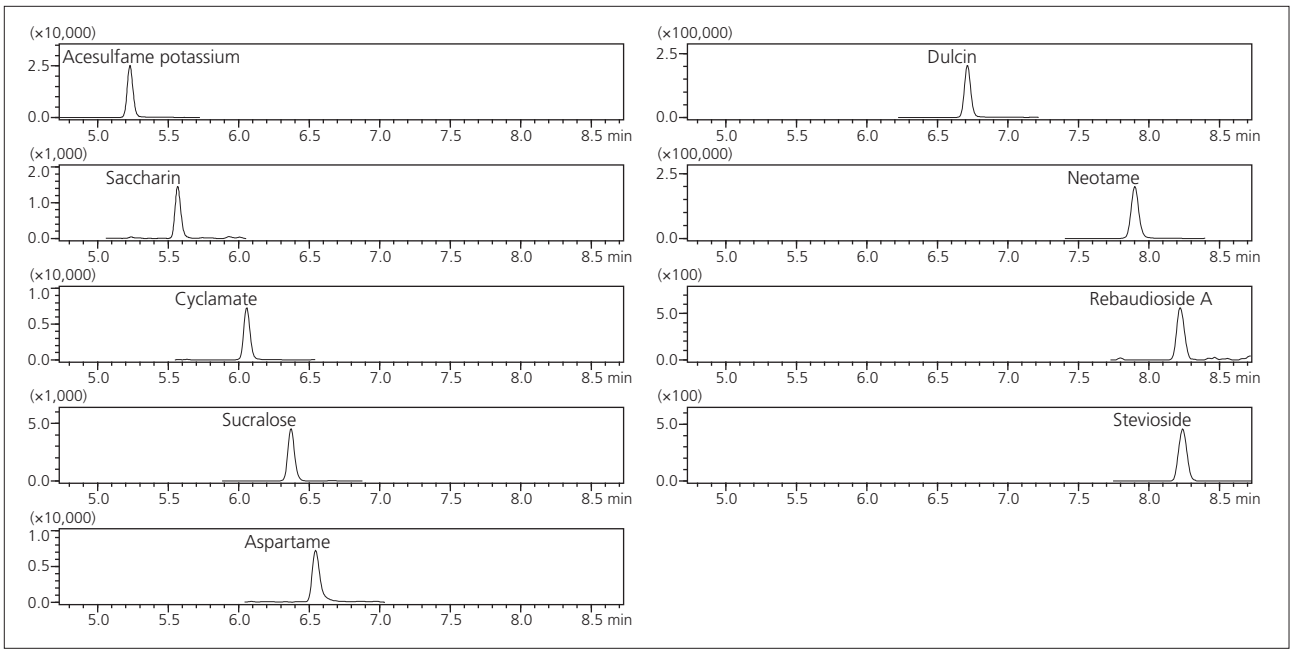

Fig. 1 Chromatograms from a 10 ng/mL Standard Mixture of Nine Sweeteners

Table 1 Analytical Conditions

Column	: Unison UK-C18 (150 mm L. × 3.0 mm I.D., 3.0 µm)
Mobile Phases	: A 5 mmol/L Ammonium Formate - Water
	: B Methanol
Gradient	: B Conc. 0 % (0.0 - 2.0 min) → 70 % (4.5 min) → 90 % (8.0 - 12.0 min) → 0 % (12.01-15.0 min)
Flowrate	: 0.2 mL/min
Column Temperature	: 40 ˚C
Injection Volume	: 5 µL
Probe Voltage	: + 4.5 kV (ESI-positive mode) / -3.5 kV (ESI-negative mode)
DL Temperature	: 300 ˚C
Block Heater Temperature	: 500 ˚C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 15 L/min

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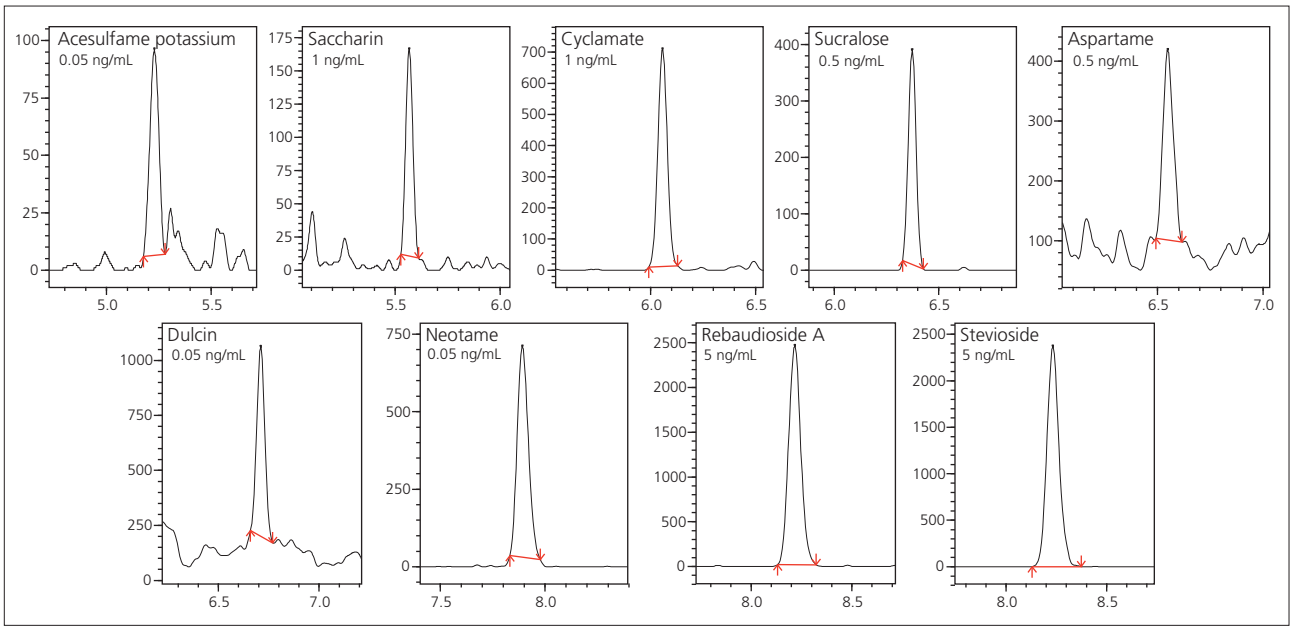

Fig. 2 Chromatograms of Nine Sweeteners at Around LLOQ

Table 2 Linearity of Nine Sweeteners

Compound Name	Polarity	Transition	Retention Time (min)	Calibration Curve Range (ng/mL)	Correlation Coefficient
Acesulfame potassium	–	162.00 > 82.10	5.228	0.05 – 100	0.997
Saccharin	–	182.00 > 42.00	5.561	1 – 100	0.999
Cyclamate	–	178.00 > 80.00	6.057	1 – 100	0.998
Sucralose	+	413.90 > 199.00	6.370	0.5 – 500	0.999
Aspartame	–	293.10 > 261.10	6.543	0.5 – 1000	0.999
Dulcin	+	181.20 > 108.10	6.712	0.05 – 10	0.999
Neotame	+	379.10 > 172.20	7.898	0.05 – 1000	0.999
Rebaudioside A	–	965.30 > 803.40	8.220	5 – 1000	0.999
Stevioside	+	822.30 > 319.20	8.238	5 – 1000	0.999

Recovery from Actual Samples

Seven sweeteners were added to foods (curry paste, rice cake flavored with mugwort, and sponge cake) pretreated by dialysis (Fig. 3), and the matrix effect was evaluated. The recovery of each added sweetener is shown in Table 3. Dulcin was the only sweetener for which the recovery was calculated based on a 1000-fold dilution of the solution after dialysis treatment, while the recovery of all other sweetener samples was calculated based on 100-fold dilution. The recovery was good with all samples, ranging from 85 to 125 %.

Table 3 Recovery of Seven Added Sweeteners

Compound Name	Added Concentration	Recovery (%)		
		Curry Paste	Rice Cake Flavored with Mugwort	Chocolate Sponge Cake
Acesulfame potassium	5 µg/mL	100.8	94.2	93.7
Saccharin		97.0	87.7	88.3
Cyclamate		99.6	89.3	92.0
Sucralose		96.2	89.6	82.6
Aspartame		94.0	89.4	87.2
Dulcin		110.2	99.5	99.5
Neotame		122.5	106.9	110.0

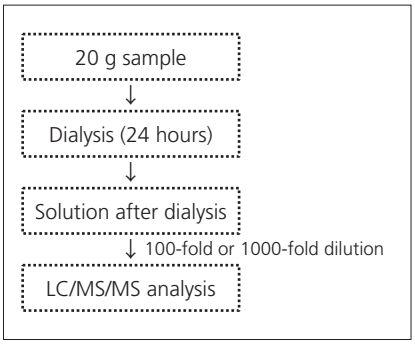

Fig. 3 Workflow of Pretreatment

This Application News was prepared with the cooperation of Tokyo Food Sanitation Association, who provided samples and guidance.

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Application News

No.C133

Liquid Chromatography Mass Spectrometry

Simultaneous Analysis of 16 Sweeteners Using Triple Quadrupole LC/MS/MS [LCMS-8050]

Artificial sweeteners such as aspartame, sucralose, and acesulfame potassium fall under the category of designated additives according to Japan's Food Sanitation Act, and prescribed standards are in place for their use in some foods and quantities.
Cyclamate and other artificial sweeteners used in some regions outside Japan are included among undesignated additives in Japan, and inspection is required in specific imported foods.
Consequently, quantitation for large numbers of sweeteners, including not only permitted in Japan but also undesignated, are needed.
Application News C121 described the simultaneous analysis of nine artificial sweeteners including both designated and undesignated additives using an LCMS-8040 triple quadrupole LC/MS/MS system. In this article, we introduce an example of simultaneous analysis of 16 sweeteners using an LCMS-8050.

Standard Mixture Analysis

MRM analysis was performed on 16 sweeteners using the analytical conditions shown in Table 1. Chromatograms of each compound near their lower limit of quantitation are shown in Fig. 1, with calibration curve ranges and correlation coefficients shown in Table 2. Results that met an accuracy of 100 % ±20 % and area repeatability (%RSD) of within 20 % were used for calibration point. Good linearity was obtained for all compounds, with correlation coefficients of 0.997 or higher.

Table 1 Analytical Conditions

Column	: Unison UK-C18 (150 mm L. × 3.0 mm I.D., 3.0 µm)	Injection Volume	: 1 µL
Mobile Phases	: A 5 mmol/L Ammonium formate - Water	Probe Voltage	: + 4.0 kV (ESI-positive mode) / -3.0 kV (ESI-negative mode)
	: B 5 mmol/L Ammonium formate - Methanol	Nebulizing Gas Flow	: 3 L/min
Gradient	: B.Conc. 0 % (0.0-2.0 min) → 70 % (4.5 min) → 90 % (8.0-12.0 min) → 0 % (12.01-15.0 min)	Heating Gas Flow	: 10 L/min
		Interface Temperature	: 300 °C
		DL Temperature	: 150 °C
Flowrate	: 0.4 mL/min	Block Heater Temperature	: 250 °C
Column Temperature	: 40 °C	Drying Gas Flow	: 10 L/min

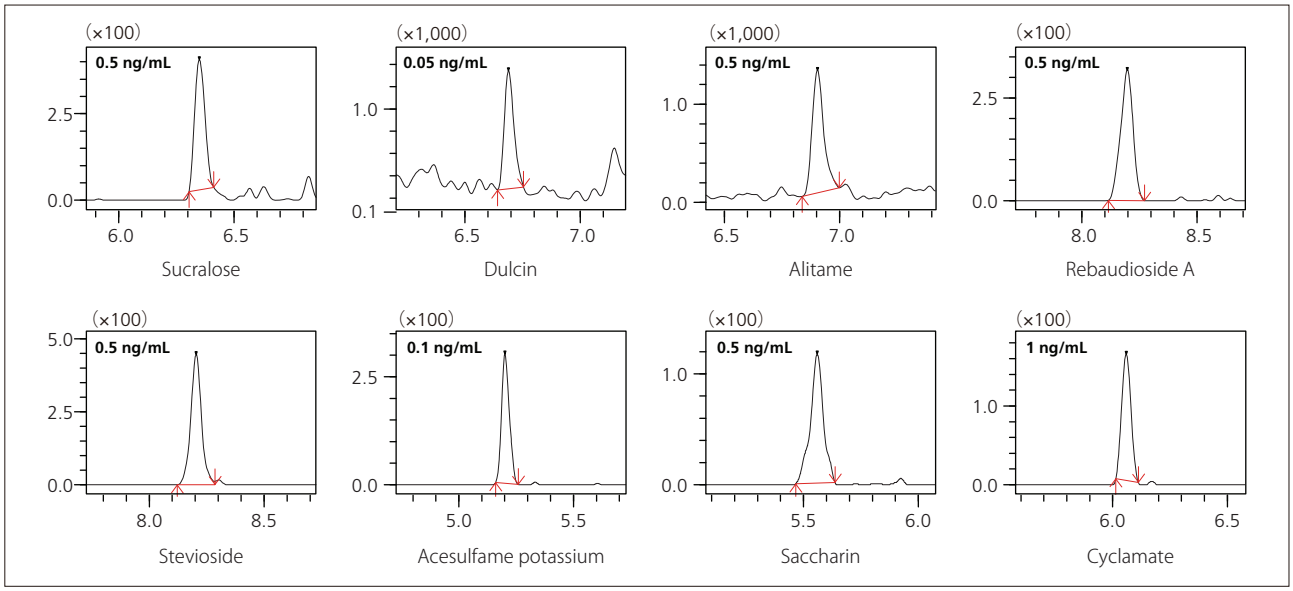

Fig. 1-1 Chromatograms of 16 Sweeteners

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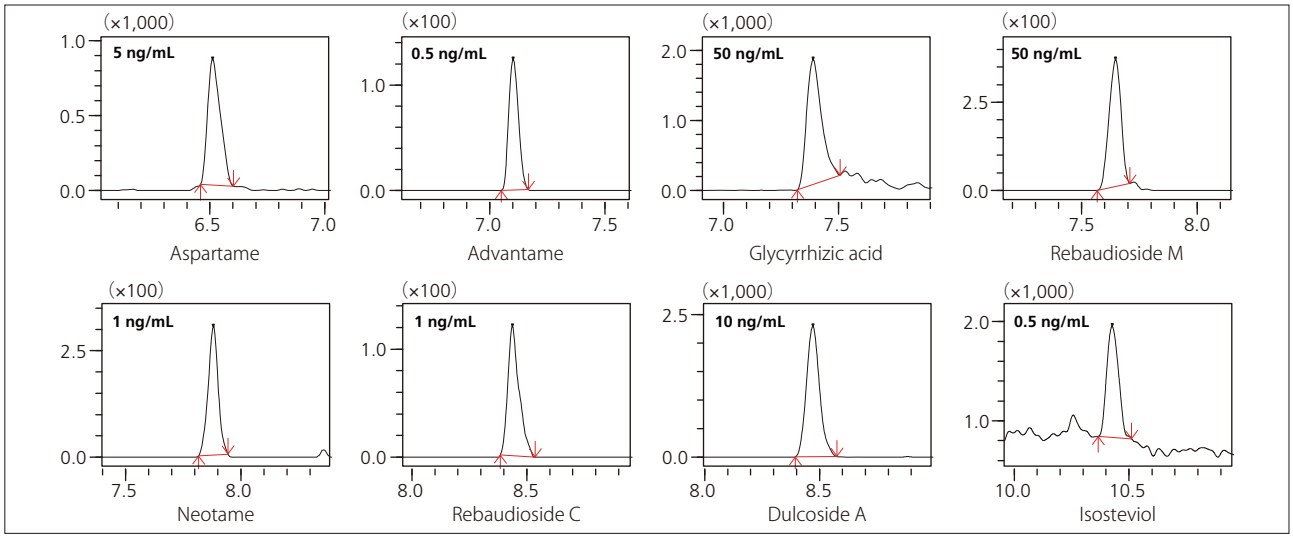

Fig. 1-2 Chromatograms of 16 Sweeteners (continued)

Table 2 Linearity of 16 Sweeteners

Compound Name	Polarity	Transition	Holding Time (min)	Calibration Curve Range (ng/mL)	Correlation Coefficient
Sucralose	+	414.00>199.10	6.36	0.5 - 100	0.999
Dulcin	+	181.20>108.10	6.70	0.05 - 10	0.999
Alitame	+	332.20>129.00	6.92	0.5 - 100	0.999
Rebaudioside A	+	984.50>325.10	8.21	0.5 - 100	0.999
Stevioside	+	822.00>319.30	8.23	0.5 - 100	0.999
Acesulfame potassium	−	161.90>82.00	5.23	0.1 - 10	0.999
Saccharin	−	181.90>42.00	5.58	0.5 - 50	0.997
Cyclamate	−	178.00>80.00	6.08	1 - 100	0.999
Aspartame	−	293.40>261.10	6.53	5 - 100	0.999
Advantame	−	457.30>200.30	7.12	0.5 - 100	0.999
Glycyrrhizic acid	−	821.20>351.10	7.41	50 - 1000	0.999
Rebaudioside M	−	1289.60>802.90	7.66	50 - 1000	0.999
Neotame	−	377.30>200.00	7.90	1 - 100	0.999
Rebaudioside C	−	949.50>787.20	8.46	1 - 100	0.999
Dulcoside A	−	787.50>625.20	8.50	10 - 1000	0.999
Isosteviol	−	317.30>317.30	10.46	0.5 - 1000	0.999

Recovery from Real World Samples

Sweeteners were added to sample solutions prepared according to the procedure shown in Fig. 2, and recovery of these additives was verified by measuring the samples after 100-fold or 1000-fold dilution. The results are shown in Table 3.

Dialysis and solid phase extraction are common methods used in sample pretreatment for sweetener analysis, but these operations have the drawback of being complex, time-consuming, and laborious. Pretreatment by solvent extraction requires no special equipment, and can be performed quickly and simply.

Table 3 Recovery

Compound Name	Additive Concentration	Real World Sample	Dilution Ratio	Recovery (%)
Glycyrrhizic acid	100 µg/mL	Soy sauce	100	85.20
Acesulfame potassium	10 µg/mL	Powdered soft drink (café au lait)	1000	81.21
Aspartame				104.2
Neotame	10 µg/mL	Ketchup	100	108.5

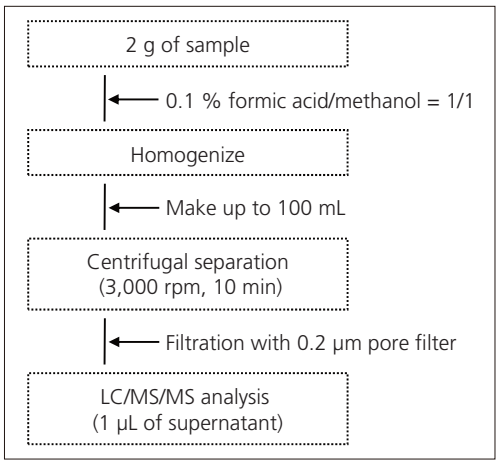

Fig. 2 Pretreatment Workflow

This Application News was prepared with the cooperation of Japan Food Research Laboratories, who provided samples and guidance.

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Application News

No.C141

Liquid Chromatography Mass Spectrometry

High Sensitivity Analysis of Peanut Allergen in Cumin and Spice Mix [LCMS-8060]

Food allergens are a major public health concern. Among them, peanut allergy is one of the common food allergies. To avoid unexpected contact with food allergens, food labels are strictly used to indicate the presence of specific allergens. With the increasing awareness of food allergies, the presence of undeclared peanut in cumin lead to huge recalls in recent years. Although ELISA is the most commonly used technique to detect allergens, its false-positive rate is a major concern due to its cross-reactivity. We developed a method with high specificity and sensitivity to overcome this issue by using a high sensitivity triple quadrupole mass spectrometer to detect peanut allergen Ara h1 (Fig.1) in commercially available spices and seasonings.

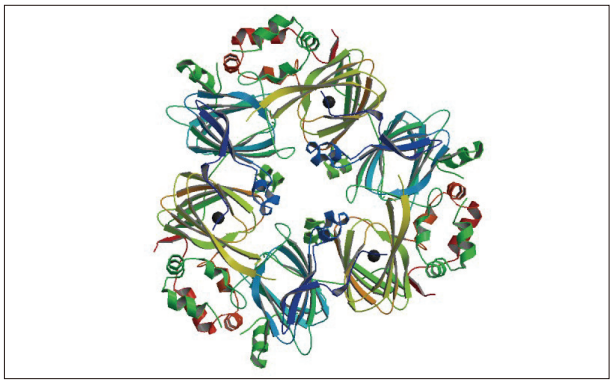

Fig. 1 Structure of Ara h1 [3S7I] (68kDa) Vicilin Like Protein

Sample Preparation

Commercially available defatted peanut flour was purchased and used for the initial development work. The test samples were ground and protein content was enriched by liquid-liquid extraction. Extracted proteins were denatured, reduced and alkylated before subjecting to tryptic digestion to obtain peptides that were quantitated as proxies of original protein abundance.

Cinnamon, cumin, chilli pepper, ginger, garlic, mustard seed, nutmeg, oregano, rosemary, sage, turmeric and thyme were selected as test food samples for evaluating cross-reactivity and sensitivity of the developed method. Food samples were pretreated as above with or without 2 ppm peanut powder.

Selection of MRM Transitions Using Skyline

Ara h1 is known as is known as the sensitizing allergen in 95 % of peanut allergy. Tryptic digest of protein extracted from peanuts were analyzed by monitoring theoretically calculated transitions of peptides based on amino acid sequences of two clones P17 and P41B of Ara h1.

MRM transitions for each clone was determined by using Skyline (MacCoss Lab Software). The transition list, which contained more than ten peptides for each clone, was reviewed by removing several peptides that could be susceptible by post translational modification and Maillard reaction during food processing.

Finally, nine peptides including three common peptides to both clones were selected based on sensitivity. Three transitions were set for each peptide.

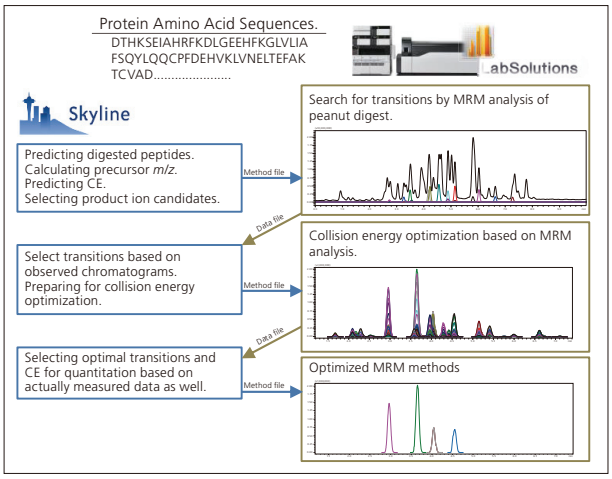

Fig. 2 Workflow of MRM Transition Optimization Using Skyline

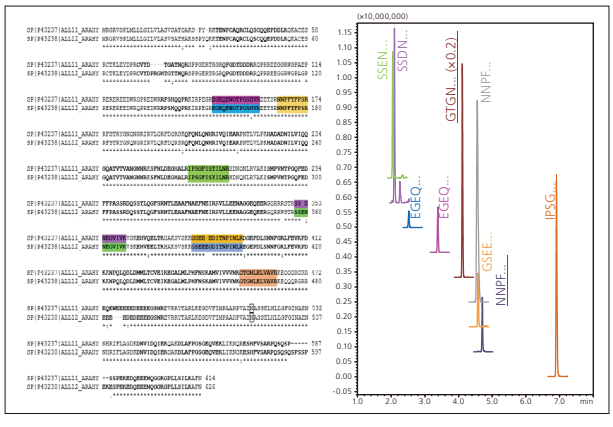

Fig. 3 AA Sequences of P17/P41B and Nine MRM Chromatograms

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Table 1 Analytical Conditions

System	: Nexera X2	System	: LCMS-8060
Column	: Shim-pack XR-ODS Ⅱ (50 mm L. × 2 mm I.D., 1.6 µm)	Ionization	: Heated ESI
		Probe Voltage	: +1 kV (positive ionization)
Column Temperature	: 40 °C	Temperature	: Interface: 250 °C Desolvation Line: 150 °C Heater Block: 200 °C
Mobile Phases	: A: Water + 0.1 % formic acid B : Acetonitrile		
Flowrate	: 500 µL/min	Gas Flow	: Nebulizing Gas: 3 L/min Heating Gas: 20 L/min Drying Gas: 5 L/min
Gradient	: 2 %B (0.00 min) > 25 %B (7.00 min) > 95 %B (7.10-8.00 min) > 2 %B (8.10-10.00 min)		
Injection Volume	: 10 µL		

Table 2 MS/MS Acquisition Parameters

MRM Transitions	Name	Polarity	Quan	Qual1	Qual2
	EGEQEWGTPGSEVR	+	780.85 > 802.40	780.85 > 644.35	780.85 > 316.10
	NNPFYFPSR	+	571.25 > 669.35	571.25 > 506.25	571.25 > 229.10
	IPSGFISYILNR	+	690.40 > 765.45	690.40 > 211.15	690.40 > 502.25
	SSDNEGVIVK	+	524.25 > 515.35	524.25 > 359.25	524.25 > 175.05
	GSEEEDITNPINLR	+	793.90 > 726.45	793.90 > 612.40	793.90 > 402.25
	GTGNLELVAVR	+	564.80 > 686.40	564.80 > 557.40	564.80 > 444.30
	EGEQEWGTPGSHVR	+	784.85 > 652.35	784.85 > 555.30	784.85 > 316.10
	SSENNEGVIVK	+	588.30 > 515.35	588.30 > 359.25	588.30 > 246.20
	GSEEEGDITNPINLR	+	822.40 > 726.45	822.40 > 612.40	822.40 > 402.25

Dwell Time	: 41 to 130 msec depending upon the number of concomitant transitions to ensure to have at least 15 points per peak (max total loop time 400 msec).
Pause Time	: 3 msec
CID Pressure	: 300 kPa
Quadrupole Resolution	: Q1: Unit Q3: Unit

Interface Optimization

Ionization parameters optimization was performed using companion software ISSS (Interface Setting Support Software, Shimadzu Corp.). As a result, sensitivity was improved more than twofold compared to default values.

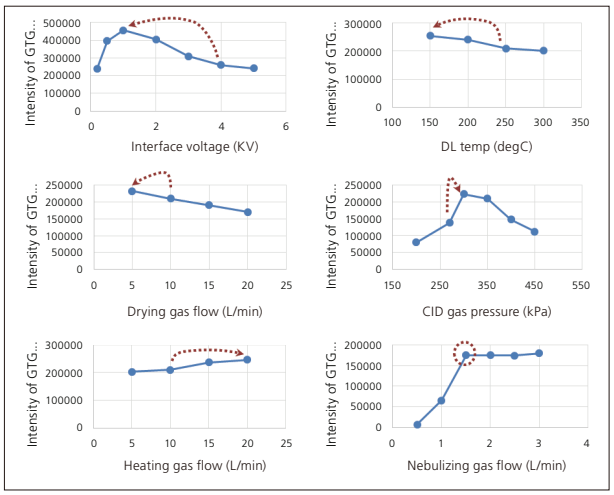

Fig. 4 Interface Optimization Results

Effect of Surfactant During Digestion

A higher intensity of peptides by addition of a surfactant during tryptic digestion was expected due to improved digestion efficiency. However, the intensity of peptides were relatively worse by adding surfactant. Thus, no surfactant was used for tryptic digestion.

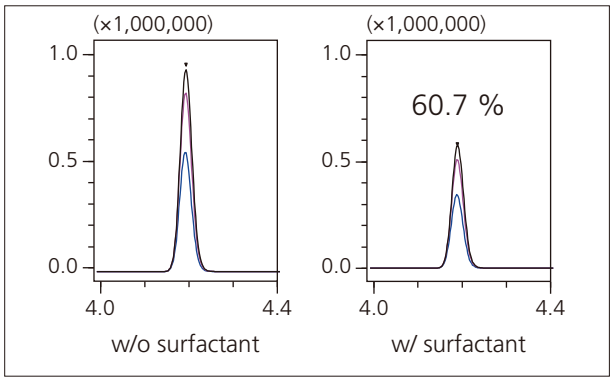

Fig. 5 Difference of the Chromatograms of Peptide GTG... by Addition of Surfactant

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Peanut Allergen in Other Nuts

Walnuts, cashew nuts, and almonds were analyzed to test specificity. These nuts were spiked with 2 ppm (2 mg/kg) of peanut before sample preparation. The spiked peanut peptides were successfully detected and any obvious peak was detected in blank samples.

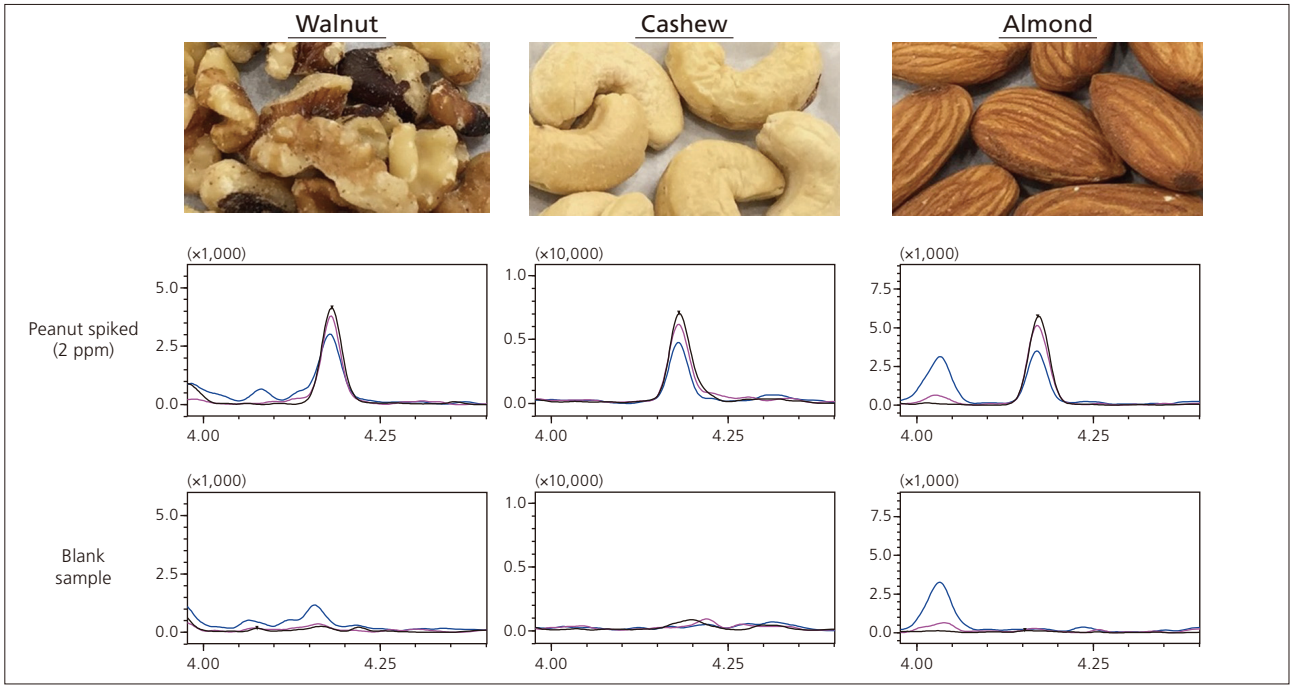

Fig. 6 Chromatograms of Peptide GTG… in Other Kind of Nuts With or Without Spiking with Peanuts

Detection of ARA h1 in Spice Mixes and Seasonings

Several spice mixes and seasonings were analyzed using sample preparation and analytical conditions described here. Peaks of tryptic peptides of Ara h1 from samples without spiking of peanut peptides were detected.

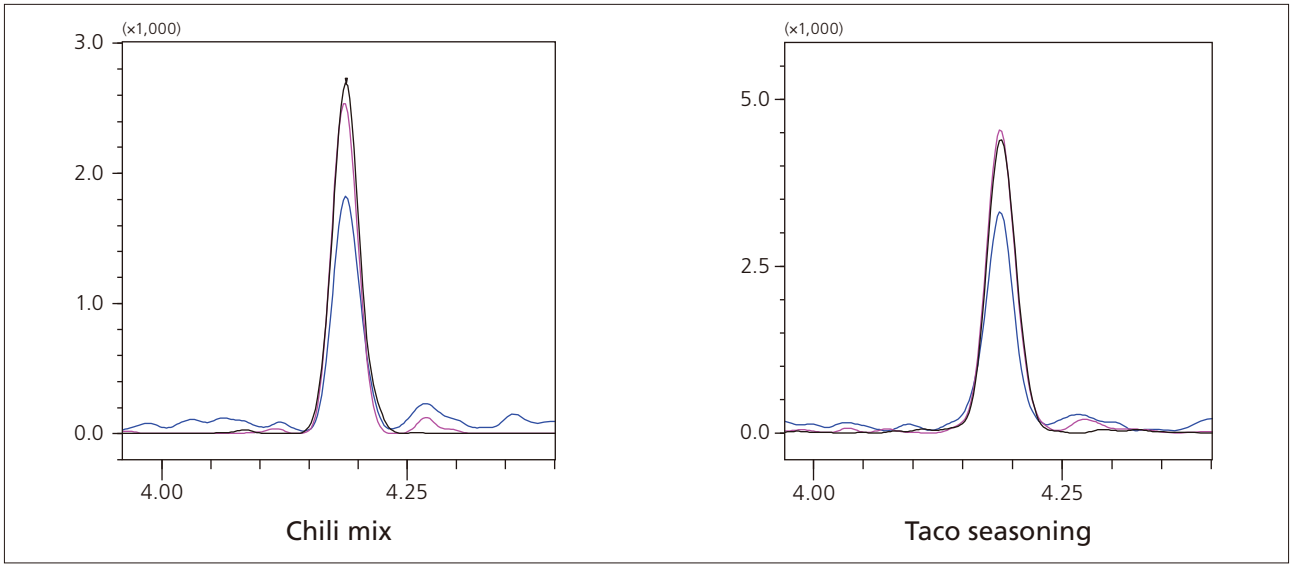

Fig. 7 Detected Peaks of Peptide GTG… in Chili Mix and Seasoning

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Peanut Allergen in Spices

Contaminated spice samples were prepared and analyzed to confirm that the low amount of peanuts added into the various spices can be detected. Peptides of Ara h1 were successfully observed from the spice samples spiked with 2 ppm of peanuts. It was also confirmed that there are no obvious false-positive peaks from the blank samples.

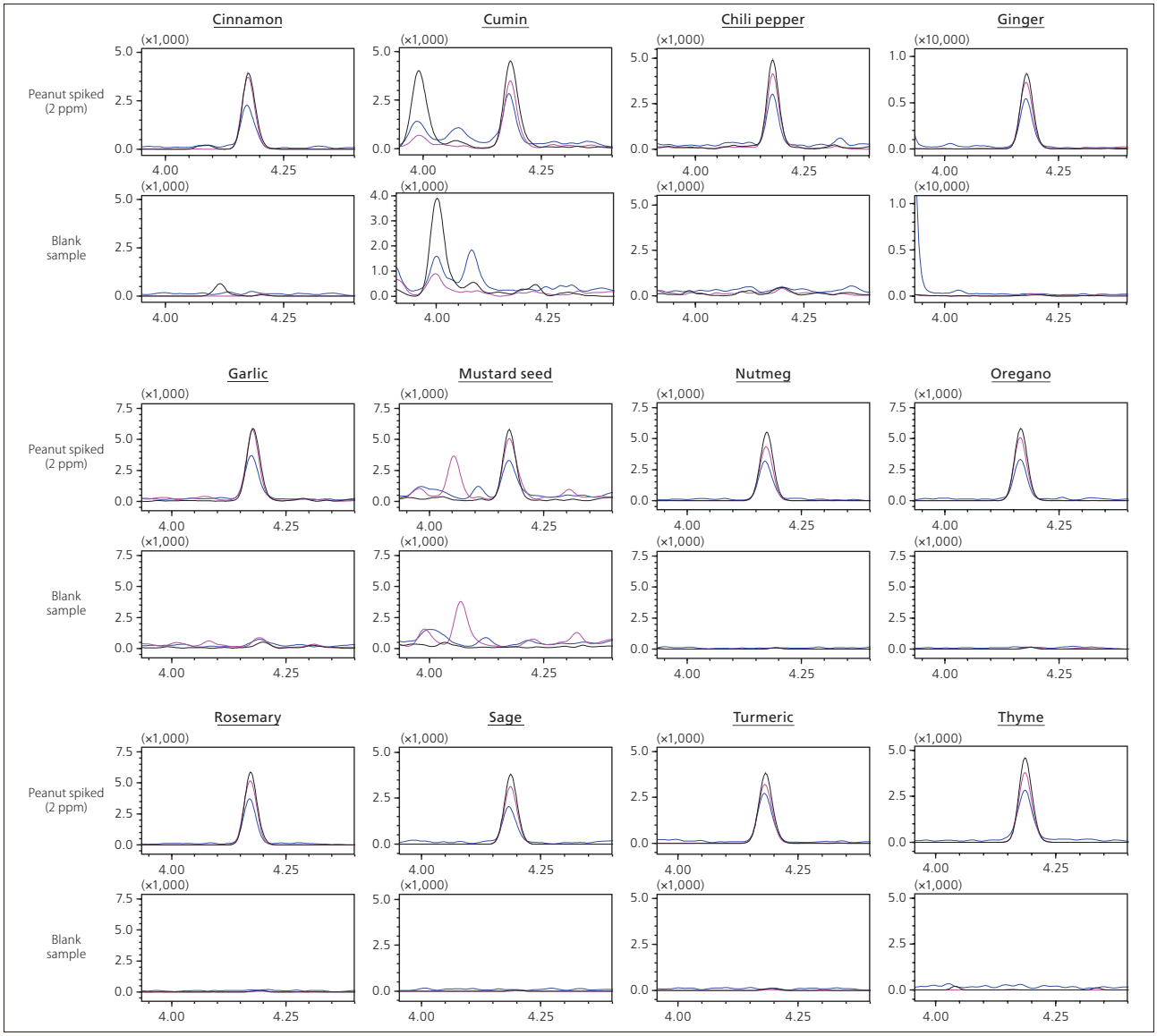

Fig. 8 Chromatograms of Peptide GTG... in Spices With or Without Spiking with Peanuts

Conclusion

A method for the analysis of Ara h1 in spices and seasonings was successfully developed.
The combination of the developed method and a high sensitivity triple quadrupole mass spectrometer enabled the detection of 2 ppm or lower of peanut allergen Ara h1 in spices and seasonings.

First Edition: Dec. 2016

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Application News

No. C164

Liquid Chromatograph Mass Spectrometry

Ultra-High-Speed Analysis of Melamine in Powdered Milk Using LDTD-MS/MS

The deliberate contamination of powdered milk and pet food with melamine has become a serious social issue. If melamine is contained in food at high concentrations together with cyanuric acid, which is produced in the manufacturing process of melamine, contamination can lead to kidney stones and even kidney failure. In many cases, melamine is added for producing adulterated products, and when added, is done so at very high concentrations. In order to stop these sorts of adulterated products at the border, high-speed screening analysis that can be performed together with easy sample preparation is required. A widely reported analysis technique for melamine in powdered milk involves using LCMS and GCMS after performing pretreatment to remove impurities. This article describes an ultra-high-speed analysis of melamine in powdered milk without column separation by using a laser diode thermal desorption (LDTD) ion source together with the LCMS-8060.

An ion source for ultra-high-speed screening analysis developed by Phytronix Technologies Inc. (https://phytronix.com/) in Canada was employed as the LDTD ion source. Mass spectrometry can be completed within a few seconds by sample vaporization using laser irradiation and subsequent APCI ionization. By applying samples to 96-well plates, up to 10 plates can undergo consecutive analysis. When using the LDTD ion source together with a Shimadzu LCMS-8060, each instrument can be utilized as necessary, such as for direct analysis using LDTD or for LC/MS analysis with column separation, simply by loading a method file with no need to disconnect the LDTD ion source from the LCMS-8060 (Fig. 1). This allows for MRM optimization of the compound for analysis on the LCMS-8060 and then ultra-high-speed analysis with LDTD using the determined MRM transitions. Conversely, polyspecimen analysis screening using ultra-high-speed analysis with LDTD can be performed first, and then using the results, LC/MS analysis can be performed with respect to a particular sample. In this way the combination of the LDTD ion source and LCMS-8060 can be used to switch between two completely different analysis methods according to the purpose of analysis.

In this research, we connected an LDTD ion source, performed MRM optimization of melamine using DUIS (dual ion sources of ESI and APCI), and then used the obtained MRM transitions in ultra-high-speed analysis by LDTD-MS. In performing ultra-high-speed analysis by LDTD-MS, we used a mass spectrometry system comprising an LDTD ion source and the LCMS-8060 and used samples prepared by adding melamine to powdered milk and collecting the melamine using liquid-liquid extraction. The following introduces an example of analyzing melamine in powdered milk by switching between the two analysis systems of LCMS and LDTD-MS.

MRM Optimization Using LC-MS with an LDTD System Connected

First, MRM optimization was performed in DUIS mode using a standard sample of melamine. The LC conditions used in optimization were the MRM optimization conditions used for general flow injection analysis (FIA). Fig. 2 shows the MS/MS spectrum (CE: −25 V) obtained when optimizing melamine in DUIS mode. Of the MRM transitions (*m/z* 126 > 85, 127 > 68, and 127 > 43) identified under these conditions, the MRM transition (*m/z* 127 > 68) with low background noise in LDTD-MS analysis was used to perform the analysis of melamine in powdered milk with LDTD-MS.

T. Nakanishi

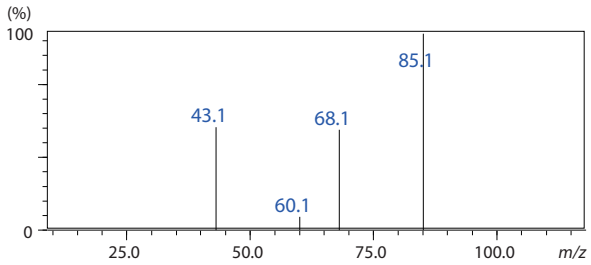
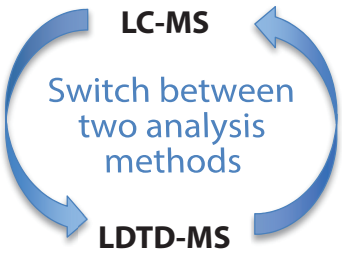


Fig. 2 MS/MS Spectrum of Melamine Using DUIS Mode



While independently utilizing the three ionization methods of ESI, APCI, and DUIS, MRM optimization of target components can be performed to ensure a smooth start to ultra-high-speed analysis using LDTD, and in cases of complex analysis samples, detailed analysis by LC/MS can be performed following the LDTD analysis.

Easy application of samples to 96-well plates for LDTD-MS allows ultra-high-speed analysis (four second ionization) of multiple components by LDTD-MS.

Fig. 1 Two Methods of Analysis Using LC-MS and LDTD-MS

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Extraction of Melamine Added to Powdered Milk

Commercially-available powdered milk was weighed out (125 mg portions) and transferred to 1.5 mL Eppendorf tubes. Next, 0.5 mL of ultra pure water and 0.5 mL of acetonitrile were added and the mixtures were thoroughly agitated for one minute. Then, 12.5 µL of 0, 5, 10, 25, 50, 100, 500, and 1000 µg/mL melamine solutions prepared in advance were added to each powdered milk suspension. These correspond to the concentrations of 0, 0.5, 1, 2.5, 5, 10, 50, and 100 ppm in the powdered milk. Further agitation was performed for another minute to ensure that the added melamine was sufficiently mixed into each solution. Powdered milk components were precipitated by centrifugal separation (14,000 g, room temperature, 5 min) and 200 µL of supernatant containing melamine was collected and transferred to new tubes. Next, 200 µL of sodium carbonate buffer solution (saturated NaCl, pH 10) was added and thoroughly agitated, and then 1 mL of ethyl acetate was added and sufficiently agitated. Since this separates into an aqueous layer and organic layer, centrifugal separation was performed. From the organic layer which contains melamine, 4 µL was taken and dispensed into a LazWell plate (96 well) and then dried. The LazWell plate was set into the LDTD ion source and batch analysis was performed on each sample.

Table 1 LDTD-MS Analysis Conditions

LDTD Analysis Conditions	
Laser pattern	: 65 % laser power, 2 seconds
Gas flow rate	: 3.0 L/min
MS Analysis Conditions	
Mode	: MRM (pos)
Interface	: APCI
DL temperature	: 250 °C
Heat block temperature	: 400 °C

LDTD-MS Analysis of Melamine Added to Powdered Milk

Table 1 summarizes the LDTD-MS analysis conditions. Fig. 3 shows MRM chromatograms of melamine added to powdered milk (corresponding to 0.5, 5, and 50 ppm concentrations in the powdered milk). It is apparent that the LDTD ion source ionized the melamine within just six seconds (within 0.1 minute). Also, analysis at n = 3 of the samples with melamine added at each concentration resulted in favorable repeatability as shown in Fig. 3. These results indicate that ultra-high-speed analysis by LDTD-MS has unparalleled throughput and is capable of quantitative analysis with high repeatability that is comparable to LCMS analysis. Next, the peak area for each additive concentration of melamine was graphed based on the analysis results of each sample concentration (Fig. 4). A linearity of $R^2 = 0.998$ was verified from these analysis results. From these results we can see that LDTD-MS enables ultra-high-speed analysis with both high repeatability and linearity, even for samples that contain many impurities, such as melamine in powdered milk.

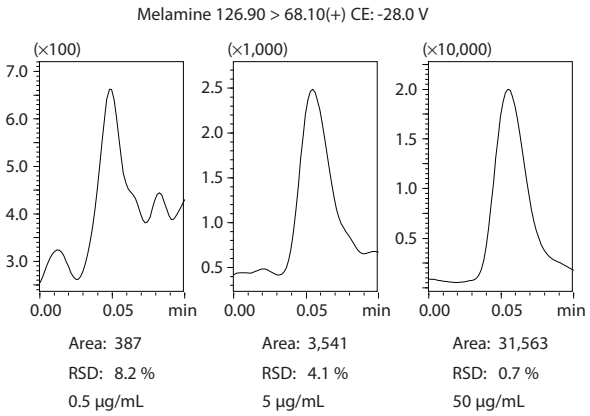

Fig. 3 MRM Chromatograms of Melamine Added to Powdered Milk

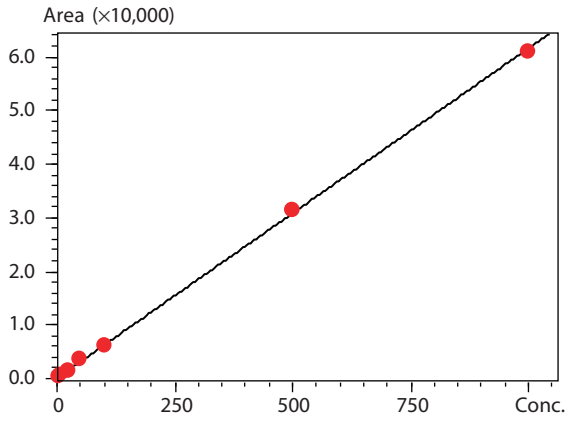

Fig. 4 Linearity of Melamine Added to Powdered Milk

In this research, we performed MRM optimization in DUIS mode on the LCMS-8060 followed by ultra-high-speed analysis using LDTD-MS with respect to melamine added to powered milk, and verified the level of repeatability and linearity. As demonstrated, the combination of the LCMS-8060 with an LDTD ion source allows easy switching of the analysis system according to the purpose of analysis, thereby allowing multicomponent optimization by LCMS, or LCMS analysis of complex analysis samples as necessary based on the results of simple ultra-high-speed screening analysis by LDTD. These two characteristic analysis methods can be utilized as necessary.

First Edition: Dec. 2017

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Application News

No. C203

Liquid Chromatography Mass Spectrometry

Food Metabolomics Analysis of Deterioration Characteristics of Alcoholic Drinks Using LC/MS/MS

Recently, metabolomics technology has become a hot topic due to its ability to comprehensively analyze in vivo metabolites. Food metabolomics has grown out of this technology allowing its application to food products. Conventionally, sensory analysis conducted by human assessors to evaluate flavors, aroma, deliciousness, grades, etc. has been the main method used in food evaluation. Food metabolomics is used to more scientifically "evaluate/predict the quality" of food and "explore functional ingredients" by comprehensively analyzing the metabolites in food and comparing the findings against those from evaluations conducted by humans such as sensory analysis.

This report describes an analysis method used to determine the deterioration characteristics of foods based on food metabolomics. The samples, commercially available Japanese rice wine (sake) and white wine, were stored under adverse conditions and then separated by high performance liquid chromatography mass spectrometry (LC/MS/MS), followed by multivariate analysis, to comprehensively investigate the changes in hydrophilic metabolites, including amino acids, organic acids, nucleosides, and nucleotides.

N. Kato Y. Inohana

Samples and Deterioration Experiment

The samples were commercially available alcoholic drinks, including two types of sake (kept refrigerated) and a white wine. The characteristics of these samples are shown in Table 1. To perform accelerated deterioration testing, the samples were stored under each of the test conditions shown in Table 2. Alcoholic drinks are currently distributed domestically and internationally and large volumes are imported and exported. Consequently, the ability to transport these beverages without a negative impact on quality is recognized as very important if the value of the products is to be maintained. The experimental conditions used in this study were designed to reproduce the conditions under which the quality of the products might be adversely affected during transportation, including exposure to the sun, high temperatures, and vibration.

Every sample stored under each of the specified conditions was separated by centrifugation at 12,000 rpm for 5 min, and the supernatant was diluted 100-fold with ultrapure water so it could be analyzed by LC/MS/MS.

Table 1. Characteristics of Test Samples

	Samples
Sake No. 1	Junmai-daiginjoshu, rice-polishing ratio = 50%, Alcohol by volume (ABV) = 15%
Sake No. 2	Ginjoshu, brewer's alcohol added, rice-polishing ratio = 50%, Alcohol by volume (ABV) = 15%
White wine	Produced in Australia, antioxidant (sulfite) added, Alcohol by volume (ABV) = 13%

Table 2. Experimental Conditions for Accelerated Deterioration Testing

	Storage Conditions
A	Stored in a refrigerator protected from light for 2 weeks
B	Stored at room temperature exposed to light for 2 weeks
C	Stored in a refrigerator protected from light for 2 weeks, followed by heating to 50° C while protected from light for 24 hours
D	Stored in a refrigerator protected from light for 2 weeks, followed by shaking at room temperature while protected from light for 24 hours.

Analysis Conditions

Using the ion-pairing free LC/MS/MS method of the LC/MS/MS Method Package for Primary Metabolites Ver. 2, the analysis was conducted with LCMS™-8060 (Fig. 1). The analysis method included in the package enables the simultaneous analysis of the 97 hydrophilic metabolites, which are known to be important in metabolome analyses in the field of life science. The HPLC and MS analysis conditions are shown in Table 3.

Table 3. Analysis Conditions

[HPLC conditions] (Nexera™ X2)		
Column	:	Reversed-phase column
Mobile phases	:	A) 0.1% Formic acid in water
		B) 0.1% Formic acid in acetonitrile
Mode	:	Gradient elution
Flow rate	:	0.25 mL/min
Injection volume	:	3 µL
[MS conditions] (LCMS-8060)		
Ionization	:	ESI (Positive and negative mode)
Mode	:	MRM
Nebulizing gas flow	:	3.0 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

Fig. 1. Nexera™ X2 and LCMS™-8060

Metabolome Analysis

Each sample was measured by LC/MS/MS, and then principal component analysis (PCA) and one-way analysis of variance (one-way ANOVA) were conducted using the areas of each component with Traverse MS software.

When PCA was performed, no apparent difference was observed between the samples stored under different conditions for any of the types of alcoholic drinks tested. In contrast, detailed examination of ANOVA results revealed that some of the components increased or decreased according to the type of alcoholic drink and/or storage conditions. As an example, the results of ANOVA for the effects of storage conditions on sake No. 1 are shown in Fig. 2. The green frames indicate the results for components which showed significant differences ($p<0.05$) between the samples stored under different conditions.

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Fig. 2. Results of ANOVA for the Effects of Storage Conditions on the Components in Sake No. 1

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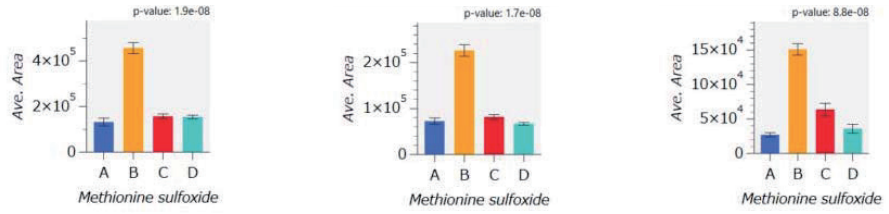
In this experiment, some of the components measured in the samples of sake No. 1 stored under condition B were significantly different from those of the samples stored under the other conditions. A similar trend was observed for sake No. 2 and the white wine, showing that some of the experimental conditions, such as heating to 50° C or shaking for around 24 hours, were not sufficient to have a significant impact on hydrophilic compounds, including amino acids and organic acids. It is difficult to draw definitive conclusions based solely on the findings of this study. However, the results suggest that even if the products are accidentally exposed to conditions such as heating and shaking, just for a short period during transportation and/or storage after purchasing the product, this is unlikely to have a significant impact on the quality of the product.

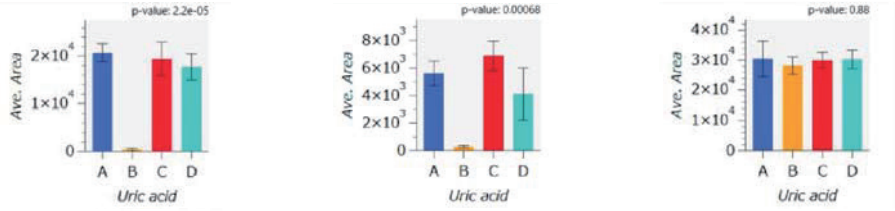
In the samples of sake No. 1, cysteine, methionine sulfoxide, and uric acid were the components which showed significant differences ($p<0.05$) between the samples stored under condition B and those stored under the other conditions. Additionally, there were several other components which showed, for example, a different trend only in the white wine samples. The results of comparing these components in each alcoholic drink tested are shown in Figs. 3 to 6.



Sake No. 1 Sake No. 2 White wine

Fig. 3. Results of ANOVA for Methionine Sulfoxide

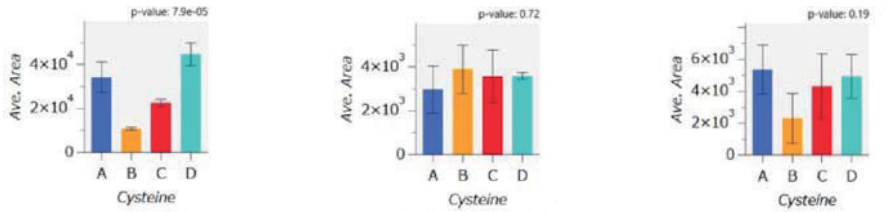
The results of statistical analysis for the methionine sulfoxide contained in each alcoholic drink tested are shown in Fig. 3. The analysis revealed that regardless of the type of alcoholic drink, the level of this component in the samples stored under condition B was markedly higher than those stored under the other conditions. Methionine is known to be an amino acid residue which is more susceptible to aging-associated oxidation and thus considered to be a cause of the increased in vivo oxidative protein damage, and is promptly oxidized to methionine sulfoxide under intracellular oxidative stress conditions. The results of this study suggest the possibility of using methionine sulfoxide as a marker of oxidation of the components of alcoholic drinks.



Sake No. 1 Sake No. 2 White wine

Fig. 4. Results of ANOVA for Uric Acid

Results of statistical analysis for uric acid contained in each alcoholic drink tested are shown in Fig. 4. The analysis revealed that only in the sake samples was the level of this component in those stored under condition B lower than those stored under the other conditions. Uric acid is highly susceptible to oxidation, allowing it to exert a strong antioxidant effect comparable to that of ascorbic acid, which is a known physiological role. It was assumed that uric acid contained in the wine samples was unlikely to undergo oxidation during the storage period because the white wine used in this study had sulfite added as an antioxidant.



Sake No. 1 Sake No. 2 White wine

Fig. 5. Results of ANOVA for Cysteine

Results of statistical analysis for cysteine contained in each alcoholic drink tested are shown in Fig. 5. The analysis revealed that only in sake No. 1 was the level of this component in the samples stored under condition B significantly lower than those stored under the other conditions. Besides methionine, cysteine is known as a precursor of dimethyl trisulfide (DMTS), a major malodorous component of deteriorated sake. Given that the lowered level of cysteine was associated with an increase in DMTS production, it is assumed that addition of brewers alcohol, which was a substantial difference between the samples of sake No. 1 and No. 2, may be a factor that could change the susceptibility of sake to deterioration.

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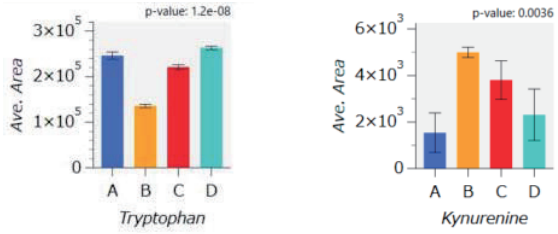

Fig. 6. Results of ANOVA for Tryptophan and Kynurenine in the White Wine Samples

Results of statistical analysis for tryptophan and kynurenine in the white wine samples are shown in Fig. 6. The analysis revealed that the levels of tryptophan and kynurenine in the samples stored under condition B were lower and higher, respectively, than those stored under the other conditions. A similar trend for tryptophan and kynurenine was observed in the samples stored under condition C, although the degree was small. Tryptophan is known to be metabolized to kynurenine through one of its known metabolic pathways, the kynurenine pathway (Fig. 7). Thus, the changes in these components observed in this study appear to correspond to the changes predicted from their relationship to this pathway.

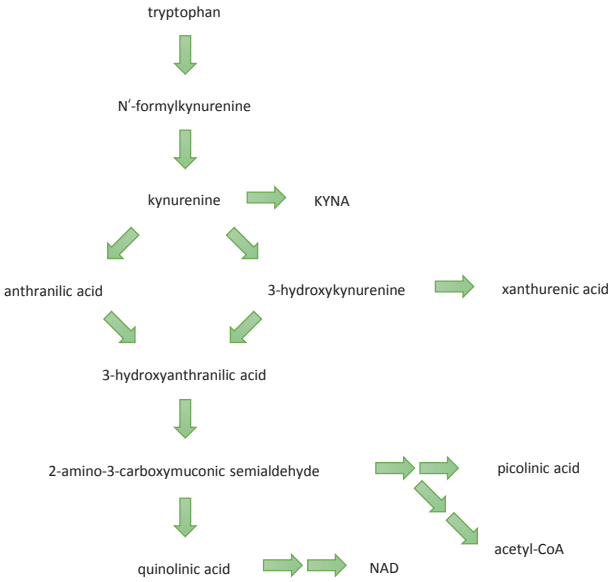

Fig. 7. Kynurenine Pathway

Summary

In conclusion, food metabolomics using LC/MS/MS enabled a comprehensive exploratory analysis of the component(s) that characterize the deterioration of alcoholic drinks.

First Edition：Nov. 2019

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Watch the product video

Enhanced performance

Sensitivity and Robustness

ANALYTICAL INTELLIGENCE

- Automated support functions utilizing digital technology, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher productivity and maximum reliability.
- Allows a system to monitor and diagnose itself, handle any issues during data acquisition without user input, and automatically behave as if it were operated by an expert.
- Supports the acquisition of high quality, reproducible data regardless of an operator's skill level for both routine and demanding applications.

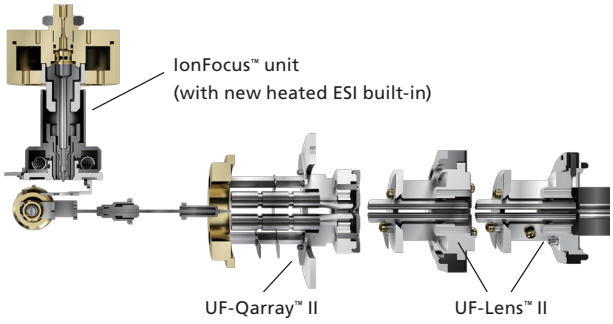
Liquid Chromatograph
Mass Spectrometer

LCMS-8060NX

The LCMS-8060NX is a triple quadrupole mass spectrometer with world-class sensitivity and detection speeds. It boasts increased robustness and ease of use as well as Analytical Intelligence functions to maximize your laboratory's output.

World-class sensitivity and speed

Inheriting the excellent speed and sensitivity of the LCMS-8060, the LCMS-8060NX improves the desolvation efficiency through increasing the ESI heat transfer efficiency and the maximum gas flow rate. Optimum ionization conditions can be set for a wider range of compounds, enabling even higher sensitivity in analysis.



High robustness minimizes downtime

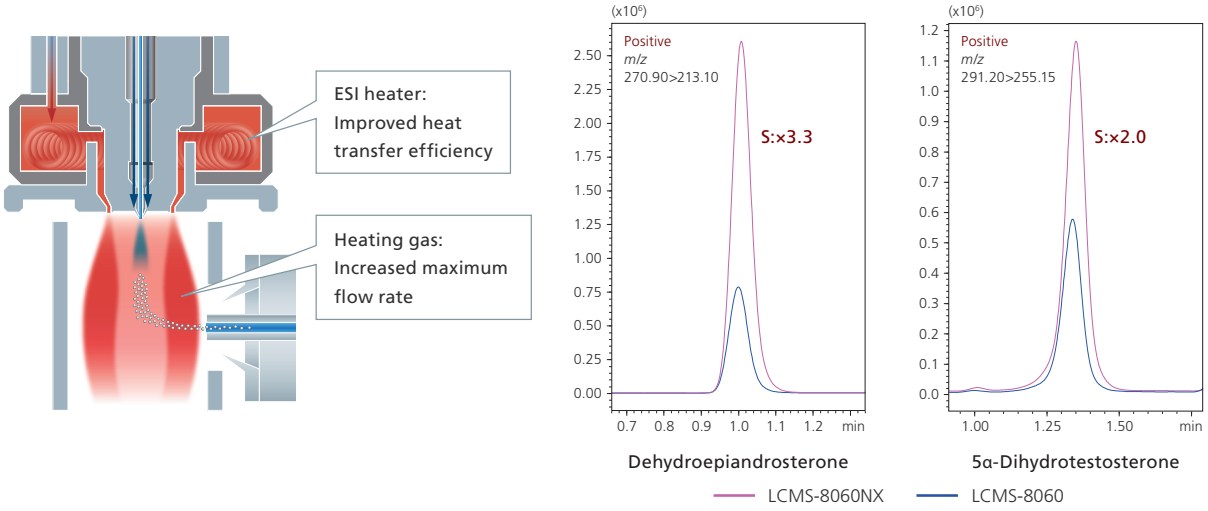
The newly-developed IonFocus™ unit introduces ions into the mass spectrometer with greater efficiency while expelling unneeded neutral particles, reducing matrix effects and contamination inside the instrument. The new ion guide UF-Qarray™ II and the UF-Lens™ II increase the robustness of the instrument while maintaining a high ion transmission rate.

Excellent ease-of-use for greater workflow efficiency

New parameters enable high sensitivity without manual optimization, while features such as automated start-up and shutdown (with LabSolutions Connect™ MRM) allow unattended operation. Combining the MS with the Nexera™ series UHPLC provides multiple Analytical Intelligence functions to further increase the efficiency of your overall workflow.

Increased desolvation efficiency for higher sensitivity

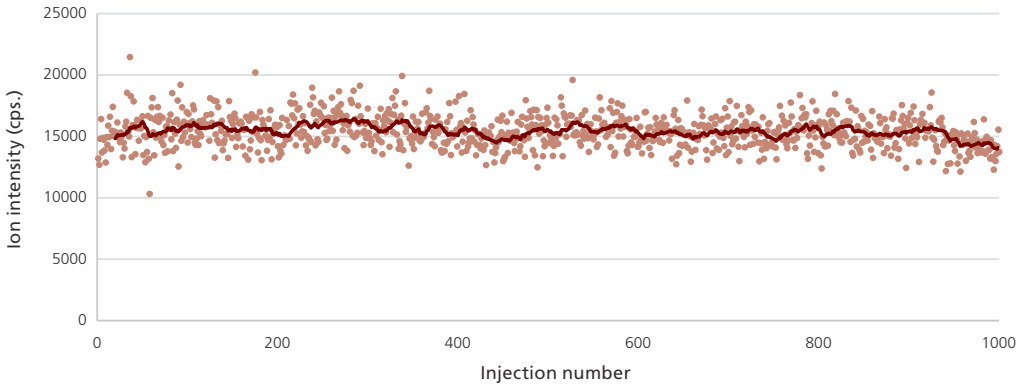
A new heat-assisted design improves the desolvation efficiency and dramatically enhances the sensitivity for challenging molecules such as steroid hormones.



Superior robustness

The LCMS-8060NX employs new ion guides, the UF-Qarray II and the UF-Lens II, to increase robustness while maintaining high ion transmission efficiency. Improved robustness means higher laboratory throughput as maintenance interval times increase.

The urine metabolite data shown below provides confidence for the analysis of biological matrices. After the analysis of 1000 urine samples, prepared only by dilution with water, the LCMS-8060NX delivers superior data stability without loss of sensitivity.



Analytical results of repeated injections of metabolites in urine

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